A small step towards understanding impaired walking development in children with cerebral palsy

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A SMALL STEP TOWARDS UNDERSTANDING IMPAIRED WALKING DEVELOPMENT IN CHILDREN WITH CEREBRAL PALSY

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Amsterdam Movement Sciences

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A SMALL STEP TOWARDS UNDERSTANDING IMPAIRED WALKING DEVELOPMENT IN CHILDREN WITH CEREBRAL PALSY

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Annike Bekius

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No one is too small to make a difference

Greta Thunberg

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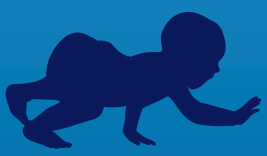


1

General introduction

Many of us walk without conscious effort. Neonates are able to perform a stepping pattern just after birth. Yet, in typically developing (TD) children it takes about a year before they can walk without support. After that, while the body grows, the locomotor patterns develop further and gait is refined and optimized towards a mature pattern. In children with cerebral palsy (CP) walking development is often delayed and may remain incomplete. In my thesis I sought to extend our knowledge of the underlying mechanisms accompanying impaired walking development in children with or at high risk of CP.

Cerebral palsy

Malformation or lesions of an immature brain before birth, around birth or during early post-natal development may give rise to CP. Possible causes for these lesions include loss of oxygen, i.e., hypoxia-ischemia, cortical malformation, infection, and hemorrhage (Zhou, Butler, & Rose, 2017). Premature birth is one of the main risk factors for the development of CP (Odding, Roebroeck, & Stam, 2006). CP affects around 2 in 1000 live births, making it the most common movement disorder in children (Himmelmann & Uvebrant, 2018). The presence of motor asymmetry, delayed motor milestones and/or gait abnormalities, in combination with atypical muscle tone and posture in young children are prime indicators for diagnosis (Wu, Day, Strauss, & Shavelle, 2004).

CP is defined as a group of permanent, albeit not fixed, disorders of the development of movement and posture. It comes with a wide clinical spectrum, from mild to severe motor impairments, with heterogenous expressions, due to variation in the nature and location of brain lesions (Rosenbaum et al., 2007). The variability in functional mobility can be classified using the Gross Motor Function Classification System (GMFCS), with GMFCS I-III indicating that the child is able to walk independently or with support, while in GMFCS IV-V the child uses a wheelchair. There are three motor subtypes of CP: spastic, dyskinetic, and ataxic. The most common one is spastic CP, affecting around 85% of the children with CP. About 12% have dyskinetic CP, and 3% ataxic CP (Himmelmann & Uvebrant, 2018). The distribution of spastic CP can be unilateral or bilateral (Bax et al., 2005). Either way, the type of CP is classified based on clinical features and seemingly depends on the location of the brain lesion.

Neuromuscular deficits in CP include abnormalities such as muscle weakness, spasticity, dystonia impaired selective motor control, and coordination and balance problems. These original impairments can yield secondary musculoskeletal changes including shortened and stiff muscles as well as bony deformations. Together with the rapid body growth of young children, these factors can contribute to walking impairments in CP (Meyns et al., 2016). There is an arsenal of treatments seeking

to maintain or improve functional mobility in CP (Novak et al., 2013), including physical therapy, serial casting (Weide et al., 2019), botulinum toxin type A (BoNT-A) injection (Schless et al., 2019), single-event multilevel orthopedic surgery (SEMLS, Ancillao et al., 2017; Haberfehlner et al., 2018), and selective dorsal rhizotomy (SDR, Bolster et al., 2013; Romei et al., 2018; Summers et al., 2019). Yet, most of these treatments are after the age of two years, to optimize motor development.

The critical period for early interventions may be before the age of two years, when the brain is more plastic and the corticospinal tract is still maturing (Cappellini et al., 2020b; Hadders-Algra, 2014; Novak et al., 2017; Yang et al., 2013). On average, the diagnosis of CP is made around a corrected age of 12 months, with a wide variation (Granild-Jensen, Rackauskaite, Flachs, & Uldall, 2015; Hubermann, Boychuck, Shevell, & Majnemer, 2016). To be able to start interventions early, it is important to identify children at a young age who are at risk of developing CP. A high risk of CP (Hielkema et al., 2010) is determined by the presence of one of the following: cystic periventricular leukomalacia, diagnosed on serial ultrasound assessments of the brain (de Vries, Eken, & Dubowitz, 1992), unilateral or bilateral parenchymal lesion of the brain (de Vries et al., 2001), diagnosed using MRI, term/near-term asphyxia resulting in Sarnat 2 or 3 (Sarnat & Sarnat 1976) with brain lesions on MRI and/or with neurological dysfunction during infancy suggesting the development of CP, or other structural brain damage. Detecting high risk of CP at an early age is important to start interventions early (Morgan et al., 2021), and improve functional mobility at a later stage in these children.

Walking development

Walking development can be described as the maturation of locomotor patterns over time from supported to independent (unsupported) walking. Immediately after birth, neonates can perform unloaded steps. While their body weight is supported they display neonate stepping, which is an irregular pattern with clear alternation between flexors and extensors and a high degree of co-contraction. This stepping pattern typically disappears around 4-6 weeks postnatally and reappears at around 6-8 months as supported walking. Subsequently, it develops towards independent walking (Dominici et al., 2011; Forssberg, 1985; Thelen & Cooke, 1987; Yang, Stephens, & Vishram, 1998). During walking development, body size and mass change, therewith also biomechanical requirements for walking.

Locomotor patterns from infants during the supported walking period, from neonate stepping up to the onset of independent walking, show hyperflexion in hip and knee, resulting in a high foot lift during the swing phase (Dominici, Ivanenko, & Lacquaniti, 2007; Forssberg, 1985; Ivanenko, Dominici, Cappellini, & Lacquaniti, 2005b). TD children start to walk independently between 9 to 18 months of age.

Walking without support requires maintaining balance that, in turn, requires postural control. Toddlers, just after the onset of independent walking, achieve this through short steps with outward pointing toes, a wide base of support, flat foot strike, and/or arms held in a fixed high position. The plantigrade characteristics of the adult locomotor pattern, such as ankle dorsiflexion at heel strike, knee flexion during stance phase, and joint coordination along the gait cycle, are not present during neonate stepping, supported walking, and newly acquired independent walking (Forssberg, 1985). The foot trajectory (i.e., the time course of the vertical foot displacements; Dominici et al., 2007) in toddlers shows a single-peak foot lift, and intersegmental coordination (i.e., the plane of covarying leg elevation angles) is still immature, showing a deviation from planarity and a wider gait loop than in adults (Bianchi, Angelini, Orani, & Lacquaniti, 1998; Borghese, Bianchi, & Lacquaniti, 1996; Cheron et al., 2001; Dominici, Ivanenko, Cappellini, Zampagni, & Lacquaniti, 2010; Ivanenko, Dominici, & Lacquaniti, 2007). During the development of the toddler locomotor pattern towards a mature adult gait pattern, one can observe a maturation of the foot trajectory towards a double-peaked trajectory with minimum foot clearance during mid-swing (Dominici et al., 2007; Forssberg, 1985), as well as rapid maturation of intersegmental coordination of the lower limb segments in the first few months after the onset of independent walking (Cheron et al., 2001; Dominici et al., 2011; Ivanenko et al., 2004a, 2007).

Muscles activation patterns from infants during the supported walking period show wide muscle contraction durations and excessive antagonist muscle co-contraction during stance, as well as a large peak around 20-30 ms after foot contact in most muscles (Leonard, Hirschfeld, & Forssberg, 1991). This peak may be a result of hyperactivity of stretch reflexes (Forssberg, 1985), or of nonplantigrade gait (Ivanenko et al., 2007). After the onset of independent walking, muscle contraction durations decrease, and the timing and amplitude of activation per muscle across the gait cycle changes, resulting in more asynchronous and reciprocal muscle activations (Cappellini et al., 2016; Dominici et al., 2011; Ivanenko et al., 2013; Leonard et al., 1991; Okamoto, Okamoto, & Andrew, 2003).

Developmental stages of locomotion usually occur at later ages in children with CP compared to TD children (Largo, Molinari, Weber, Pinto, & Duc, 1985; Meyns et al., 2012a). Maturation of the foot trajectory, intersegmental coordination (Cappellini et al., 2016), and muscle activation patterns (Berger, 1998; Cappellini et al., 2016) is delayed or less pronounced in children with CP who walk independently. The locomotor and muscle activation patterns in these children were found to be similar to TD children during supported walking (Leonard et al., 1991). After the onset of independent walking, children with CP often do not mature towards an adult walking pattern, but they retain some characteristics of the immature toddler

pattern, for example a flat foot landing, a lack of dorsiflexion during swing, and increased coactivation of the antagonist muscles (Berger, Altenmueller, & Dietz, 1984; Berger, Quintern, & Dietz, 1982; Cappellini et al., 2016; Leonard et al., 1991).

The maturation of locomotor patterns is thought to result from the maturation of gait-specific neural circuitries, involving sensory integration of the supraspinal and spinal network (Yang & Gorassini, 2006; Yang et al., 1998). Moreover, environmental feedback and musculoskeletal constraints may impact the maturation of locomotor patterns (Thelen & Cooke, 1987; Thelen, Ulrich, & Wolff, 1991). The maturation of locomotor patterns may be a combination of changes in gait kinematics, as described above, together with changes in neuromuscular control (Dewolf, Sylos-Labini, Cappellini, Lacquaniti, & Ivanenko, 2020).

Neuromuscular control

Walking requires refined activation of numerous muscles. In order to coordinate the activation, the central nervous system (CNS) is hypothesized to group muscles and, hence, to control (a small number of) muscle groups rather than individual muscles, drastically reducing the dimensionality of neuromuscular control. Such muscle groups are referred to as muscle synergies, motor primitives or motor modules. That is, a synergy contains multiple muscles though a muscle can contribute to multiple synergies (Bizzi & Cheung, 2013; Dominici et al., 2011; Hart & Giszter, 2010; Ivanenko, Cappellini, Dominici, Poppele, & Lacquaniti, 2005a). Muscle synergies can be extracted from electromyography (EMG) data that reflects the activation of a single muscle using, e.g., non-negative matrix factorization (NMF, Lee & Seung, 1999). NMF decomposes the EMG signal of multiple muscles into modes that contain a temporal activation pattern and corresponding spatial weighting coefficients for every muscle, i.e., synergy weights (Figure 1.1).

Muscle synergy analysis served to investigate neuromuscular control during walking in both healthy and patient populations. In TD children, the number of synergies increases from two during neonate stepping to four in children who just started to walk independently (Dominici et al., 2011; Sylos-Labini et al., 2020), suggesting that primitive locomotor patterns in TD children during supported walking are retained, while new patterns are added during development. Healthy adults seem to recruit four or five muscle synergies during walking, also accounting for different speeds and stride-to-stride variability (Cappellini, Ivanenko, Poppele, & Lacquaniti, 2006; Clark, Ting, Zajac, Neptune, & Kautz, 2010; Ivanenko, Poppele, & Lacquaniti, 2004b; Tang et al., 2015). In post-stroke individuals, the number of muscle synergies appears reduced compared to unimpaired individuals, likely reflecting a 'simplified' control strategy of the CNS after stroke (Clark et al., 2010). Likewise, children with CP have been reported to recruit fewer synergies

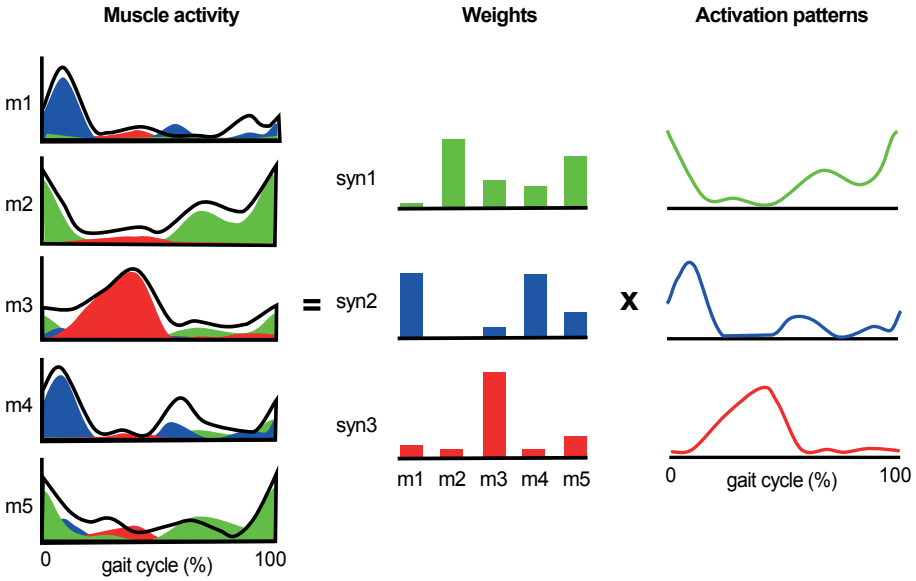


Figure 1.1 Schematic of muscle synergy extraction. The muscle activity along the gait cycle of five muscles (m1-m5) is decomposed into three muscle synergy modes (syn1, syn2, and syn3) containing spatial weighting coefficients for every muscle (weights) and temporal activation patterns. Adapted from data acquired in Bach, Daffertshofer, and Dominici (2021b).

compared to TD children (Goudriaan et al., 2018; Hashiguchi et al., 2018; Kim, Bulea, & Damiano, 2018b; Short, Damiano, Kim, & Bulea, 2020; Shuman et al., 2016; Shuman, Goudriaan, Desloovere, Schwartz, & Steele, 2018, 2019; Shuman, Schwartz, & Steele, 2017; Steele, Munger, Peters, Shuman, & Schwartz, 2019; Steele, Rozumalski, & Schwartz, 2015; Tang et al., 2015; Yu et al., 2019). As opposed to stroke survivors who can typically walk before suffering the attack, children with CP still have to learn to walk after their brain injury, which most likely affects the development of neuromuscular control. It remains unknown how neuromuscular control develops in children with CP from supported to independent walking.

Increased knowledge of the maturation of locomotor patterns, as identified by gait kinematics and neuromuscular control, is of clinical relevance. The earlier CP can be detected in children with early brain lesions, the earlier interventions to improve motor function in these children can be started. Previous research on the effectiveness of neuromuscular control as a predictor of treatment outcomes in children with CP showed mixed results (Oudenhoven et al., 2019b; Shuman et al., 2016, 2018), leaving the question whether gait kinematics and neuromuscular control are directly related or not. In other words, can we improve gait kinematics by adapting neural pathways or vice versa. Evidence for the effectiveness of existing

early interventions seems limited (Damiano & Longo, 2021; Hadders-Algra, Boxum, Hielkema, & Hamer, 2017; Morgan et al., 2016, 2021), due to limited data quantity and methodological quality, and large heterogeneity in study samples. This calls for gaining more knowledge about the underlying mechanisms of early motor development in children at high risk of CP (Cappellini et al., 2020b).

RESEARCH QUESTIONS

My overarching aim was to identify mechanisms that underlie impaired walking development in young children with early brain lesions, who have CP, or who are considered at high risk of developing CP. To achieve this aim, I sought to answer the following research questions:

- How is the development of neuromuscular control affected by early brain lesions in very young children with or at high risk of CP?
- What is the relation between the maturation of gait kinematics and neuromuscular control in children with or at high risk of CP?
- How do methodological choices impact the investigation of neuromuscular control?

I consider answering these questions essential for early detection of motor deficits in CP, and the knowledge gained may open up new vistas on novel early interventions to improve walking ability in children with CP.

OUTLINE THESIS

My thesis contains a set of experimental assessments. Commonly, locomotor patterns and muscle activity have been investigated in terms of kinematics and EMG. The latter served to study neuromuscular mechanisms of early walking development, with muscle synergy analysis as core method.

In **Chapter 2** I systematically reviewed the literature to provide the current status of the research on muscle synergies during walking in children with CP. Findings on quantification, structure and variability of muscle synergies are considered, and the potential of muscle synergy analysis as a clinical method to quantify altered neuromuscular control and to predict clinical outcomes is discussed.

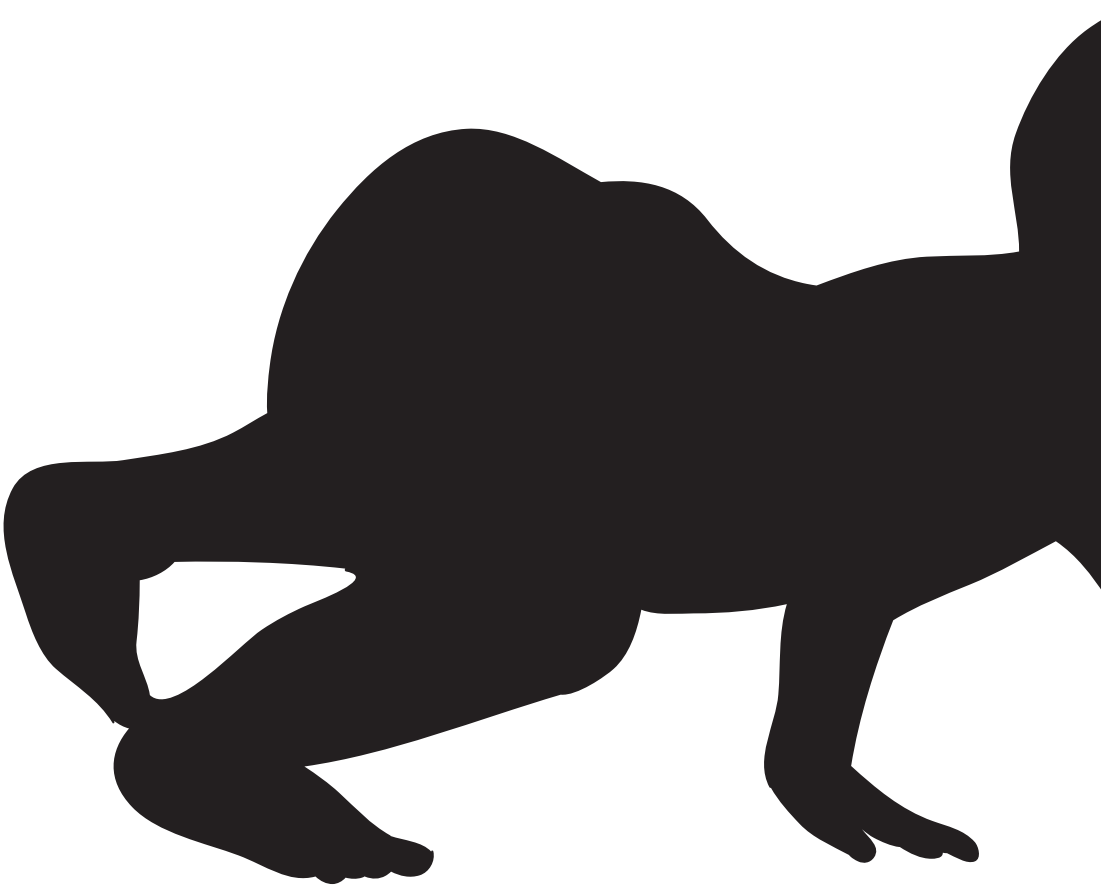
In **Chapter 3** I assessed altered neuromuscular control already in the early phase of motor development, in children with or at high risk of CP. In a cross-sectional design, muscle synergy analysis served to compare neuromuscular control

in children with CP and TD children before and after the onset of independent walking, and to quantify differences between the most and least affected side in children with unilateral and asymmetric bilateral CP.

Chapter 4 builds further on this but I employed a longitudinal design, combining gait kinematic and EMG measures to study walking maturation in three children at high risk of CP, with divergent developmental trajectories. The focus of this research was on development starting before the onset of independent walking, covering a period of one to two years, with the aim to record the emergence of the first independent steps.

Chapter 5 is a methodological advance that supplies data towards a broader scope on top of the conventional muscle synergy analysis. In addition to the calculation of muscle synergies, intermuscular coherence spectra were identified in adults during walking using network analysis during changes in interlimb coordination.

My main findings are summarized and discussed in **Chapter 6**, where I also elaborate on clinical implications of these findings and propose suggestions for future research.



2

Muscle synergies during walking in children with cerebral palsy: a systematic review

Bekius, A., Bach, M. M., van der Krogt, M. M., de Vries, R., Buizer, A. I., and Dominici N. (2020). *Frontiers in Physiology, 11,* 632

ABSTRACT

Background Walking problems in children with cerebral palsy (CP) can in part be explained by limited selective motor control. Muscle synergy analysis is increasingly used to quantify altered neuromuscular control during walking. The early brain injury in children with CP may lead to a different development of muscle synergies compared to typically developing (TD) children, which might characterize the abnormal walking patterns. The overarching aim of this review is to give an overview of the existing studies investigating muscle synergies during walking in children with CP compared to TD children. The main focus is on how muscle synergies differ between children with CP and TD children, and we examine the potential of muscle synergies as a measure to quantify and predict treatment outcomes.

Methods Bibliographic databases were searched by two independent reviewers up to 22 April 2019. Studies were included if the focus was on muscle synergies of the lower limbs during walking, obtained by a matrix factorization algorithm, in children with CP.

Results The majority (n = 12) of the 16 included studies found that children with CP recruited fewer muscle synergies during walking compared to TD children, and several studies (n = 8) showed that either the spatial or temporal structure of the muscle synergies differed between children with CP and TD children. Variability within and between subjects was larger in children with CP than in TD children, especially in more involved children. Muscle synergy characteristics before treatments to improve walking function could predict treatment outcomes (n = 3). Only minimal changes in synergies were found after treatment.

Conclusions The findings in this systematic review support the idea that children with CP use a simpler motor control strategy compared to TD children. The use of muscle synergy analysis as a clinical tool to quantify altered neuromuscular control and predict clinical outcomes seems promising. Further investigation on this topic is necessary, and the use of muscle synergies as a target for development of novel therapies in children with CP could be explored.

INTRODUCTION

Walking is the most common form of locomotion adopted by humans and limbed animals, and it requires the activation of numerous muscles. It has been theorized, that in order to coordinate this complex behavior, the central nervous system controls basic building blocks, referred to as muscle synergies or motor modules, rather than individual muscles. Muscle synergies are defined as temporal basic activation patterns of different groups of muscles with a corresponding weighting coefficient for every muscle. Each synergy contains multiple muscles and every muscle can contribute to multiple synergies (Bizzi & Cheung, 2013; Dominici et al., 2011; Hart & Giszter, 2010; Ivanenko et al., 2005a).

Over the past years, researchers applied muscle synergies as a framework to analyze neuromuscular control in both healthy subjects and individuals with neurological disorders. Generally, muscle synergies are extracted from electromyography (EMG) using matrix factorization algorithms, like the nonnegative matrix factorization (NMF), independent component analysis, or factor analysis (Lee & Seung, 1999). In the healthy population, four or five synergies extracted from a large number of EMGs are required during normal walking and these synergies also account for stride-to-stride variability and various speeds (Cappellini et al., 2006; Clark et al., 2010; Ivanenko et al., 2004b; Tang et al., 2015). The muscle synergies of healthy adults ('mature synergies') are often used as a template to compare the results of synergy analyses of pathological gait. Muscle synergies appear to be altered in the adult population after brain injury. It has been shown that the number of muscle synergies in post-stroke individuals during walking is reduced compared to unimpaired individuals due to merging of the 'mature synergies' observed in healthy adults (Clark et al., 2010). These findings correlate with the degree of motor impairment which might reflect a simplified control strategy of the central nervous system in moderate to severely impaired post-stroke individuals. However, it is unclear whether and how this change in synergy organization can be generalized to other clinical populations and how it relates to gait abnormality.

Cerebral palsy (CP) is a neurodevelopmental motor disorder caused by non-progressive lesions in an immature brain (Himmelmann, Hagberg, Beckung, Hagberg, & Uvebrant, 2005). CP has a wide clinical spectrum, with mobility varying from walking without aids, to being completely wheelchair dependent. The Gross Motor Function Classification System (GMFCS) is used to classify functional mobility in CP. Various diagnostic subtypes exist, based on motor type and distribution of CP, that is, spastic, dyskinetic, or ataxic, and unilateral or bilateral CP, respectively (Bax et al., 2005). Individuals with CP who learn to walk, do so after their brain injury, in contrast to adult stroke survivors who have years of

walking experience prior to the brain lesion. In typically developing (TD) children, the number of basic muscle activation patterns increases from two in stepping neonates to four in toddlers, just after their first independent steps (Dominici et al., 2011). The early brain injury in children with CP may lead to a different development of muscle synergies, which might be an underlying factor of abnormal walking patterns. Studies on muscle synergies in children with CP are scarcer than in stroke and have used diverse methods to calculate synergies. Methodological choices in factorization methods, filtering conditions, the number of muscles recorded, and the recording quality appear to influence the outcomes of the synergy calculations (Santuz, Ekizos, Janshen, Baltzopoulos, & Arampatzis, 2017; Shuman et al., 2017; Steele, Tresch, & Perreault, 2013).

Several types of treatment exist to improve gait quality and functional mobility in children with CP. Recent research has identified the possibility that muscle synergies can predict effectiveness of therapies in children with CP (Oudenhoven, et al., 2019b; Shuman et al., 2016, 2018). A better insight into the neuromuscular control mechanisms underlying the altered muscle activation patterns in children with CP could possibly help to improve therapy choices and functional mobility outcomes. In addition, more knowledge about these mechanisms can be important for the interpretation of clinical signs of CP at an early age, improve indication for therapy in individual patients, and might even be used to develop new diagnostic tools (Cheung et al., 2012).

The present systematic review aims to give an overview of the existing studies investigating muscle synergies in children with CP during walking to evaluate the current knowledge on this topic. The primary aim is to examine how muscle synergies in children with CP differ from those exhibited by TD children during walking by investigating the quantification and structure of synergies, and the variability of synergies between and within children with CP. Second, we aim to examine the predictability of treatment outcomes using muscle synergy characteristics, and the effect of treatment on muscle synergies in children with CP.

MATERIALS AND METHODS

A systematic review protocol was developed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-statement (www.prisma-statement.org). It is registered on PROSPERO and can be accessed online (number: CRD42019149109).

Search strategy

A comprehensive search was done in the bibliographic databases PubMed, Embase.com, and Web of Science (Core collection), in collaboration with a medical librarian (RV). Databases were searched up to 22 April 2019. The following terms were used including synonyms and closely related words as index terms or free-text words: "Muscle synergy", "Cerebral palsy", "Typically developing", "Children", "Walking". The search was performed without date, language, or publication status restriction.

Study selection

Studies were eligible for inclusion if they met the following inclusion criteria: (1) children with CP younger than 19 years old, and in case of mixed populations: the majority of the investigated population younger than 19, (2) the focus of the study was on muscle synergies of the lower limb during walking, (3) use of a matrix factorization algorithm to obtain the muscle synergies. Studies were excluded if, (a) it was a conference abstract, (b) it was a conference paper, but a full paper was published afterwards, (c) the study focused on muscle synergies of the upper limb, and (d) the article was a review or protocol.

After exclusion of duplicate articles, two independent reviewers (AB and MB) performed a title and abstract screening on the residual articles. Thereafter, the reviewers assessed the eligibility of the remaining articles in a full-text screening. Any in- and exclusion conflict among the reviewers was discussed until a consensus was reached. Study designs were defined as being either a cross-sectional, case-control, or retrospective cohort study. Methodological quality and risk of bias of the included articles was assessed using the Downs and Black checklist by the same two independent reviewers (Downs & Black, 1998). In the original scale it is possible to score up to 32 points, but we used a modified version that was applicable for the types of studies included in this systematic review, as has been done in other reviews using the Downs and Black scale (Gorber, Tremblay, Moher, & Gorber, 2007; Hebert-Losier, Newsham-West, Schneiders, & Sullivan, 2009). This left a maximum total score of 14 points for cross-sectional studies, and 15 points for case-control and cohort studies. Each study was assigned a grade of "excellent" (13–15 points), "good" (10–12 points), "fair" (7–9 points) or "poor" (<7 points). Any disagreements in grading among the reviewers was discussed until consensus was reached. Articles were not excluded based on poor quality, but this played a role in the overall assessment of the article in the review.

Data extraction and analysis

Data extraction of the included articles was performed independently by AB and MB. Subject characteristics (age, CP type and distribution, GMFCS), study methods (number of strides analyzed, number of muscles recorded, EMG pre-processing steps, analysis criteria), and outcome measures (muscle synergies) were summarized in a table. The main outcome measures analyzed in this review were: (1) quantification of muscle synergies during walking, such as total number of synergies, VAF_1 and walk-DMC, and (2) the spatial and temporal structure of muscle synergies during walking. These outcome measures were assessed in both children with CP and TD children, and pre- and post-treatment in some studies. In addition, variability in number and structure of synergies between and within subjects in the group of children with CP was evaluated.

RESULTS

Study selection

The electronic search in the cited databases and manual searching of reference lists identified 1127 articles, plus 2 references via additional sources (Figure 2.1). After duplicate removal, 682 articles were screened on title and abstract, from which 617 were excluded, mostly because of differing target populations (e.g. animals, other diagnosis, or age) or study design (i.e. no muscle synergy analysis during walking involved). Full-text screening of 65 articles left a total of 16 articles that were selected for this review, reasons for exclusion of 49 articles are noted in Figure 2.1.

Study characteristics

Twelve of the 16 articles compared children with CP with TD children, four included only children with CP. The studies varied in sample size, from 3 to 549 children with CP and 8 to 84 TD children. All studies included children with age ranged from 1 to 16 years, in only one study (Steele et al., 2015) older individuals with CP were also included. All studies included children with spastic CP, except for one that included one dyskinetic child (Tang et al., 2015), and GMFCS levels varied from I to IV. An overview of all studies is given in Table 2.1.

Risk of bias

Results of the methodological quality assessment are presented in Table 2.2. Eight studies used a cross-sectional design (Cappellini et al., 2016, 2018; Goudriaan et al., 2018; Hashiguchi et al., 2018; Kim et al., 2018b; Tang et al., 2015; Torricelli et al., 2014; Yu et al., 2019), five used case-control designs (Shuman et al., 2016,

2017, 2019; Steele et al., 2015, 2019), and three were retrospective cohort studies (Oudenhoven et al., 2019b; Schwartz et al., 2016; Shuman et al., 2018). Quality scores ranged from 5 to 13, one study received the grade "poor" (Torricelli et al., 2014), ten studies "fair" (Cappellini et al., 2016, 2018; Hashiguchi et al., 2018; Kim et al., 2018b; Shuman et al., 2016, 2018, 2019; Steele et al., 2019; Tang et al., 2015; Yu et al., 2019), three studies "good" (Goudriaan et al., 2018; Shuman et al., 2017; Steele et al., 2015), and two studies "excellent" (Oudenhoven et al., 2019b; Schwartz et al., 2016).

Calculation of synergies

All studies used NMF to obtain the muscle synergies from the original (processed) muscle activity. Muscle activity was recorded during over-ground walking using surface EMG in all cases, 4 to 11 muscles were included per leg, as specified in Table 2.3. The raw EMG data was most commonly processed using the following steps: high-pass filtered, demeaned (optional), rectified, low-pass filtered, amplitude scaled, and time-normalized. NMF has non-negative constraints, meaning that the original EMG data cannot be negative, and the most used algorithm is the "multiplicative update rule" algorithm presented by Lee and Seung (1999).

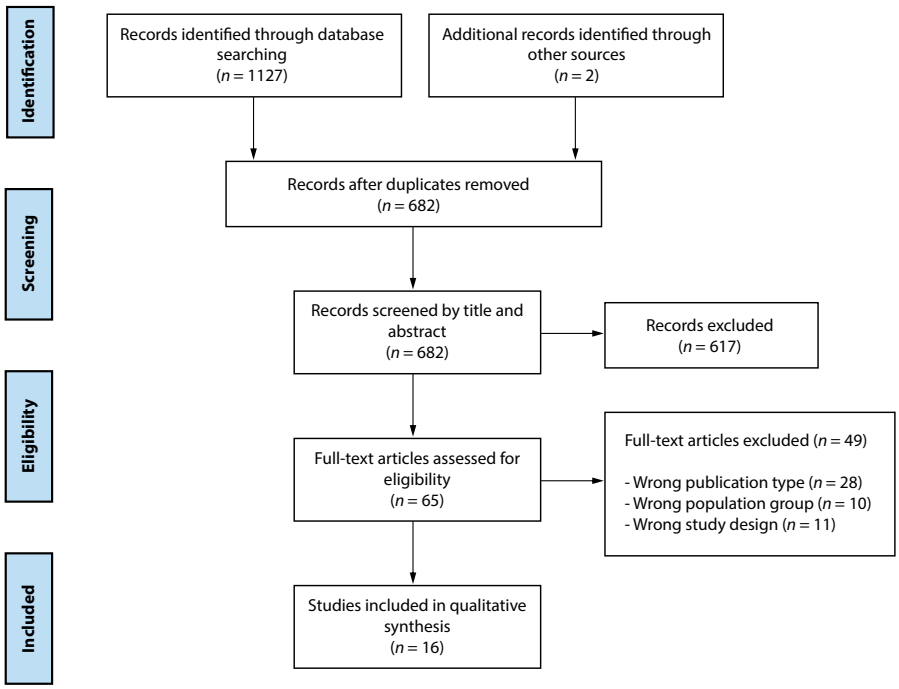


Figure 2.1 Flow chart article selection

Table 2.1 Summary of the selected study characteristics

Study	Subjects	Age (yrs)	CP type & distribution	GMFCS	N strides analyzed	N muscl (=total)	EMG pre-processing	Analysis criteria	Synergies			
									Total N	VAF_1 (%)	Walk-DMC	Structure
Cappellini et al. (2016)	CP: 35 TD: 33	CP: 2.3-11.8 TD: 1.0-11.8	Spastic (16 uni, 19 bi)	I, II, III	CP: 50±24 TD: 72±28	11 bi (=22)	HP: 30 Hz, Demeaned, LP: 10 Hz	RMSE of VAF vs. n curve<10^{-4}	CP: 4 TD: 4	-	-	Temporal: CP ≠ TD
Cappellini et al. (2018)	CP: 14 TD: 14	CP: 3.0-11.1 TD: 3.3-11.8	Spastic (5 uni, 9 bi)	I, II	CP: 35±5 TD: 27±3	11 bi (=22)	HP: 30 Hz, LP: 10 Hz	RMSE of VAF vs. n curve<10^{-4}	CP: 4 TD: 4	-	-	Temporal: CP ≠ TD
Hashiguchi et al. (2018)	CP: 13 TD: 10	CP: 12.8±3.8 TD: 13.4±0.5	NG	I, II, III	5	8 uni (=8)	BP: 20-250 Hz, LP: 10 Hz	VAF>90%	CP: 55%=2, 30%=3, 15%=4 TD: 10%=3, 60%=4, 30%=5	-	-	-
Tang et al. (2015)	CP: 12 TD: 8 AD: 10	CP: 5.8 (3.7-9.0) TD: 6.1 (4.5-9.2) AD: 24.5 (23-26)	Spastic (2 uni, 9 bi) 1 Dysk	I, II, III, IV	>20	8 bi (=16)	HP: 50 Hz, Demeaned, LP: 10 Hz	VAF>95%	CP: 37.5%=2*, 29.2%=3*, 33.3%=4* TD: 31.2% =3, 68.8% =4 AD: 100% =4	-	-	CP ≠ TD&AD SCA: CP=57.0±16.8, TD=84.2±11.8, AD=95.7±2.0
Yu et al. (2019)	CP: 18 TD: 8	CP: 4.4 (2.3-6.5) TD: 4.4±1.4	Spastic (bi)	I, II, III	8 (NMF on each stride separately)	8 bi (=16)	HP: 50 Hz, Demeaned, LP: 10 Hz	VAF_4	CP: GMFCS I/II=4, GMFCS III=3 TD: 4	-	-	Spatial: CMFCS I/II = TD GMFCS III TD Temporal: CP ≠ TD
Torricelli et al. (2014)	CP: 3	15, 14, 14	Spastic (bi)	II	>3	8 bi (=16)	BP: 20-400 Hz, Demeaned, LP: 5 Hz	VAF>90%	2	-	-	CP ≠ AD
Shuman et al. (2017)	CP: 113 TD: 73	CP: I: 10.4±4.8, II: 10.9±5.8, III: 12.2±9.4 TD: 10.3±3.5	Spastic (bi)	I, II, III	NG	5 bi (=10)	HP: 40 Hz, LP: 4, 6, 8, 10, 20, 30, 40 Hz	VAF>90% VAF_1 Walk-DMC Different LP cut-offs	**LP 4 Hz** CP: 2.1±0.6 TD: 2.9±0.4 **LP 40 Hz** CP: 2.9±0.4 TD: 3.4±0.5	**LP 4 Hz** CP: I=80†, II=84†, III=88† TD: 72 **LP 40 Hz** CP: I=75†, II=78†, III=82† TD: 62.4	**LP 4 Hz** CP: I=82†, II=75†, III=64† TD: 100 **LP 40 Hz** CP: I=84†, II=77†, III=67† TD: 100	-

(Continued)

Table 2.1 Continued

Study	Subjects	Age (yrs)	CP type & distribution	GMFCS	N strides analyzed	N muscl (=total)	EMG pre-processing	Analysis criteria	Synergies			
									Total N	VAF_1 (%)	Walk-DMC	Structure
Steele et al. (2019)	CP: 20	10.4 (6.2-13.6)	Spastic (bi)	I, II, III	>3	5 bi (=10)	HP: 25 Hz, LP:10 Hz	VAF>95% VAF_1	3.1 (range 2-4)	81.4±5.5	-	-
Shuman et al. (2019)	CP: 147 TD:31	CP: BoNT-A: 6.8±2.9, SDR: 9.3±2.0, SEMLS: 12.1±3.1 TD: 9.3±2.8	Spastic (33 uni, 144 bi)	I, II, III	NG	8 bi (=16)	HP: 20 Hz, LP: 10 Hz	VAF>90% VAF_1 Walk-DMC	CP: 2.8±0.6 TD: 4.2±0.4	CP (pre-treatment): BoNT-A: 79.1±6.2, SDR: 80.1±4.9, SEMLS: 80.2±5.9 TD: 64.4±3.1	Improved post-treatment	Spatial & temporal: Pre-treatment CP ≠ TD
Oudenhoven et al. (2019b)	CP: 36	7.2 (4-13)	Spastic (bi)	I, II, III	3	5 bi (=10)	HP: 20 Hz, LP: 2 Hz	VAF>90%	Higher N = better treatment outcomes	No correlation with treatment outcomes	-	-
Kim et al. (2018b)	CP: 20 TD: 8	CP: 12.5±3.3 TD: 12.0±2.6	Spastic (17 uni, 3 bi)	I, II	5 (NMF on each stride separately)	8 bi (=16)	HP: 35 Hz, LP: 5 Hz	VAF>90% VAF_1 Walk-DMC	Mean per stride CP: 3.4±0.3 TD: 3.8±0.2	CP: 71±4 TD: 61±3	CP: 65±14.2 (40.2-91.3) TD: 100±10 (85.1-113.0)	Spatial: CP = TD Temporal: CP ≠ TD
Steele et al. (2015)	CP: 549 TD: 84	CP: 9.8 (7.4-13.3)# TD: 10.3 (7.6-13.0)#	Spastic (122 uni, 427 bi)	I, II, III, IV	1	5 bi (=10)	BP: 20-400 Hz, LP: 10 Hz	VAF>90%	CP: >80%=1 or 2 TD: >60%=3	CP: 84.2 (83.7-84.7) TD: 74.6 (71.3-76.1)	CP: 86.2 (85.5-86.9) TD: 100 (97.9-102.1)	Spatial: CP = TD Temporal: CP ≠ TD
Shuman et al. (2016)	CP: 5 TD: 6	CP: 10.2 (6.0-13.0) TD: 10.3 (6.0-13.0)	Spastic (2 uni, 3 bi)	I	CP: 47.5±19.6 (24-81) TD: 44.8±15.9 (25-78)	8 bi (=16)	HP: 40 Hz, LP: 4 Hz	VAF_1	-	CP: 77.2±4.1 TD: 68.4±2.3	-	-
Goudriaan et al. (2018)	CP: 15 TD: 15 DMD: 15	CP: 8.9 (7.6-9.8)# TD: 8.6 (7.3-10.0)# DMD: 8.7 (6.8-9.9)#	Spastic (8 uni, 7 bi)	I, II	10	8 bi (=16)	BP: 20-450 Hz, LP: 10 Hz	VAF_1	-	CP: 74 TD: 65 DMD: 60	-	-
Schwartz et al. (2016)	CP: 473	7.7±3.3	NG	I, II, III	>4	8 bi (=16)	NG	Walk-DMC	-	-	Higher walk-DMC pre- treatment = better outcomes	-

(Continued)

Table 2.1 Continued

Study	Subjects	Age (yrs)	CP type & distribution	GMFCS	N strides analyzed	N muscl (=total)	EMG pre-processing	Analysis criteria	Synergies			
									Total N	VAF_1 (%)	Walk-DMC	Structure
Shuman et al. (2018)	**Centre 1** CP: 473 TD: 84 **Centre 2** CP: 163 TD: 12	**Centre 1** CP: 7.5±3.4 **Centre 2** CP: 9.3±2.7	NG	I, II, III	NG	**Centre 1** 8 bi (=16) **Centre 2** 4 bi (=8)	HP: 20 Hz, LP: 10Hz	Walk-DMC	-	-	CP < TD Higher walk-DMC pre-treatment = better outcomes	-

Abbreviations: CP, cerebral palsy; TD, typically developing; AD, adults; DMD, duchenne muscular dystrophy; Uni, unilateral; Bi, bilateral; Dysk, dyskinetic; GMFCS, gross motor function classification system; N, number; muscl, muscles; HP, high-pass filter, LP, low-pass filter; NMF, non-negative matrix factorization; VAF, variance accounted for; VAF_1, variance accounted for by one synergy (%); RMSE, root mean square error; Walk-DMC, dynamic motor control index during walking; SCA, synergy comprehensive assessment; NG, not given; BoNT-A, Botulinum Toxin Type A; SDR, Selective Dorsal Rhizotomy; SEMLS, Single-Event Multilevel Surgery. Age and number of strides are given as mean (± 1 standard deviation or the range when provided by the authors) unless marked by a #, as this signifies the median (25^{th}-75^{th} percentile). * Values in figure and text are not in agreement, so these values are extracted from the figure; † Signifies that values are extracted from graphical representations and are not precise.

Table 2.2 Results of methodological quality assessment

Study	Study design	Reporting										Ext. validity			Int. validity - bias							Int. validity - confounding						Power	Total score	Grade
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27		
Cappellini et al. (2016)	Cross-sectional	1	1	1			1	1			0	0*	0*				1		1		1	1	0*					0	9	Fair
Cappellini et al. (2018)	Cross-sectional	1	1	1			1	1			0	0*	0*				1		1		1	1	0*					0	9	Fair
Goudriaan et al. (2018)	Cross-sectional	1	1	1			1	1			0	0*	0*				1		1		1	1	0*					1	10	Good
Hashiguchi et al. (2018)	Cross-sectional	1	1	1			1	1			0	0*	0*				1		1		1	1	0*					0	9	Fair
Kim et al. (2018b)	Cross-sectional	1	1	1			1	1			1	0*	0*				1		1		1	0*	0*					0	9	Fair
Tang et al. (2015)	Cross-sectional	1	1	1			1	1			0	0*	0*				1		1		1	0*	0*					0	8	Fair
Torricelli et al. (2014)	Cross-sectional	1	1	0			1	0			0	0	0				1		0		1	0*	0*					0	5	Poor
Yu et al. (2019)	Cross-sectional	1	1	1			1	1			1	0*	0*				1		1		1	0*	0*					0	9	Fair
Shuman et al. (2016)	Case-control	1	1	0			1	1			1	0*	0*				1	0	1		1	0*	0*					0	8	Fair
Shuman et al. (2017)	Case-control	1	1	1			1	1			0	0*	1				1	0*	1		1	1	0*					0	10	Good
Shuman et al. (2019)	Case-control	1	1	0			1	1			1	0*	0*				1	0*	1		1	1	0*					0	9	Fair
Steele et al. (2015)	Case-control	1	1	1			1	1			0	1	1				1	0*	1		1	1	1					0	12	Good
Steele et al. (2019)	Case-control	1	1	0			1	1			0	0*	0*				1	1	1		1	1	0*					0	9	Fair
Schwartz et al. (2016)	Retrospective cohort	1	1	1			1	1			0	1	1				1	1	1		1	1	1					0	13	Excellent
Shuman et al. (2018)	Retrospective cohort	1	1	1			1	1			0	0	0*				1	0*	1		1	1	0*					0	9	Fair
Oudenhoven et al. (2019b)	Retrospective cohort	1	1	1			1	1			0	1	1				1	1	1		1	1	1					0	13	Excellent

1: yes; 0:no; 0*: unable to determine; Ext.: external; Int.: internal; maximum total scores: cross-sectional = 14; case-control and cohort = 15.

Table 2.3 Overview of the recorded and analyzed muscles

Study	Muscles	Recorded sides	Analyzed sides	N analyzed muscles in NMF
Cappellini et al. (2016)	TA, SOL, GL, GM, RF, VM, VL, ST, BF, TFL, GLMax	Bi	Both legs separately	11 for each leg
Cappellini et al. (2018)	TA, SOL, GL, GM, RF, VM, VL, ST, BF, TFL, GLMax	Bi	Both legs separately	11 for each leg
Hashiguchi et al. (2018)	TA, SOL, GL, RF, VM, ST, BF, GLMed	Uni	Most affected leg	8
Tang et al. (2015)	TA, SOL, GL, VL, RF, ST, BF, TFL	Bi	Both legs separately	8 for each leg
Yu et al. (2019)	TA, SOL, GL, VL, RF, ST, BF TFL	Bi	Both legs separately	8 for each leg
Torricelli et al. (2014)	TA, GM, VM, VL, RF, AL, ST, BF	Bi	Both legs separately	8 for each leg
Shuman et al. (2017)	TA, GM, RF, ST, BF	Bi	Random leg	5
Steele et al. (2019)	TA, GM, VL, RF, ST	Bi	Both legs separately	5 for each leg
Shuman et al. (2019)	TA, SOL, GM, RF, VL, ST, BF, GLMed	Bi	Most affected or random leg	8
Oudenhoven et al. (2019b)	TA, GM, RF, VL, ST	Bi	Most affected leg	5
Kim et al. (2018b)	TA, GM, RF, ST	Bi	Both legs combined	8
Steele et al. (2015)	TA, GM, RF, ST, BF	Bi	Uni CP: most affected leg Other individuals: random leg	5
Shuman et al. (2016)	TA, SOL, GL, RF, VL, ST, BF, GLMed	Bi	Uni CP: most affected leg TD & Bi CP: both legs	8 or 16
Goudriaan et al. (2018)	TA, GM, RF, ST, GLMed	Bi	Most affected or involved leg	5
Schwartz et al. (2016)	TA, GM, RF, ST	Bi	NG	8
Shuman et al. (2018)	**Centre 1:** TA, GM, RF, ST **Centre 2:** TA, SOL, GM, RF, VL, ST, BF, GLMed	Bi	Most affected or random leg	**Centre 1:** 4 **Centre 2:** 8

Abbreviations: N, number; TA, tibialis anterior; SOL, soleus; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; RF, rectus femoris; VM, vastus medialis; VL, vastus lateralis; ST, semitendinosus; BF, biceps femoris; TFL, tensor fasciae latae; AL, adductor longus; GLMax, gluteus maximus; GLMed, gluteus medius; Uni, unilateral; Bi, bilateral; NMF, non-negative matrix factorization; NG, not given.

Quantification of synergies

The quantification of synergies was often done post-hoc based on the variance of EMG activity accounted for (VAF). VAF is a measure of the quality of the EMG reconstruction based on the selected number of muscle synergies. Twelve of the sixteen studies included in this review reported the total number of synergies during walking using a certain VAF threshold (Cappellini et al., 2016, 2018; Hashiguchi et al., 2018; Kim et al., 2018b; Oudenhoven et al., 2019; Shuman et al., 2017, 2019; Steele et al., 2015, 2019; Tang et al., 2015; Torricelli et al., 2014; Yu et al., 2019). Out of these articles, nine compared the number of synergies between individuals with CP and unimpaired individuals (Cappellini et al., 2016, 2018; Hashiguchi et al., 2018; Kim et al., 2018b; Shuman et al., 2017, 2019; Steele et al., 2015; Tang et al., 2015; Yu et al., 2019), while the remaining three only included children with CP in their study (Oudenhoven et al., 2019b; Steele et al., 2019; Torricelli et al., 2014). Despite a difference in VAF threshold, number of subjects, and number of recorded muscles (see Table 2.3), the majority of studies found that children with CP recruited fewer synergies (range 1-4) compared to TD children (range 3-4) or healthy adults (all 4) on average when comparing the number of synergies during walking (Hashiguchi et al., 2018; Kim et al., 2018b; Shuman et al., 2017; Steele et al., 2015; Tang et al., 2015; Torricelli et al., 2014; Yu et al., 2019). In contrast, Cappellini et al. (2016, 2018) found that both children with CP and TD children recruited 4 synergies. They used a linear regression procedure that plots the VAF against the number of synergies and finds the smallest number for which the root mean square error of the corresponding linear fit is smaller than 10^{-4} (d'Avella, Portone, Fernandez, & Lacquaniti, 2006). The authors show that this corresponds to a VAF > 80% for all subjects.

Six studies reported VAF_1, the variation of EMG activity that can be explained by just one synergy, which is another parameter computed to study the complexity of the locomotor behavior (Goudriaan et al., 2018; Kim et al., 2018b; Shuman et al., 2016, 2017; Steele et al., 2015, 2019). Five of the six studies found that the average VAF_1 was significantly larger in children with CP (range 71.0-84.2%) compared to TD children (range 61.0-74.7%, see Table 2.1). Steele et al. (2019) did not compare with TD children, but showed that VAF_1 was 81.4 ± 5.5% for children with CP.

Three studies reported the Dynamic Motor Control index during walking (walk-DMC; Kim et al., 2018b; Shuman et al., 2017; Steele et al., 2015), which is associated to VAF_1, for comparisons of muscle synergies between children with CP and TD children. Walk-DMC transforms VAF_1 to a z-score with respect to TD children. A score of 100 signifies the average walk-DMC of TD children and each 10-point interval is one standard deviation. Steele et al. (2015) proposed this measure as a clinical tool to quantify altered neuromuscular control, in order to plan treatments and

predict clinical outcomes. In agreement with the results on VAF_1, all three studies found significantly lower walk-DMC values in children with CP (range of averages 65.0-86.2) compared to TD children (average 100; Kim et al., 2018b; Shuman et al., 2017; Steele et al., 2015). One of these studies showed that an increase in low-pass filter cut-off frequency from 4 to 40 Hz caused an increase in the total number of synergies, and a decrease in VAF_1 in both children with CP and TD children. However, it had no effect on walk-DMC, since this measure normalizes VAF_1 to a z-score (Shuman et al., 2017).

Structure of synergies

Eight studies compared the structure of synergies in terms of the results on temporal and spatial patterns between children with CP and controls (Cappellini et al., 2016, 2018; Kim et al., 2018b; Shuman et al., 2019; Steele et al., 2015; Tang et al., 2015; Torricelli et al., 2014; Yu et al., 2019). Two studies found that the spatial structure of synergies of children with CP was different from healthy adults ('mature synergies'; Tang et al., 2015; Torricelli et al., 2014), as was assessed by Tang et al. (2015) using a model called synergy comprehensive assessment. In addition, Tang et al. (2015) showed that the spatial structure of synergies in children with CP was different from TD children, and that a large variation in synergy structure was present in the CP group. The majority of children with CP showed a combination of 'mature synergies' and synergies specific to CP, however none of the affected legs in children with unilateral CP showed merely 'mature synergies'.

Six studies found that the spatial structure of synergies in both children with CP and TD children was related to that of 'mature synergies', but that the temporal structure differed between children with CP and TD children (Cappellini et al., 2016, 2018; Kim et al., 2018b; Shuman et al., 2019; Steele et al., 2015; Yu et al., 2019). These studies found differences in the duration and shifts of the peaks of the temporal patterns within the gait cycle in children with CP compared to TD children. In addition, Yu et al. (2019) showed larger co-activation between synergies and higher variability of the temporal patterns within groups (GMFCS I and II), in children with CP compared to TD children.

Between-subject variability

Five studies discussed the muscle synergy differences within the heterogenous CP group (see Table 2.4). The relation between the severity of CP and muscle synergies was examined comparing between different distribution of CP, i.e. uni- or bilateral (Steele et al., 2015; Tang et al., 2015), and levels of impairment of functional mobility, as represented by GMFCS scores and/or Gillette Functional Assessment Questionnaire (Novacheck, Stout, & Tervo, 2000) scores (Hashiguchi et al., 2018;

Table 2.4 Severity of CP

Study	Distribution of CP		Level of impairment of functional mobility		
	Total N	Walk-DMC	Total N	Walk-DMC	Structure
Tang et al. (2015)	Uni: 4 both legs Bi: 2 or 3 per leg	-	GMFCS I: 2=50%; 3=25%; 4=25% GMFCS II: 2=50%; 3=17%; 4=33% GMFCS III: 2=25%; 3=75% GMFCS IV: 2=100%	-	-
Steele et al. (2015)	-	He: 89.2 (87.8-90.6) Di: 86.9 (85.9-87.9) Tri: 84.4 (82.5-86.3) Quad: 81.4 (80.0-82.8)	-	GMFCS I: 92.4 (91.1-93.7) GMFCS IV: 79.2 (77.5-80.9) FAQ=10: 90.9 (89.2-92.6) FAQ<7: 80.0 (78.7-81.3)	Higher GMFCS level = more synergy structures that are specific to CP
Yu et al. (2019)	-	-	GMFCS I/II=4; GMFCS III=3	-	GMFCS I: DAM=29.8; GMFCS II: DAM=30.6; TD: DAM=26.4
Hashiguchi et al. (2018)	-	-	No correlation with GMFCS levels: χ^2 =4.06, p=0.40	-	-
Kim et al. (2018b)	-	-	No correlation with GMFCS levels	-	GMFCS level was correlated with normalized cluster number: r=0.51, p=0.01

Abbreviations: CP, cerebral palsy; N, number of synergies; Walk-DMC, dynamic motor control index during walking; Uni, unilateral; Bi, bilateral; He, hemiplegic; Di, diplegic; Tri, triplegic; Quad, quadriplegic; GMFCS, gross motor function classification system; DAM, deviation of activation matrix (to identify variability of activations patterns between subjects).

Kim et al., 2018b; Schwartz et al., 2016; Steele et al., 2015; Tang et al., 2015; Yu et al., 2019).

Children with CP that were bilaterally affected recruited fewer synergies, as identified by lower walk-DMC scores (Steele et al., 2015), a lower total number of synergies, and synergy structures more specific to the CP group (Tang et al., 2015). In addition, higher GMFCS levels in children with CP were related to lower walk-DMC scores (Schwartz et al., 2016; Steele et al., 2015) and a lower total number of synergies (Tang et al., 2015; Yu et al., 2019). In contrast, Hashiguchi et al. (2018) and Kim et al. (2018b) did not find a correlation between the total number of synergies and GMFCS level, although Hashiguchi et al. (2018) found that a higher level of spasticity in children with CP, as assessed by the modified Ashworth Scale, was correlated with a lower number of synergies. The temporal structure of synergies was shown to differ between the affected and less affected side of children with unilateral CP and children with bilateral CP (Cappellini et al., 2016), and higher synergy variability was found in children with higher GMFCS levels (Kim et al., 2018b; Yu et al., 2019).

Within-subject variability

No systematic differences in number, and spatial or temporal structure of synergies were found between days (Shuman et al., 2016; Steele et al., 2019). However, muscle synergies were found to be variable between strides in both children with CP and TD children (Kim et al., 2018b; Shuman et al., 2016). Kim et al. (2018b) used a cluster analysis based on a combination of iterative *k*-means clustering and intraclass correlation coefficient analyses to identify stride-to-stride variability of muscle synergies (Kim, Bulea, & Damiano, 2016). The authors found that children with CP had a higher normalized cluster number, meaning that they showed more distinct clusters across strides, although they recruited fewer synergies. Thus, children with CP had higher variability in spatial and temporal synergy structure between strides compared to TD children, for various VAF thresholds (see Table 2.5).

Treatment

Three studies investigated whether muscle synergy characteristics in children with CP before treatment are predictive of the effect of different treatments, including selective dorsal rhizotomy (SDR), single-event multilevel orthopedic surgery (SEMLS), single-level orthopedic surgery, botulinum toxin type A (BoNT-A) injection or conservative treatment (physical therapy). Higher walk-DMC values before treatment were associated with improved gait quality, as defined by the Gait Deviation Index and walking speed, after several treatments (Schwartz et al., 2016; Shuman et al., 2018). A higher total number of synergies before treatment

Table 2.5 Variability of synergies

Study	Between strides		Between days	
	VAF_1 (%)	Structure	VAF_1 (%)	Structure
Shuman et al. (2016)	**Mean std:** CP=5.0% (range 2.5-7.5%); TD=4.9% (range 3.7-6.5%) **Mean max difference:** CP=19.1%; TD=18.2%	-	**Day 1:** CP=77.4±5.3%; TD=68.4±5.0% **Day 2:** CP=76.9±4.8%; TD=68.4±4.7%	-
Steele et al. (2019)	-	-	**Average change day 1 to day 2:** 4.24±3.09%	**CS (day 1 vs. day 2):** 2 syn: w=0.89±0.10; act=0.93±0.06 3 syn: w=0.83±0.11; act=0.91±0.06 4 syn: w=0.90±0.08; act=0.92±0.05
Kim et al. (2018b)	-	**Normalized cluster number*** for VAF >80%: CP=0.38±0.06; TD=0.33±0.04 >85%: CP=0.37±0.07; TD=0.31±0.05 >90%: CP=0.41±0.05; TD=0.34±0.08 >95%: CP=0.39±0.05; TD=0.34±0.02	-	-

Abbreviations: CP, cerebral palsy; TD, typically developing; VAF_1, variance accounted for by one synergy (%); std, standard deviation; max, maximum; CS, cosine similarity; syn, synergies; w, weights; act, activations.

was associated with an improved knee angle at initial contact and midstance after SDR, but not with an improvement of overall gait quality, as quantified by the Edinburgh visual gait score (Oudenhoven et al., 2019b).

Shuman et al. (2019) investigated whether muscle synergies change after treatment, and whether these changes were associated with treatment outcomes. They found no changes in the number of synergies, or synergy weights, and only minimal changes in VAF_1 after BoNT-A and SDR. Temporal structure of synergies changed only after SDR, towards being more different from TD children. Children with CP whose synergies had a temporal structure more similar to TD children after treatment showed improved gait quality.

DISCUSSION

Walking problems in children with cerebral palsy (CP) can in part be explained by limited selective motor control, i.e. the impaired ability to use the correct muscle group to move a joint independently from other joints in a limb during movement (Desloovere et al., 2006). Muscle synergy analysis is increasingly used to quantify altered neuromuscular control during walking. This systematic review analyzed 16 studies investigating muscle synergies in children with CP during walking, and aimed to examine how these synergies differ from those exhibited by TD children.

Quantification of synergies

The majority of studies found that children with CP require fewer synergies during walking compared to TD children, either based on a certain VAF threshold, VAF_1, or walk-DMC (Goudriaan et al., 2018; Hashiguchi et al., 2018; Kim et al., 2018b; Schwartz et al., 2016; Shuman et al., 2016, 2017, 2018, 2019; Steele et al., 2015, 2019; Tang et al., 2015; Torricelli et al., 2014; Yu et al., 2019). The authors of these studies suggest that neuromotor control is altered or less complex in children with CP. The number of synergies for children with CP and TD children varied between studies. Cappellini and colleagues were the only ones that did not find a difference in terms of number of synergies between children with CP and TD children (Cappellini et al., 2016, 2018).

The differences in findings between studies may be a consequence of the varying functional mobility levels of subjects included by the different studies. Cappellini et al. (2016, 2018) included children with CP with a relatively high functional mobility level (77-79% GMFCS I) compared to the other studies (range 22-67% GMFCS I), with the exception of (Shuman et al., 2016) (100% GMFCS I). It is plausible that the functional mobility of children with CP and TD children was too similar in Cappellini et al. (2016, 2018) to find a difference in the number of synergies between groups.

The use of different methods to define the total number of synergies may also

impact synergy outcomes between studies (Hug, Turpin, Dorel, & Guevel, 2012; Russo, D'Andola, Portone, Lacquaniti, & d'Avella, 2014). Most studies in this review used VAF to define the total number of synergies, several of these defined a specific VAF threshold, but there is no agreement on the optimal height of this threshold. Consequently, the VAF thresholds ranged from 80% to 95% across studies, affecting the total number of synergies that are considered. However, this only influences comparisons of the number of synergies between studies, but differences between groups within one study can still be observed. No systematic differences in number of synergies were found between studies in this review, using different VAF thresholds. To avoid the impact of this threshold, some studies used VAF_1 or the related measure walk-DMC, and found results comparable to the VAF threshold. Cappellini et al. (2016, 2018) were the only ones using a different method to define the number of synergies, namely the 'best linear fit' method. However, it is unlikely that the use of this method explains the similarity in total number of synergies between children with CP and TD children found in Cappellini et al. (2016, 2018), since the authors verified that their results agreed with a VAF > 80%. None of the studies in this review considered the added variance of the following synergy as a measure to define the total number of synergies (Clark et al., 2010). The added variance could be an extra tool in the future to define the total number of synergies as it negates the risk that a synergy does not contribute sufficiently to the muscle activation pattern of interest.

The variation in synergy outcomes between studies could also be explained by the different number of muscles recorded. According to previous research, a low number of muscles used for analysis could lead to an over-estimation of VAF (Damiano, 2015; Steele et al., 2013; Zelik, La Scaleia, Ivanenko, & Lacquaniti, 2014). Several studies used NMF to decompose four to eight muscles into two to four synergies, but it is debatable whether this reduction aids enough in terms of easing the interpretation of the data from a statistical point of view. Yet, since it is not feasible to measure all muscles involved in walking, a decomposition will always approximate true neural signaling. Cappellini and colleagues (2016, 2018) were the only ones recording a large number of bilateral muscles, 11 per leg, which may result in a more precise estimation of the muscle synergies involved during walking. This could possibly explain in part why they did not find differences between CP and TD, while others did.

In addition, processing methods of the EMG data, such as filters and amplitude scaling, have been shown to influence muscle synergy outcomes (Shuman et al., 2017). The majority of studies included in this review used a low-pass filter with a cut-off frequency of 10 Hz, but some studies used low-pass filters of 2 Hz (Oudenhoven et al., 2019b), 4 Hz (Shuman et al., 2016), and 5 Hz (Kim et al.,

2018b; Torricelli et al., 2014). The lower the low-pass cut-off frequency, the more data is attenuated, which has been shown to result in a lower number of synergies (Shuman et al., 2017; van der Krogt et al., 2016), and smaller increases in VAF post-treatment (van der Krogt et al., 2016). There is no consensus yet on the best cut-off frequency for a low-pass filter. Different filter types and filter orders are used across studies, but these choices appear to be less significant than the low-pass cut-off frequency (Devaprakash, Weir, Dunne, Alderson, & Donnelly, 2016). The influence of methodological choices on muscle synergies is especially important to consider when comparing results across studies or between centers, using different ways to process their data. Overall, despite differences in the number and choice of muscles, and EMG preprocessing methods, studies found similar results. Moreover, the methods were the same in the CP and TD group within all studies and should therefore have an equal effect on the muscle synergies of all groups. Consequently, these factors are not likely to explain the lack of difference in number of synergies between children with CP and TD children found by Cappellini and colleagues (2016, 2018).

Structure of synergies

A subset of the included studies examined differences in the structure of muscle synergies between children with CP and TD children (Cappellini et al., 2016, 2018; Steele et al., 2015; Tang et al., 2015; Torricelli et al., 2014), but they showed different results. Some studies found differences in the spatial structure, i.e. muscle weights, between children with CP and TD children (Tang et al., 2015; Torricelli et al., 2014), whereas others only found differences in the temporal structure, i.e. timing and duration of the peaks of the temporal activation patterns (Cappellini et al., 2016, 2018; Kim et al., 2018b; Steele et al., 2015; Torricelli et al., 2014; Yu et al., 2019).

Variation in the use of amplitude scaling methods could result in a different weighting of the synergies per muscle. Scaling to unit variance appears to reduce these differences in muscle weights, with more consistent synergy structures across low-pass filters and at a lower number of calculated synergies compared to peak amplitude scaling (Shuman et al., 2017). Although the differences were small, this finding might be specifically interesting for research investigating muscle synergies in clinical populations, which recruit fewer synergies compared to TD children. Moreover, normalization to individual maxima could distort the relative muscle weights due to variable weakness in CP, which can result in inconsistent findings on the spatial structure of synergies across studies (Damiano, 2015).

Deviation from the structure of 'mature synergies' in children with CP was found (Tang et al., 2015; Torricelli et al., 2014), and could be a result of the lack of fractionation of synergies, i.e. splitting of one synergy into more, during

development. Previous research in stroke patients suggests that a lower number of synergies could result from merging of the synergies of healthy controls (Cheung et al., 2012; Clark et al., 2010). Merging and fractionation of synergies influenced the longitudinal changes of walking patterns in patients after subacute stroke, whereas the number of synergies did not (Cheung et al., 2012; Hashiguchi et al., 2016). Therefore, it might add value to examine the structure including possible fractionation of synergies.

The studies used different methods to quantify similarity between synergy structure. Torricelli et al. (2014) compared the temporal activation patterns using adult data (Winter, 1991), not specifying the method they used, while Tang et al. (2015) and Yu et al. (2019) used Pearson's correlation coefficients, and the other studies used cluster analyses to compare the structure between subjects (Cappellini et al., 2016, 2018; Kim et al., 2018b; Steele et al., 2015). These cluster analyses identified comparable patterns across subjects. Three studies isolated the synergies that where not consistent across children as 'Not Classified' (Cappellini et al., 2016, 2018; Steele et al., 2015). This means that the synergies that were specific to one child were not considered, and the authors did not quantify how many synergies were removed from each subject. Consequently, differences in synergies within the group of children with CP, and between children with CP and TD children were possibly lost, which could be a reason why these studies did not find (large) differences in synergy structure between children with CP and TD children. Kim et al. (2018b) did allow synergy structures to be assigned to more clusters, and they also found similar synergy structures between children with CP and TD children. However, children with CP recruited fewer synergies per stride, and the use of these structures was less consistent across strides. This means that relative to the number of synergies per stride, children with CP could access more synergy structures than TD children, which suggests that children with CP exhibit the same complexity of synergy structures, but the control of these structures might be decreased. In order to confirm this idea, more studies using the same clustering method are necessary.

Cappellini et al. (2016) found similarities in temporal structure of synergies between children with CP and TD toddlers (1-1.2 years of age) who just started to walk independently. This suggests that muscle synergies in children with CP lag behind in development compared to TD children, which agrees with previous research showing similarity between the walking pattern in children with CP and early gait in TD children (Berger et al., 1982, 1984; Leonard et al., 1991).

Variability of synergies

The variation in findings between studies on the number and structure of synergies might be related to the differences in distribution and levels of functional mobility in CP. Children with more severe types of CP, defined by either more distributed CP or higher GMFCS levels, were found to use fewer synergies (Steele et al., 2015; Tang et al., 2015), with different spatial (Tang et al., 2015) and temporal (Cappellini et al., 2016; Shuman et al., 2019; Steele et al., 2015) structures compared to less affected children. These results might reflect a simpler motor control strategy during walking with increasing severity of CP.

In contrast, Hashiguchi et al. (2018) and Kim et al. (2018b) did not find a relationship between number of synergies and GMFCS level, possibly because of the small sample size, which limits the variability in a group. Tang et al. (2015) and Yu et al. (2019) also included a limited group of children and they did find an effect of GMFCS level on the number of synergies. Thus, the relationship between the severity of CP and muscle synergies is shown in studies with a sufficient number of subjects (Cappellini et al., 2016; Shuman et al., 2019; Steele et al., 2015), but small sample sizes can coincidentally not show it.

One study found a higher stride-to-stride variability in muscle synergies in children with CP (Kim et al., 2018b). This may represent a more immature walking pattern (Hausdorff, Zemany, Peng, & Goldberger, 1999). High stride-to-stride variability can influence VAF values and thus impact the decomposition of the data into muscle synergies. Only four studies used the minimum of about 20 strides that is necessary according to Oliveira, Gizzi, Farina, and Kersting (2014) to create optimal reconstructions of the data and minimize the influence of the variability between strides (Cappellini et al., 2016, 2018; Shuman et al., 2016; Tang et al., 2015). Based on the low amount of studies in this review assessing specifically this aspect we cannot infer whether a lower number of analyzed strides could have an effect on a lower number of synergies.

Considering the high diversity within the group of children with CP, it is not surprising that many studies found larger variability in number and structure of muscle synergies in children with CP compared to TD children. In some studies children with more severe types of CP walked with an assistive device or trunk or hand support (Hashiguchi et al., 2018; Kim et al., 2018b; Oudenhoven et al., 2019b; Shuman et al., 2019; Tang et al., 2015; Yu et al., 2019). In addition, children with more severe types of CP generally walk slower compared to less affected and TD children. Walking speed is an important factor to consider when evaluating muscle synergies, as previous research found that both number and structure of synergies were affected by walking speed in healthy adults (Kibushi, Hagio, Moritani, & Kouzaki, 2018; Yokoyama, Ogawa, Kawashima, Shinya, & Nakazawa, 2016a) and TD

children (Steele et al., 2015). These findings suggest that different walking speeds require different control from the central nervous system. However, others found that muscle synergies were robust across different walking speeds in healthy adults (Chvatal & Ting, 2012; Ivanenko et al., 2004b) and children with CP (Hashiguchi et al., 2018; Tang et al., 2015). Although findings are inconsistent, walking speed as a possible confounding factor in comparisons of muscle synergies between children with CP and TD children should be considered during muscle synergy analysis. In addition, the quality of EMG data and the absence of task-independent normalization may have caused variation in muscle synergy results between studies, and should be considered in the future.

Treatment

The finding that muscle synergies before treatment were correlated with the effect of treatment in children with CP (Oudenhoven et al., 2019b; Shuman et al., 2016, 2018), suggests that knowledge about muscle synergies in children with CP before treatment could help predict whether children will benefit from a specific treatment, and therefore potentially assist in treatment decisions. Walk-DMC has been proposed as a possible measure to quantify altered neuromuscular control pre-treatment, since it has been shown to be correlated with improvement of gait kinematics and walking speed after treatment (Schwartz et al., 2016; Shuman et al., 2018). Importantly, EMG processing methods, and number and type of muscles have limited impact on walk-DMC values. Therefore, this measure could be useful as a comparison of muscle synergy analyses across studies or different clinical centers using different EMG protocols. However, walk-DMC values are highly variable in a heterogeneous population like CP (Shuman et al., 2018; Steele et al., 2015). Although the mean results of walk-DMC values using a large sample size might be a good predictor of treatment outcome, caution should be taken when using individual walk-DMC values in treatment prediction.

Besides the use of muscle synergies as a predictor of treatment outcomes, muscle synergies may also be a target for treatment themselves. Younger children with CP might be more sensitive to interventions (Yang et al., 2013), because their brain is highly plastic and their corticospinal tract is still maturing. Future research should examine the opportunities of specific therapies that target the neural level and adapt muscle synergies, to improve the walking pattern of children with CP. Previous research in unimpaired individuals showed that both the spatial and temporal structure of muscle synergies can change due to intense training in elite athletes (Kim, Kim, Kim, & Yoon, 2018a; Sawers, Allen, & Ting, 2015), and with the use of ankle exoskeletons (Jacobs, Koller, Steele, & Ferris, 2018; Steele, Jackson, Shuman, & Collins, 2017). However, current treatments studied in CP were found

to have no effect on the spatial structure and merely an effect on the temporal structure of muscle synergies (Shuman et al., 2019). These results suggest that the number and spatial structure of synergies may be hard to change in children with CP, but that the temporal structure of synergies could be a target for treatment. However, normalization of the EMG data is an important factor that may have influenced the results on the spatial structure of synergies. It remains to be further investigated whether novel treatments, such as feedback training (Booth, van der Krogt, Harlaar, Dominici, & Buizer, 2019), or therapeutic electrical stimulation of muscles, tendons (í Dali et al., 2002; Sommerfelt, Markestad, Berg, & Saetesdal, 2001; Stackhouse et al., 2007; Wright, Durham, Ewins, & Swain, 2012), or spinal cord (Solopova et al., 2017), could improve muscle synergies, eventually leading to walking improvement.

Future directions

The number of studies currently available on this topic is limited, which makes it difficult to draw additional conclusions. With this systematic review we hope to inform researchers about the current research status and to guide them towards better research in the future.

The large variation in number and structure of muscle synergies derived from children with CP appears to reflect the diversity of CP and the ability of walking. However, methodological factors also seem to play a role in the determination of muscle synergies. On the one hand, it will be helpful when studies investigating muscle synergies in children with CP use consistent methods across different studies, in order to compare results. On the other hand, this would limit researchers to explore and use novel technologies. At least, researchers could consider recording a number of muscles that is representative for the muscle activation during walking, as well as a sufficient number of strides, in order to make a proper decomposition of muscle synergies. To achieve consistency in EMG data processing steps across studies, researchers should be informed about the choice of filters and factorization methods. The determination of a suitable method to process EMG data of children with CP during walking, for example with a standard EMG processing pipeline, is an important area for future research. If the group of children with CP is heterogeneous, muscle synergy analysis should be performed on separate groups based for example on different distribution of CP (i.e. uni- or bilateral CP) or different functional mobility levels with sufficient sample sizes, in order to examine the diversity in the CP group. In addition, study of the influence of walking speed on muscle synergies in children with CP and TD children could be useful in the interpretation of the results found in the studies included in this review. Irrespective of the differences in data collection and analysis, the majority

of the studies included in this review found similar results, which indicates that the difference in muscle synergies between CP and TD we observe is robust. These corresponding findings from different studies and research groups, provide strong evidence that the observations are related to neural control, and do not merely reflect methodological choices.

It is worth to mention that all the studies reported in this review used the so-called synchronous synergy model (time-invariant synergy approach) to investigate muscle synergies during walking in children with CP. However, various other models such as the time-varying synergy model, first introduced by d'Avella and Tresch (2002), or the space-by-time model (Delis, Panzeri, Pozzo, & Berret, 2014) exist, and could be implemented to study muscle activation modularity in children with CP.

Investigation of the longitudinal development of muscle synergies within subjects would minimize the inter-subject variability and give more insight in the developmental changes in children with CP. Moreover, nothing is known about the development of muscle synergies in very young children at high risk of CP compared to TD children. A longitudinal design with consecutive measurements within subjects could give new insights in the development of muscle synergies during walking in children with CP, and might open up new paradigms for early interventions in CP.

Despite the increasing number of studies investigating muscle synergies, the underlying mechanisms of muscle synergies remain unknown. It is still a topic of debate whether muscle synergies have a neural or non-neural origin (Bizzi & Cheung, 2013; Zandvoort, van Dieën, Dominici, & Daffertshofer, 2019). Muscle synergies in neonates were shown to mainly reflect spinal cord and brainstem activity, with an increase of the integration of supraspinal and sensory control during development (Dominici et al., 2011). Even though children with CP have cortical lesions, the differences in muscle synergies compared to TD children might also depend on changes in the brainstem and/or spinal cord. In addition, it is debatable whether the use of fewer muscle synergies necessarily reflects less complex motor control, as is suggested in most studies, or whether it is merely caused by higher variability in the EMG data in children with CP. Further research on the underlying mechanisms of muscle synergies is required to answer these questions.

Conclusions

In conclusion, the majority of studies found that children with CP use fewer synergies than TD children, and differences in both spatial and temporal structure of synergies were found. In addition, large variability of muscle synergies was found

in the group of children with CP, which might be due to the heterogeneity in this group with different functional mobility levels of CP. The inter-subject variability in number and structure of synergies was higher in children with more severe CP, and within subjects the stride-to-stride variability was higher in children with CP compared to TD children, which is known to influence VAF values and thus impact the decomposition of the EMG data into muscle synergies.

The findings in this systematic review support the idea that children with CP use a simpler motor control strategy compared to TD children. The use of muscle synergies as a clinical tool to quantify altered neuromuscular control and predict clinical outcomes seems promising. Further investigation on this topic is necessary, and the use of muscle synergies as a target for development of novel therapies in children with CP could be explored.

3

Neuromuscular control before and after independent walking onset in children with cerebral palsy

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ABSTRACT

Background Early brain lesions which produce cerebral palsy (CP) may affect the development of walking. It is unclear whether or how neuromuscular control, as evaluated by muscle synergy analysis, differs in young children with CP compared to typically developing (TD) children with the same walking ability, before and after the onset of independent walking.

Methods Here we grouped twenty children with (high risk of) CP and twenty TD children (age 6.5–52.4 months) based on their walking ability, supported or independent walking. Muscle synergies were extracted from electromyography data of bilateral leg muscles using non-negative matrix factorization. Number, synergies' structure and variability accounted for when extracting one (VAF_1) or two (VAF_2) synergies were compared between CP and TD.

Results Children in the CP group recruited fewer synergies with higher VAF_1 and VAF_2 compared to TD children in the supported and independent walking group. The most affected side in children with asymmetric CP walking independently recruited fewer synergies with higher VAF_1 compared to the least affected side.

Conclusion Our findings suggest that early brain lesions result in early alterations of neuromuscular control, specific for the most affected side in asymmetric CP.

INTRODUCTION

Cerebral palsy (CP) is a neurodevelopmental disorder caused by brain lesions before birth or early in life (Bax et al., 2005; Himmelmann & Uvebrant, 2018). It covers a wide clinical spectrum, from children who manage to walk independently, to children being completely wheelchair dependent. Children can be affected symmetrically (bilateral CP) or asymmetrically (unilateral CP or asymmetric bilateral CP, Cans, 2000). Topography and severity of CP can be difficult to predict in infancy (Novak et al., 2017).

Typically, infants take their first independent steps between the age of 9 to 18 months, representing an important milestone in motor development (Adolph & Robinson, 2013). Reaching this milestone can be challenging for children with CP. Early interventions can be critical to improve motor functions, including walking, because the neural networks under development are still highly plastic (Yang et al., 2013). To improve interventions that promote functional mobility in children with CP, it is important to identify neuromuscular mechanisms of abnormal motor development as early as possible. One possibility to assess these neuromuscular mechanisms is the use of muscle synergy analysis.

The central nervous system has been theorized to reduce the degrees of freedom in the coordination of muscle activation during walking through basic building blocks, named muscle synergies or locomotor modules, that resemble identical temporal activation patterns of groups of muscles (Bizzi & Cheung, 2013; Dominici et al., 2011; Hart & Giszter, 2010; Ivanenko et al., 2005a). The number of basic activation patterns in typically developing (TD) children increases from two during neonate stepping, to four in toddlers who have just started to walk independently (Dominici et al., 2011). Muscle synergy analysis has recently been adopted to quantify neuromuscular control during walking in school-age children with CP, and has been shown to provide a consistent measure between days (Shuman et al., 2016).

Despite a limited number of recorded muscles previous studies show that older children with CP recruit fewer synergies during walking compared to age-matched TD children (Bekius et al., 2020; Hashiguchi et al., 2018; Shuman et al., 2018; Steele et al., 2015, 2019; Tang et al., 2015). In addition, several studies reported that the walking patterns of older children with CP retain some of the characteristics of the younger TD children, by showing the excessive muscular co-contraction of only a few muscles (Berger, 1998; Leonard et al., 1991; Meyns et al., 2012a). The small number of recorded muscles and the age of the children involved in these studies limits our current understanding of neuromuscular control in very young children with early brain lesions (Cahill-Rowley & Rose, 2014; Steele et al., 2013; Yang et al., 2013). A more detailed and comprehensive assessment of multi-muscle coordinate patterns is needed (Damiano, 2015).

The time before the onset of independent walking can be a critical period for early interventions to improve motor functions including walking (Hadders-Algra, 2004; Yang et al., 2013). Previous studies compared children with CP and TD children of similar age, while it may be relevant to match these groups for developmental phase (Bekius et al., 2020). One study from Prosser, Lee, VanSant, Barbe, & Lauer (2010) compared the trunk and hip muscles in children with CP and TD with similar walking experience (an average of 28.5 months of walking experience). Nevertheless, the age range of the children with CP included in this study was quite large (2 to 9 years old), as was the walking experience (0.5 to 60 months). In addition, they did not differentiate between children able to walk independently and children who needed support, e.g., by using an assistive device, to perform the walking task.

The aim of this study was to assess whether neuromuscular control in young children with CP differs from that of TD children with the same walking ability in the early phase of motor development, i.e., before the onset of independent walking (supported walking) and just after the onset of independent walking in particular by means of the contributing muscle synergies. In addition, we examined whether there was a difference in neuromuscular control between the most and least affected side of children who were affected asymmetrically (unilateral or asymmetric bilateral CP). We hypothesized that already before or during the first years of independent walking, children with (high risk of) CP recruit fewer synergies compared to TD children and that this is specific to the most affected side in children with asymmetric CP.

MATERIALS AND METHODS

Participants

Children with early brain lesions, at high risk of CP or with an established diagnosis of CP (referred to as CP group) were recruited from the Departments of Pediatric Rehabilitation and of Child Neurology at Amsterdam University Medical Centers (Amsterdam UMC), and from the Department of Child Neurology at Maastricht University Medical Center (Maastricht UMC+); see Table 3.1 for the in- and exclusion criteria. TD children (referred to as TD group) were recruited by word of mouth. Participants were in either the supported walking (SW) or independent walking (IW) group, based on their walking ability. Children in the SW group could not walk independently, while children in the IW group could. Individual and average characteristics of both groups, as well as the clinical characteristics of the children in the CP group, are listed in Table 3.2.

The study was approved by the Ethics Committee of the Faculty of Behavioral and Movement Sciences at the Vrije Universiteit Amsterdam (VCWE-2016-082) for the TD group, and of Amsterdam UMC (NL59589.029.16) for the CP group. In Maastricht UMC+, local practicability was granted. The parents of all children were informed about the procedure of the study and provided written informed consent prior to participation in accordance with the Declaration of Helsinki for medical research involving human participants.

Table 3.1 In- and exclusion criteria for the CP group

Inclusion Criteria	Exclusion Criteria
• Diagnosis of CP based on the predominant type of motor impairment and classified according to the criteria proposed by Himmelmann et al. (2005). CP diagnosis was confirmed according to medical history, brain magnetic resonance results and clinical examination, OR, in children under 24 months: • At high risk for developing CP, based on the presence of one of the following (Hamer et al., 2016; Hielkema et al., 2010): - Cystic periventricular leukomalacia, diagnosed on serial ultrasound assessments of the brain (de Vries et al., 1992) - Unilateral or bilateral parenchymal lesion of the brain, diagnosed using MRI (de Vries et al., 2001) - Term/near-term asphyxia resulting in Sarnat 2 or 3 (Sarnat & Sarnat, 1976) with brain lesions on MRI and/or with neurological dysfunction during infancy suggesting the development of CP - Neurological dysfunction suggestive of development of CP	• Functional surgery on bones and/or muscles of the legs • Selective dorsal rhizotomy in the last 12 months • Severe epilepsy • GMFCS IV and V • Above the age of five years • Brain damage above the age of one year

Abbreviations: CP, cerebral palsy; GMFCS, gross motor function classification system; MRI, magnetic resonance imaging.

Procedure

Experiments were performed in the clinical gait laboratories of the Department of Rehabilitation Medicine at Amsterdam UMC (location VUmc) and Maastricht UMC+, and the BabyGaitLab laboratory of the Department of Human Movement Sciences at the Vrije Universiteit Amsterdam. The responsible investigators and one or both parents of the child were present during the experiments, and, for the CP group, also a pediatric physiotherapist. At the start of each experiment for the CP group, the pediatric physiotherapist performed a physical examination, to identify possible motor asymmetry in the CP group (in this case reported as asymmetrically affected child).

Children in the SW group walked with support on a treadmill (with the exception of one child who walked over-ground). Adequate support was provided by the physiotherapist, experimenter or parent that held the trunk of the child with both hands or held the hand of the child, while the other parent, or an experimenter, encouraged the child to take steps (Dominici et al., 2007; Ivanenko et al., 2005b). The treadmill speed was adjusted to induce a walking pattern and tuned to a

comfortable speed for the child. Children in the IW group walked independently over-ground, and were encouraged to walk in a straight line, at their preferred walking speed. Only sequences of steps executed naturally by the child were considered.

Muscle activity was recorded with surface electromyography (EMG) from 18 to 22 bilateral leg and trunk muscles simultaneously using Mini Wave wireless EMG systems (Cometa, Italy). The following muscles were recorded from each side: tibialis anterior (TA); gastrocnemius medialis (GM); gastrocnemius lateralis (GL); soleus (SOL); rectus femoris (RF); vastus medialis (VM); vastus lateralis (VL); biceps femoris (BF); tensor fascia latae (TFL); gluteus maximus (GLM) and erector spinae (ES) at L2 level. EMG electrode placement was performed according to the Surface Electromyography for the Non-Invasive Assessment of Muscles protocol (Hermens, Freriks, Disselhorst-Klug, & Rau, 2000), and the standard recommendations for minimizing cross-talk between adjacent muscles (Dominici et al., 2011; Ivanenko et al., 2005a; Ivanenko et al., 2004b). The skin was cleaned with alcohol and mini golden reusable surface EMG disc-electrode pairs (15 mm diameter, acquisition area 4 mm^2) were placed at the approximate location of the muscle. To minimize movement artefacts, pre-amplified EMG sensor units were attached to the skin of the child and fixed with elastic gauzes. The signals were amplified and sampled at 1000 Hz. Body kinematics and high-speed video were recorded at 100 Hz using a VICON system (Oxford, UK). 32-channel electro-encephalography (EEG) recordings were performed, but not analyzed here. Sampling of EMG, video, and kinematic data was synchronized online.

Spatiotemporal gait parameters

The step events were extracted from the video and confirmed with kinematic data for both sides. The gait cycle was defined as a cyclic movement of one leg, starting when the foot strikes the ground and ending when the foot of the same leg strikes again. The end of stance was defined as the moment when the foot lifts off the ground. Gait initiation/termination strides and jumps or turning were discarded from analysis. Stride velocity was calculated using the corresponding stride length and stride duration. The stride length was computed according to the 3D displacement of the foot marker. For the trials recorded during walking on the treadmill, the treadmill speed was taken into consideration to correct for the participants' displacements. Stance duration, i.e. from foot strike to foot off, for both legs was computed.

Muscle synergy

All analyses were conducted in MATLAB (version 2017b, Mathworks Inc., Natick, MA, USA). Raw EMG data were processed offline according to the following sequence: Notch filter (50 Hz), high pass filtering (30 Hz), full-wave rectification, and low pass filter with a fourth order Butterworth filter (10 Hz).

Muscle synergies were extracted using non-negative matrix factorization (NMF) of the pre-processed EMG data (Lee & Seung, 1999). EMG amplitudes of each muscle were normalized to the maximum of the mean value across all strides plus its standard deviation (SD) for each participant and the timescale was normalized to $t = 201$ data points per gait cycle for each limb. Briefly, the NMF was applied to the mean EMG envelopes for each participant, and decomposed the EMG data into temporal activation patterns (P) and synergy weights (W), according to the following equation:

$$EMG = \sum_{i=1}^{n} P_i W_i + \varepsilon \tag{3.1}$$

where the pre-processed EMG data ($m \times t$ matrix, where m is the number of muscles and t is the number of time points) is a linear combination of the temporal activation patterns P ($n \times t$ matrix, where $n \leq m$ is a predetermined number of synergies) and synergy weights W ($m \times n$ matrix), and ε denotes the residual error.

A set of 1–8 synergies was extracted with a restriction of 100 maximum iterations, 1000 replicates, and a threshold for convergence and completion of 10^{-4}. NMF was applied to the EMG activity of bilateral muscles (including both sides), and unilateral muscles (including one side). The results of the unilateral EMG analysis were used separately to compare the most and least affected side in children with asymmetric CP, and right and left side in children with symmetric CP and TD children.

The reconstruction accuracy of the extracted synergies was determined by the variability accounted for (VAF), which is the ratio of the sum of squared errors to the total sum of squares computed with respect to the mean (Cappellini et al., 2016; Dominici et al., 2011; Torres-Oviedo, Macpherson, & Ting, 2006). Next to VAF we also determined the synergies' contribution to the matrix (or Frobenius) norm (Bach, Daffertshofer, & Dominici, 2021a; Kerkman, Bekius, Boonstra, Daffertshofer, & Dominici, 2020; Zandvoort et al., 2019), which revealed comparable results. For the sake of legibility, we here present the conventionally used VAF. The minimum number of synergies to approximate the pre-processed EMG was defined as required for VAF to exceed 85%, or when the added VAF of the following synergy was

below 8% (Frère & Hug, 2012; Israely, Leisman, Machluf, & Carmeli, 2018; Maclellan et al., 2014). In addition, the selected number of synergies had to account for more than 80% VAF for every individual muscle (Clark et al., 2010; Roh, Rymer, Perreault, Yoo, & Beer, 2013; Torres-Oviedo et al., 2006). Since setting of a VAF threshold arguably comes with arbitrariness, we also investigated VAF by one synergy (VAF_1) in the unilateral EMG analysis, previously used as a summary measure of synergy complexity related to function and treatment outcome (Shuman et al., 2019; Steele et al., 2015), and by two synergies (VAF_2) in the bilateral EMG analysis (Dominici et al., 2011; Sylos-Labini et al., 2020). For the latter, we included an additional synergy to account for the mirror muscle activations in the contralateral side, shifted by 50% of the gait cycle.

In the bilateral EMG analysis, number of synergies and VAF_2 were compared between CP and TD for the SW and IW group. To compare temporal activation patterns and synergy weights between groups without a restriction to a certain threshold, the number of synergies was fixed to four, which is the number of synergies typically reported in healthy adults during walking (Clark et al., 2010; Dominici et al., 2011; Yu et al., 2019). The patterns were grouped and plotted according to the timing of the main peak relative to the normalized gait cycle. Average temporal activation patterns and synergy weights per group for each synergy were compared between CP and TD for the SW and IW group.

In the unilateral EMG analysis, number of synergies and VAF_1 for the most affected side in the asymmetric CP group, or a random side in the symmetric CP and TD group, were compared between CP and TD for the SW and IW group. Furthermore, the number of synergies and VAF_1 were compared between most and least affected side in the asymmetric CP, and right and left side in symmetric CP and TD group.

Statistical analysis

All data are reported as mean ± SD. An independent t-test was used when the data was normally distributed, and a one-tailed non-parametric Mann-Whitney U-test when it was not. An independent t-test in statistical parametric mapping was performed to assess the similarity of temporal activation patterns per synergy between CP and TD for the SW and IW group. Synergy weights were compared between groups using Pearson's correlation coefficients, where $r > 0.7$ represented high similarity and $r > 0.45$ marginal similarity (Torres-Oviedo & Ting, 2010). Significance threshold was set at $p < 0.05$ for all tests. For the comparison between unilateral results within groups, the Wilcoxon signed rank test was used for number of synergies, and a paired samples t-test for VAF_1.

RESULTS

Twenty children in the CP group (corrected age 6.5–45.5 months) and twenty children in the TD group (age 6.3–53.5 months) participated in this study, with ten children in the SW and IW groups (Table 3.2). Based on the physical evaluation performed in the children of the CP group at the start of each experiment by an expert pediatric physiotherapist we identified a total of $n = 5$ and $n = 7$ asymmetrically affected children in the SW and IW group. Time since onset of independent walking did not significantly differ between the CP and TD group (12.7 ± 9.8 vs. 13.9 ± 12.8 months; $p = 0.81$).

Table 3.2 Participant characteristics

Partici-pant	Gender	Age (mo)	CA (mo)	WO (mo)	Distribution	Subtype	GMFCS	Scores BD (side)	BW (kg)	N Strides	Speed (km/h)
CP1	M	11.6	10.6	-	Uni R	spastic	NS	b2 (bi)	9.7	17	0.39
CP2	F	14.8	15.1	-	Uni L	spastic	NS	5 (bi)	10.8	24	0.60
CP3	M	21.0	21.4	-	Uni L	spastic	NS	6 (uni R)	9.3	29	0.63
CP4	F	17.8	17.9	-	Bi (L > R)	spastic	NS	b2 (bi)	10.7	45	0.82
CP5	F	20.2	17.2	-	Bi (R > L)	spastic	NS	5 (uni L)	7.7	31	0.60
CP6	M	6.5	6.6	-	Bi	spastic	NS	b2 (bi)	7.3	22	0.64
CP7	M	9.8	6.5	-	Bi	spastic	NS	4 (bi)	-	27	0.80
CP8	F	8.5	8.9	-	Bi	undef	NS	b2 (bi)	8.8	43	0.61
CP9	F	42.8	41.2	-	Bi	spastic	III	4 (bi)	14	31	0.80
CP10	F	44.9	43.7	-	Bi	spastic	II	4 (bi)	11.4	40	0.62
CP SW	6 F; 4 M	19.8 (13.6)	18.9 (13.4)[#]	-	-	-	-	-	10.0 (2.1)	31 (9)	0.65 (0.13)
CP11	M	23.8	22.2	17.1	Uni R	spastic	I	5 (uni L)	11.1	35	1.77
CP12	M	35.6	35.8	16.1	Uni R	spastic	I	b1 (uni L)	13.4	27	2.62
CP13	M	41.0	38.0	16.0	Uni R	spastic	I	5 (uni L)	14.6	40	2.33
CP14	M	47.2	45.5	15.6	Uni R	spastic	I	5 (uni L)	15.2	45	3.87
CP15	F	22.3	22.3	15.0	Bi (L > R)	spastic	I	2 (bi)	10.6	42	1.66
CP16	M	27.8	26.9	19.1	Bi (R > L)	spastic	I	4 (bi)	14.1	66	2.75
CP17	M	38.6	38.9	16.1	Bi (R > L)	spastic	I	b2 (bi)	14.0	41	4.01
CP18	M	18.3	18.6	15.0	Bi	spastic	I	4 (bi)	10.9	18	4.00
CP19	F	34.4	29.9	24.4	Bi	ataxic	II	b2	10.0	59	2.37
CP20	M	34.4	29.9	26.7	Bi	spastic	II	4 (bi)	11.8	27	2.86
CP IW	2 F; 8 M	32.3 (9.1)	30.8 (8.6)	18.1 (4.1)[*]	-	-	-	-	12.6 (1.9)	40 (15)	2.82 (0.87)

(Continued)

Table 3.2 Continued

Partici-pant	Gender	Age (mo)	CA (mo)	WO (mo)	Distribution	Subtype	GMFCS	Scores BD (side)	BW (kg)	N Strides	Speed (km/h)
TD1	F	6.3	6.2	-	-	-	-	-	6.8	17	0.41
TD2	F	7.5	7.8	-	-	-	-	-	9.1	33	0.44
TD3	M	9.7	10.2	-	-	-	-	-	9.7	80	0.55
TD4	M	9.8	9.7	-	-	-	-	-	8.5	59	0.60
TD5	F	10.0	10.0	-	-	-	-	-	8.9	95	0.54
TD6	M	10.2	10.1	-	-	-	-	-	10.2	79	0.69
TD7	F	10.4	10.2	-	-	-	-	-	9.3	66	0.61
TD8	F	10.6	9.7	-	-	-	-	-	9.0	23	0.90
TD9	F	11.2	11.6	-	-	-	-	-	9.6	43	0.66
TD10	M	12.0	12.0	-	-	-	-	-	11.0	21	0.46
TD SW	6 F; 4 M	9.8 (1.7) #	9.8 (1.7)	-	-	-	-	-	9.2 (1.1)	52 (28)	0.59 (0.14)
TD11	M	16.5	16.5	10.7	-	-	-	-	11.3	27	2.40
TD12	F	17.5	17.8	11.6	-	-	-	-	10.7	38	2.76
TD13	F	19.3	19.3	12.9	-	-	-	-	-	93	1.68
TD14	F	19.7	19.6	12.9	-	-	-	-	10.4	49	2.99
TD15	F	20.1	20.1	13.9	-	-	-	-	10.3	86	2.48
TD16	M	20.8	20.4	14.9	-	-	-	-	13.0	66	3.35
TD17	F	24.4	24.3	11.7	-	-	-	-	11.3	45	1.94
TD18	M	27.5	27.3	11.3	-	-	-	-	13.0	21	3.16
TD19	F	47.1	47.2	14.3	-	-	-	-	16.0	49	3.27
TD20	M	53.5	52.4	11.3	-	-	-	-	15.5	28	3.47
TD IW	6 F; 4 M	26.6 (12.9)	26.5 (12.7)	12.6 (1.4) *	-	-	-	-	12.4 (2.2)	50 (25)	2.76 (0.63)

Distribution is based on the physical examination performed by a pediatric physiotherapist during the recording. Asymmetrically affected children in the CP group are highlighted in grey. The brain damage (BD) scores are defined according to a semi-quantitative MRI scale (Fiori et al., 2014): 2, full-term border-zone infarction; 4, periventricular leukomalacia; 5, posthemorrhagic porencephaly/venous infarction; 6, middle cerebral artery infarction; b1, developmental brain malformations; b2, non-specific lesions. The mean (SD) is reported for age, corrected age, walking onset (for the IW groups), body weight, number of strides and walking speed. # indicates a significant difference in age between CP and TD in the SW group ($p = 0.047$), and * indicates a significant difference in age at independent walking onset between CP and TD in the IW group ($p < 0.001$). Abbreviations: CP, cerebral palsy; TD, typically developing; SW, supported walking; IW, independent walking; F, female; M, male; CA, corrected age; WO, corrected age at independent walking onset; Bi, bilateral; Uni, unilateral; L, left; R, right; GMFCS, gross motor function classification system; NS, not yet specified; BW, body weight; kg, kilograms; N, number; SD, standard deviation.

Spatiotemporal gait parameters

Stride duration did not differ between CP and TD for SW (2.0 ± 0.5 vs. 2.1 ± 0.6 s) and IW (0.8 ± 0.1 vs. 0.8 ± 0.1 s). Relative stance duration in the IW group was significantly longer for TD (62 ± 4%) compared to CP (58 ± 3%; $p = 0.02$), but CP (72 ± 6%) and TD (71 ± 4%) did not differ significantly in the SW group ($p = 0.29$). Stride velocity did not differ significantly between CP and TD for SW

(0.7 ± 0.1 vs. 0.6 ± 0.1 km/h; $p = 0.15$) and IW (2.8 ± 0.9 vs. 2.7 ± 0.7 km/h; $p = 0.43$). Stance duration of the most affected leg of children with asymmetric CP in the IW group was significantly shorter compared to the least affected leg (57 ± 3 vs. 60 ± 4%; $p = 0.03$), while this was not the case when comparing legs in children with symmetric CP (57 ± 5 vs. 58 ± 4%), and TD children (62 ± 3 vs. 62 ± 4%; $p = 0.44$).

Muscle synergy

In the SW group, bilateral EMG analysis revealed that children in the CP group recruited two, three, or four muscle synergies, and in the TD group three or four synergies. In the IW group, children in the CP group recruited either three or four synergies, and in the TD group three, four, or five synergies (Figure 3.1A,B and *Appendix* Figure 3.S1). The mean number of synergies that explained the variability in the EMG data was lower in CP compared to TD for SW (2.9 ± 0.7 vs. 3.2 ± 0.4; $p = 0.14$) and IW (3.7 ± 0.5 vs. 4.1 ± 0.6; $p = 0.06$), albeit not reaching statistical significance. VAF_2 was higher in CP compared to TD for SW (70.5 ± 11.9 vs. 61.4 ± 7.4%; $p = 0.03$) and IW (60.8 ± 8.2 vs. 53.4 ± 11.0%; $p = 0.05$, Figure 3.1C). When the number of synergies was fixed to four, we did observe a significant difference between CP and TD in the temporal activation pattern P2 during early swing in the SW group (75.9 to 78.1% of the gait cycle; $p = 0.04$), and P1 during mid swing in the IW group (71.4 to 76.6% of the gait cycle; $p = 0.01$). Correlations between mean synergy weights of CP and TD were high ($r > 0.7$) for all synergies in the IW group, whereas in the SW group only W4 showed a high and W2 a moderate ($r > 0.45$) correlation (Figure 3.2).

The unilateral EMG analysis showed that the mean number of muscle synergies that explained the variability in the EMG data was significantly lower in CP compared to TD for SW (2.7 ± 0.5 vs. 3.1 ± 0.3; $p = 0.02$) and IW (3.0 ± 0.5 vs. 3.6 ± 0.5; $p < 0.01$). VAF_1 was significantly higher in CP compared to TD for SW (42.0 ± 12.9 vs. 22.0 ± 10.0%; $p < 0.001$) and IW (39.4 ± 13.3 vs. 29.5 ± 9.5%; $p = 0.03$; Figure 3.3A).

When comparing the most and least affected side in asymmetric CP, and right vs. left side in symmetric CP and TD children, we did not find any significant differences for the number of synergies and VAF_1 in the SW group (Figure 3.3B,C). However, in the IW group the mean number of synergies in asymmetric CP ($n = 7$) was significantly lower (3.0 ± 0.2 vs. 3.7 ± 0.2%; $p = 0.01$) and VAF_1 was significantly higher (36.8 ± 13.7 vs. 28.0 ± 9.2%; $p < 0.01$) for the most affected compared to the least affected side. No significant differences between sides could be identified in TD children ($n = 10$). Due to a limited number of children in the symmetric CP group ($n = 3$) no statistical comparison was performed (Figure 3.3C).

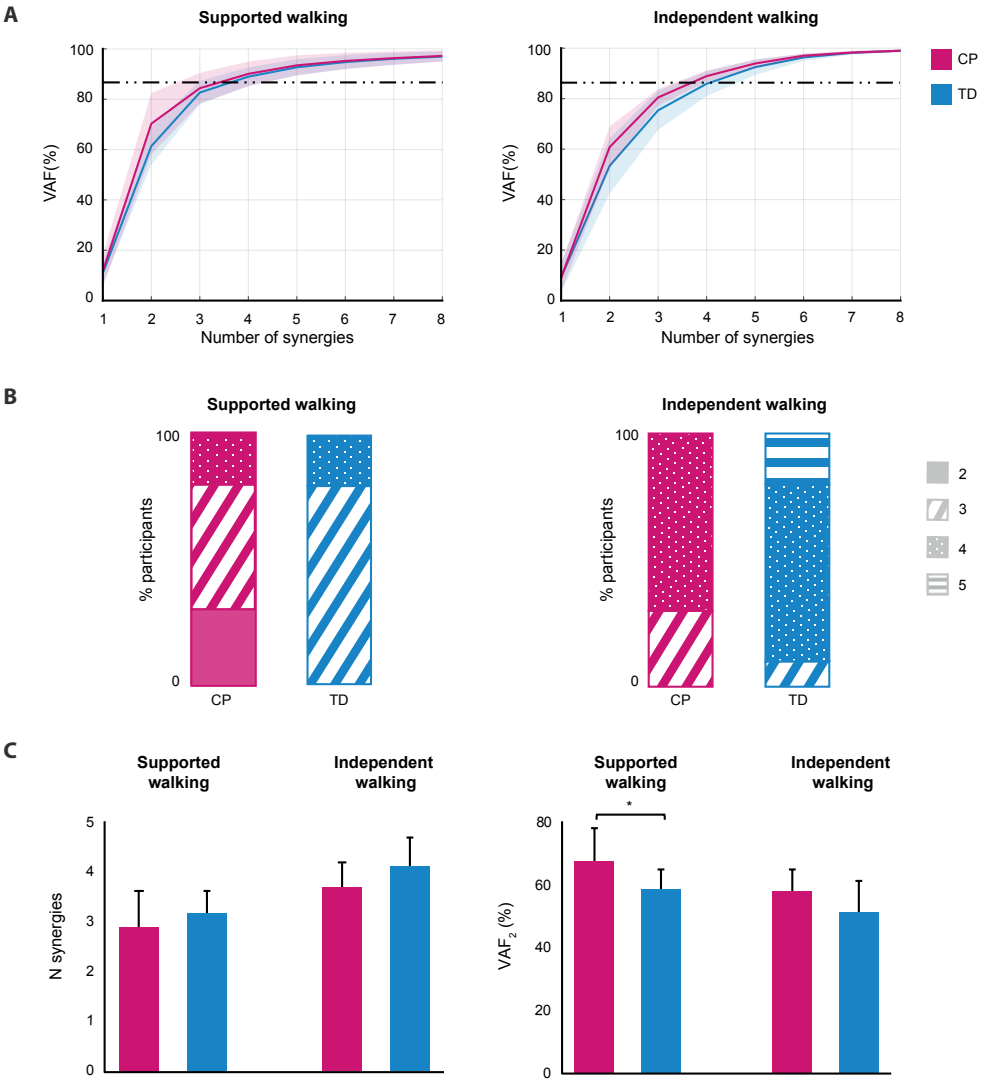


Figure 3.1 Bilateral EMG analysis results. A) Mean variability accounted for (VAF) ± SD, per synergy 1–8 for the cerebral palsy (CP) and typically developing (TD) group, for supported walking (*left*) and independent walking (*right*). **B)** Percentage number of synergies per group (n = 10) based on a VAF threshold of 85% or added VAF < 8% for the CP and TD group, for supported (*left*) and independent walking (*right*). **C)** Mean number (N) of synergies (*left*) and variability accounted for (*right*) by two synergies (VAF_2) for the CP and TD group, for supported walking and independent walking. Error bars indicate SDs, * $p < 0.05$.

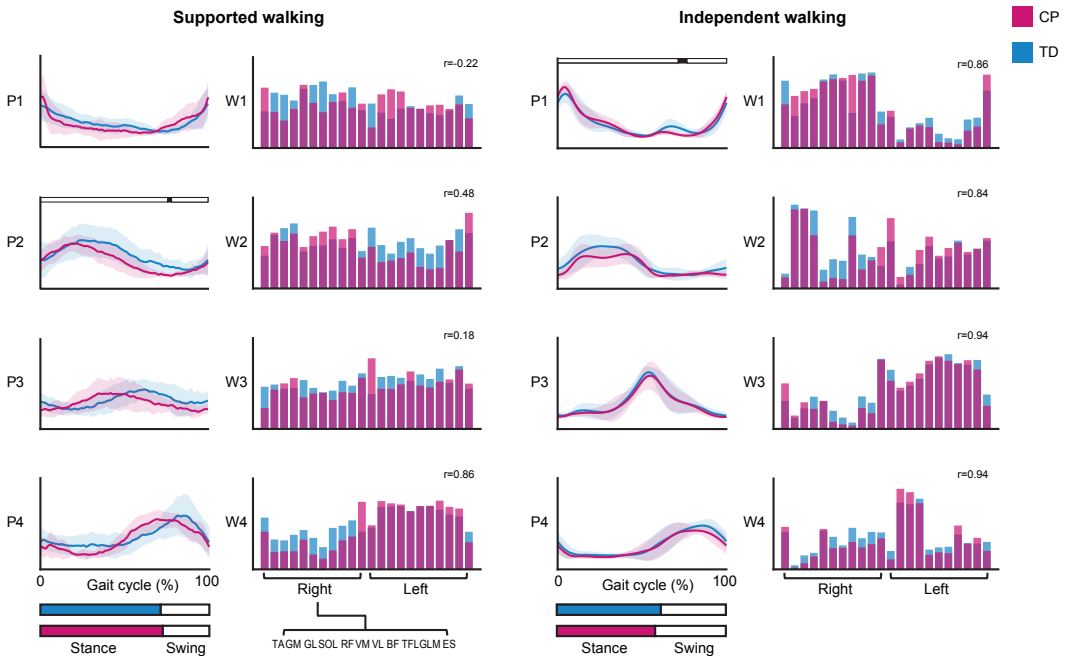


Figure 3.2 Mean activation patterns and synergy weights for a fixed number of four synergies. Bilateral EMG analysis results of the cerebral palsy (CP) and typically developing (TD) group, for supported walking (*left*) and independent walking (*right*). Lines show the mean temporal activation patterns (P) ± SD along the gait cycle, with mean stance and swing phase indicated by bar graphs at the bottom. Synergy weights (W) for the recorded muscles are depicted in a bar graph. Significant differences between activation patterns are indicated by the black bars, $p < 0.05$. Pearson's correlations coefficients (r) between mean synergy weights of the CP and TD group are given. Abbreviations: TA, tibialis anterior; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SOL, soleus; RF, rectus femoris; VM, vastus medialis; VL, vastus lateralis; BF, biceps femoris; TFL, tensor fascia latae; GLM, gluteus maximus; ES, erector spinae at L2 level.

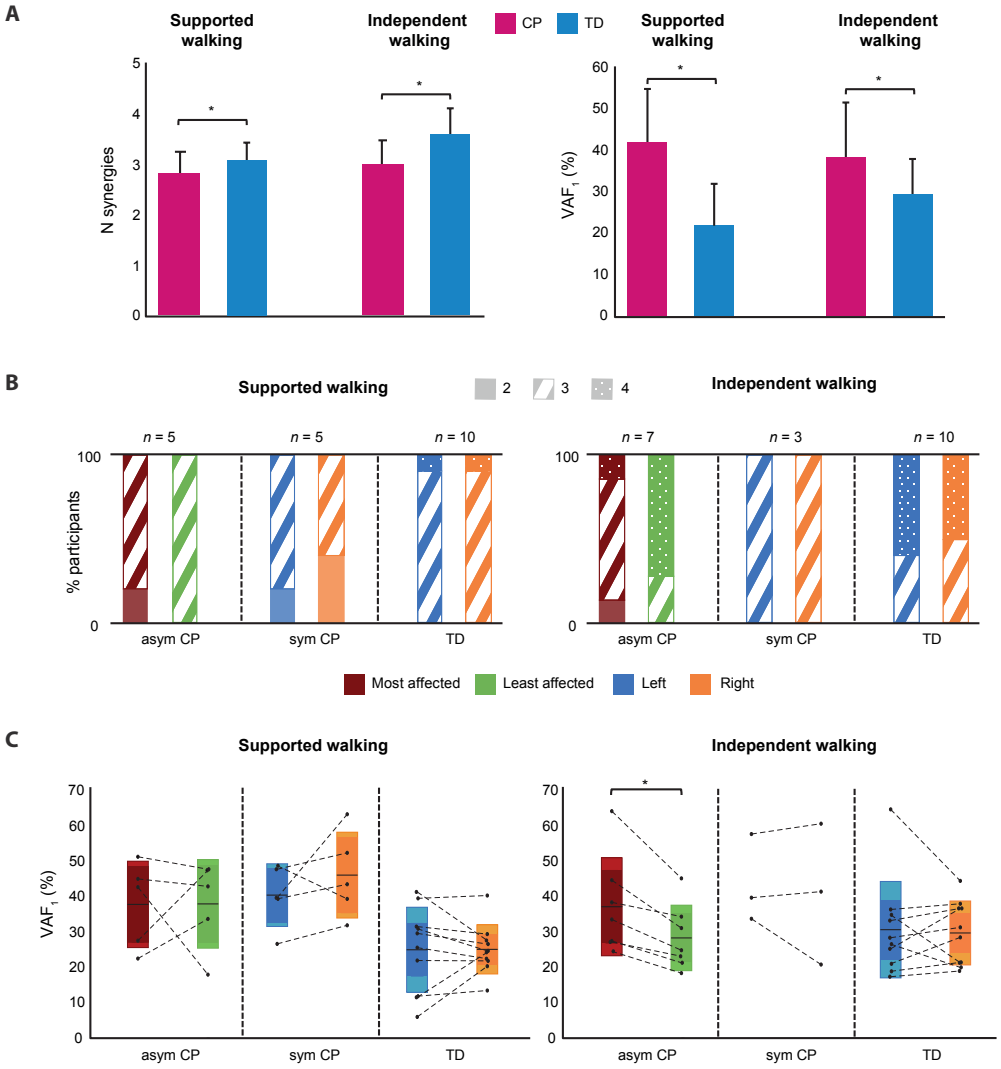


Figure 3.3 Unilateral EMG analysis results. A) Mean number (N) of synergies (*left*) and variability accounted for (*right*) by one synergy (VAF_1) for the CP and TD group, for supported walking and independent walking. Error bars indicate SDs. The unilateral EMG analysis involves the most affected side for the asymmetric CP group and a random side for the symmetric CP and TD group. **B)** Percentage number of synergies for each side. The most affected (red) and least affected (green) side for asymmetric CP, and the left (blue) and right (orange) side for symmetric CP and TD. **C)** Variability accounted for by one synergy (VAF_1) for each side. Mean VAF_1 per side is indicated by the black line in the middle of the box. The dark color of the box indicates the 95% confidence interval, and the lighter color one standard deviation. Individual participant values are indicated by black dots, and both sides of each participant are connected by broken lines. Significance between sides is indicated by a *, $p < 0.05$. Abbreviations: asym, asymmetric; sym, symmetric; *n*, number of participants.

DISCUSSION

Our study shows that children with CP recruit fewer muscle synergies compared to TD children already in the supported walking phase, and in the first years after onset of independent walking. To our knowledge, this study is the first to investigate neuromuscular control using muscle synergy analysis during supported and independent walking in such a young patient population. Our results are consistent with the hypothesis that the complexity of neuromuscular control is already reduced in very young children with CP. The critical developmental window may be before the age of two years old, when the brain is highly plastic and the corticospinal tract is still maturing (Friel, Williams, Serradj, Chakrabarty, & Martin, 2014; Hadders-Algra, 2004; Yang et al., 2013). The novelty of our findings is that the difference in neuromuscular control of walking is present in children with CP compared to TD children with the same level of walking ability during this early phase of motor development.

Unilateral EMG analysis revealed that children in the CP group recruited fewer synergies during walking, and VAF_1 was higher compared to the TD group before (during SW) and after the onset of independent walking (during IW), which is in line with previous results on VAF_1 obtained in older children (Goudriaan et al., 2018; Kim et al., 2018b; Steele et al., 2015). In these studies, however, no distinction was made between children with CP who used an assistive device to walk, and children who walked independently. In addition, the children with CP were compared with TD children of the same age, who very often had more experience of walking independently. Especially since the first independent steps, in children with CP, are typically delayed or will not occur at all. Using the walking experience, and not age, to compare motor ability in children with CP and TD children is an innovative approach. This allows for a more reasonable comparison, and to control for the improvement in walking pattern that occurs after the onset of independent walking.

In children with asymmetric CP walking independently, the number of synergies was lower, and VAF_1 higher, in the most affected compared to the least affected side. This suggests that 'simplified' neuromuscular control is specific to the most affected side in children who just start to walk independently. In bilateral EMG analysis, the least affected side in children with asymmetric CP walking independently may level out the effect of the most affected side, which can explain the lack of statistically significant differences in number of synergies between CP and TD, at least to some degree. The difference between the most and least affected side was not present in the asymmetric CP group during SW, which may have been caused by the support the children received. Alternatively, it might imply that this difference between sides occurs later in motor development.

When we removed the restriction of a threshold by fixing the number of synergies to four, we found only minor significant differences in temporal activation patterns, and synergy weights were highly correlated during IW between the CP and TD group. This confirms previous studies that reported comparable synergy structures during walking in children with CP with high functional mobility levels and TD children (Cappellini et al., 2016; Yu et al., 2019). The lower number of synergies in children with CP walking independently, as defined by a VAF threshold, possibly results from the merging of the four synergy structures (Clark et al., 2010; Hashiguchi et al., 2016). If true, this means that these children may have access to four synergies, but are not able to recruit all synergies independently. During SW, a fourth synergy did not contain additional information, since all muscles were generally active for both CP and TD, showing that on average these children could not access four synergies.

The variability in results from the EMG analysis between individuals was high. This may reflect the heterogeneity of the CP group, including children with various brain lesions and functional mobility levels, as was emphasized in previous studies (Bekius et al., 2020; Shuman et al., 2018; Steele et al., 2015). The population investigated is very young, and although we compared between children in the same developmental stage variability between children within groups may be large. Despite variability between individuals and within relatively small groups, we found significant differences between the CP and TD group during SW and IW.

Some limitations of this study have to be recognized. In young children at high risk of CP, we did not always know whether they would actually develop CP. Not all children with a high risk of CP eventually receive a diagnosis of CP, and motor types and topography may emerge and change during the first two years of life (Novak et al., 2017). Another limitation of the study was that a relatively small number of participants was included in the IW symmetric CP group, and as a consequence, we could not perform a statistical comparison between sides. Walking speed is an important factor to consider in muscle synergy analysis, since some studies found that walking speed affected the number and structure of muscle synergies (Kibushi et al., 2018; Steele et al., 2015; Yokoyama et al., 2016). The children in our study walked at their preferred and comfortable speed, which may have caused variability within the groups. However, average walking speed did not significantly differ between the CP and TD group during both SW and IW, and thus walking speed did not influence the results of the group comparison.

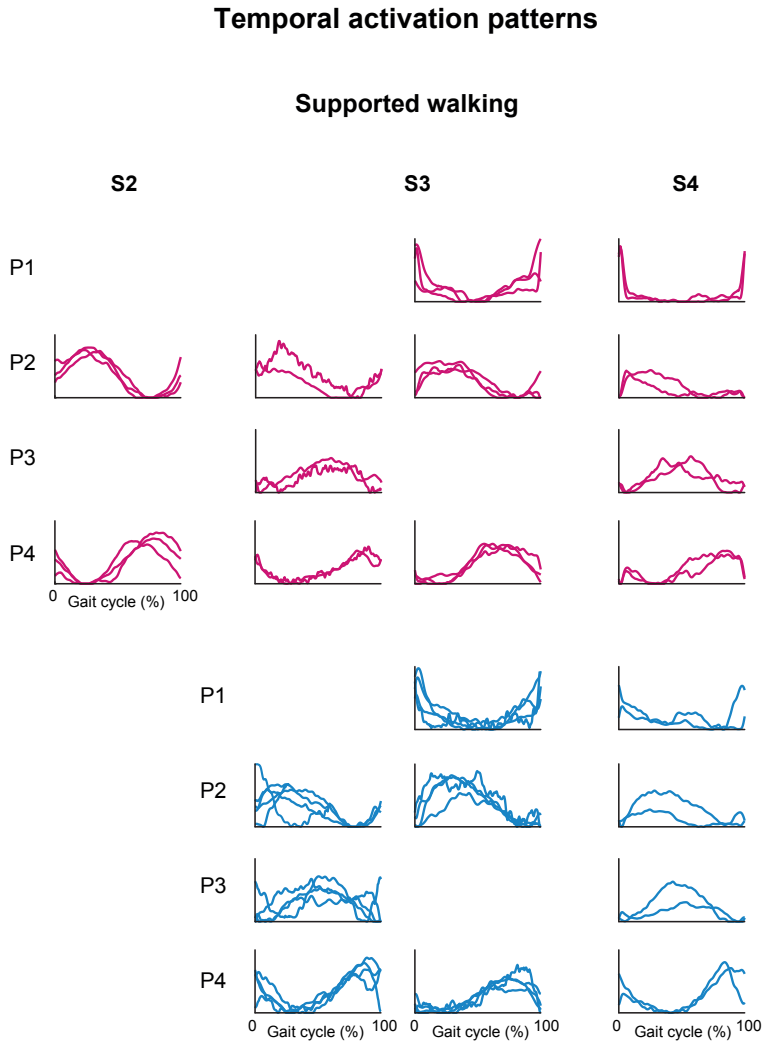
Our results encourage further investigation of the use of muscle synergy analysis as an objective tool for early detection of impaired neuromuscular control. This can help to identify candidates for targeted early interventions aimed at improving neuromuscular control and walking development. Future research

should investigate the longitudinal development of muscle synergies within children during development from supported walking to independent walking to minimize the inter-subject variability.

Conclusions

In conclusion, our study shows that young children with CP, or at high risk of CP, recruit fewer synergies compared to TD children with the same walking ability already in the early phase of motor development. The most affected side in children with asymmetric CP walking independently employed fewer synergies than the least affected side. This suggests that brain lesions in CP result in early alterations of neuromuscular control.

APPENDIX


Temporal activation patterns
Supported walking
S2
S3
S4
P1
P2
P3
P4
0
Gait cycle (%)
100

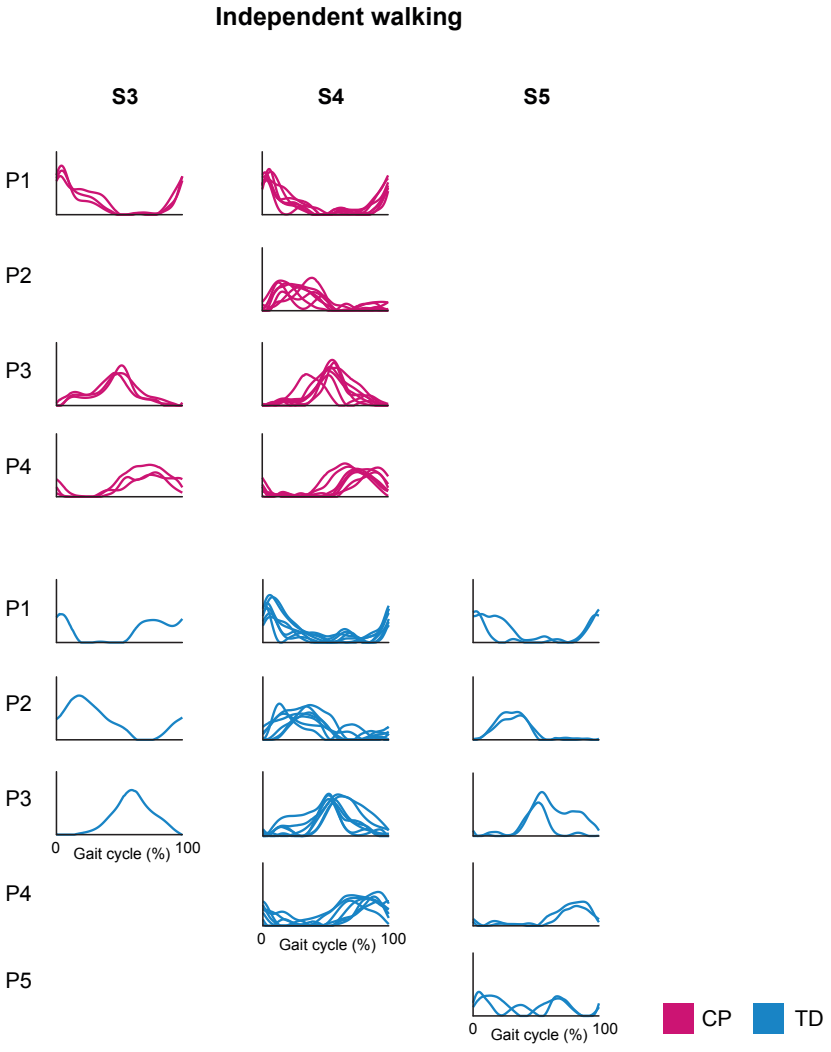


Figure 3.S1 Individual temporal activation patterns. Bilateral EMG analysis: temporal activation patterns (P1 to P5) for the number of synergies (S2 to S5) defined for each participant (separate lines) based on the variability accounted for (VAF) threshold of 85% for the cerebral palsy (CP) and typically developing (TD) group in the supported walking group (*left*) and independent walking group (*right*).

4

Early development of locomotor patterns and motor control in very young children at high risk of cerebral palsy, a longitudinal case series

Bekius, A., Bach, M. M., van de Pol L. A., Harlaar J., Daffertshofer A., Dominici N., Buizer A. I. (2021). *Frontiers in Human Neuroscience, 15,* 232

ABSTRACT

Background The first years of life might be critical for encouraging independent walking in children with cerebral palsy (CP). We sought to identify mechanisms that may underlie the impaired development of walking in three young children with early brain lesions, at high risk of CP, via comprehensive instrumented longitudinal assessments of locomotor patterns and muscle activation during walking.

Methods We followed three children (P1-P3) with early brain lesions, at high risk of CP, during five consecutive gait analysis sessions covering a period of one to two years, starting before the onset of independent walking, and including the session during the first independent steps. In the course of the study, P1 did not develop CP, P2 was diagnosed with unilateral and P3 with bilateral CP. We monitored the early development of locomotor patterns over time via spatiotemporal gait parameters, intersegmental coordination (estimated via principal component analysis), electromyography activity, and muscle synergies (determined from eleven bilateral muscles via non-negative matrix factorization).

Results P1 and P2 started to walk independently at the corrected age of 14 and 22 months, respectively. In both of them, spatiotemporal gait parameters, intersegmental coordination, muscle activation patterns and muscle synergy structure changed from supported to independent walking, although to a lesser extent when unilateral CP was diagnosed (P2), especially for the most affected leg. The child with bilateral CP (P3) did not develop independent walking and all the parameters did not change over time.

Conclusions Our exploratory longitudinal study revealed differences in maturation of locomotor patterns between children with divergent developmental trajectories. We succeeded in identifying mechanisms that may underlie impaired walking development in very young children at high risk of CP. When verified in larger sample sizes, our approach may be considered a means to improve prognosis and to pinpoint possible targets for early intervention.

INTRODUCTION

Cerebral palsy (CP) is a neurodevelopmental disorder caused by non-progressive brain lesions before birth or in the first year of life (Himmelmann & Uvebrant, 2018). The distribution of CP can be unilateral or bilateral, depending on the site of the brain lesion. CP covers a wide clinical spectrum of mobility levels, varying from walking independently to being completely wheelchair dependent. Functional mobility in CP is classified using the Gross Motor Function Classification System (Palisano et al., 1997). The levels of the GMFCS range from I to V, with children at level I and II ultimately walking without aids, and children at level III needing a walking aid. Children at level IV and V (primarily) use a wheelchair for their mobility.

Walking is the most important mode of locomotion in everyday life. Typically developing (TD) children take their first independent steps between the age of 9 and 18 months old, but this milestone is often delayed or not achieved in children with CP. Developmental stages of locomotion usually occur at a later age in children with CP compared to TD children (Largo et al., 1985; Meyns et al., 2012a). The maturation of walking patterns is reflected in both kinematic and neuromuscular measures (Dewolf et al., 2020). Understanding the mechanisms that underlie abnormal development of independent walking is important for the design of early interventions that aim at improving function mobility in children with CP.

Older children with CP appear to retain some of the characteristics of the younger TD children during the early phases of the development of walking (Berger et al., 1984; Leonard et al., 1991). In TD children the foot trajectory during swing (the time course of the vertical foot displacements) develop starting from a prominent centered single-peak foot lift in toddlers to the stereotyped double-peaked trajectory with a minimum foot clearance during mid-swing in older children and healthy adults (Dominici et al., 2007). Indeed, the mature two-peaked foot trajectory reflect the accurate endpoint control strategy that is the result of the intersegmental coordination in both limbs (Ivanenko, Grasso, Macellari, & Lacquaniti, 2002; Winter, 1992). By contrast, in children with CP a single-peak foot lift similar to TD toddlers may persist, often specific to the most affected side in unilateral CP (Cappellini et al., 2016). While in TD children the intersegmental coordination of the lower limb segments quickly develops (Cheron et al., 2001; Dominici et al., 2011; Ivanenko et al., 2004a, 2007) – typically, intersegmental coordination develops rapidly in the first few months after the onset of independent walking (Cheron et al., 2001) – in children with CP this may be less pronounced (Berger, 1998; Leonard et al., 1991). Apparently, intersegmental coordination matures less, or, much slower in children with CP. In unilateral CP, this has been shown to be specific to the most affected body side (Cappellini et al., 2016).

One way to assess neuromuscular control during walking in healthy individuals and patient populations is through muscle synergy analysis. Walking requires refined neuromuscular coordination, and the central nervous system arguably simplifies neuromuscular control during walking by the recruitment of groups of muscles, called muscle synergies or locomotor modules (Bizzi & Cheung, 2013; Dominici et al., 2011; Hart & Giszter, 2010; Ivanenko et al., 2005a). During typical development, the number of basic activation patterns increases from two during neonate stepping, to four, when children walk independently (Dominici et al., 2011; Sylos-Labini et al., 2020). Muscle activity patterns during walking in older children with CP seem to match the patterns of TD toddlers (Cappellini et al., 2016), suggesting that also the maturation of the activation of individual muscles lags behind in children with CP. School-age children with CP recruit fewer muscle synergies compared to TD children and it seems that they employ 'simpler' neuromuscular control strategies (Bekius et al., 2020; Goudriaan et al., 2018; Hashiguchi et al., 2018; Schwartz et al., 2016; Shuman et al., 2016, 2017, 2018, 2019; Steele et al., 2015; Tang et al., 2015). In children with CP, the temporal structure of muscle synergies, i.e. their activation patterns, largely agrees with that of TD toddlers, i.e. they contain wider activation bursts compared to older TD children.

Combining kinematic and neural measures can provide additional insight into motor development and may help to detect early motor deficits (Dewolf et al., 2020). Yet, previous studies investigating locomotor patterns in young children with CP had a cross-sectional design (Cappellini et al., 2016, 2018), which may limit inferences about how changes in neuromuscular control are related to the altered development of walking. In addition, a limited number of recorded muscles in previous studies (Bekius et al., 2020; Shuman et al., 2016, 2017; Steele et al., 2013; Tang et al., 2015) may limit the conclusions on the spatiotemporal structure of muscle activity patterns. A more detailed and comprehensive assessment of multi-muscle coordinated patterns is needed (Damiano, 2015). The aim of the current exploratory longitudinal study was to investigate the development of locomotor patterns and motor control during walking in very young children with early brain lesions, at high risk of CP. The focus was on development starting before the onset of independent walking, covering a period of one to two years, with the aim to record the emergence of the first independent steps. We studied locomotor patterns using kinematic analysis and motor control using muscle synergy analysis. We hypothesized (1) intersegmental dependency and muscle synergy structure to change over time after independent walking onset, and (2) the development of gait kinematics and muscle synergies to be delayed in children with a diagnosis of CP. We also expected (3) differential changes of gait kinematics on the most versus the least affected side when unilateral CP was diagnosed, and (4) the number of synergies to be reduced the more a child was affected by CP.

MATERIALS AND METHODS

Participants

Three children with early brain lesions, at high risk of CP and not yet walking independently, were included in this longitudinal study. The participants were recruited from the Department of Pediatric Rehabilitation and the Department of Pediatric Neurology at the Amsterdam University Medical Centers (Amsterdam UMC, location VUmc). The study was approved by the Medical Ethics Committee (METc) of the Amsterdam UMC (location VUmc) (NL59589.029.16). The parents of all children were informed about the procedure of the study and provided written informed consent prior to participation in accordance with the Declaration of Helsinki for medical research involving human participants.

Study design

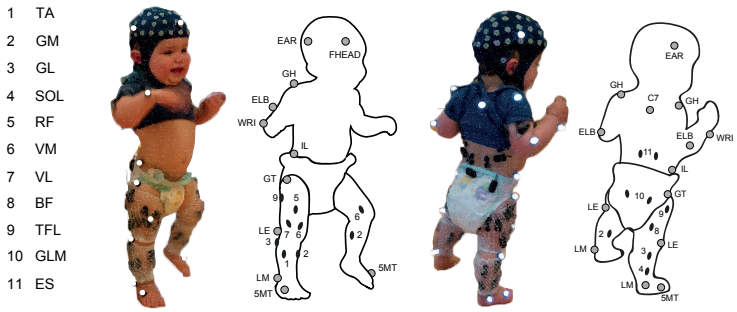
Experiments were performed in the clinical gait laboratory of the Department of Rehabilitation Medicine at the Amsterdam UMC, location VUmc. The laboratory setting and experimental procedures were adapted to the children, such that the risks were equal or lower to that of walking at home. The responsible investigators, one or both parents of the child, and a pediatric physiotherapist were present during the experiments.

Every participant underwent five consecutive gait analysis sessions within a period of one to two years, to record development from supported walking (SW), to first independent steps (FS), to independent walking (IW). SW sessions were conducted prior to independent walking onset. There, the pediatric physiotherapist, experimenter, or parent supported the child during stepping attempts by its trunk or arms. FS sessions were recorded when the children performed their first unsupported steps. They were scheduled within two weeks of first unaided walking experience, as reported by the parents. All sessions recorded after the FS session were labelled IW (Table 4.1).

All children walked barefoot on a treadmill during SW sessions and over-ground during FS and IW sessions. If children wore ankle-foot orthoses in daily life, these were removed during the experiment. The treadmill speed was adjusted to induce a walking pattern and tuned to a comfortable speed for the child. During FS and IW sessions, children were encouraged to walk in a straight line at a comfortable walking speed.

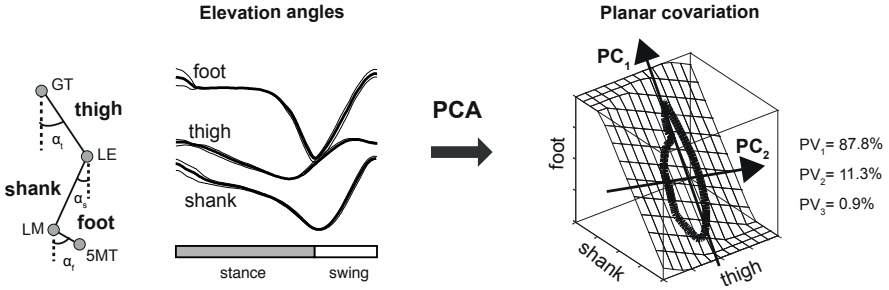
A

EMG and marker locations



B

Intersegmental coordination



C

Session overview

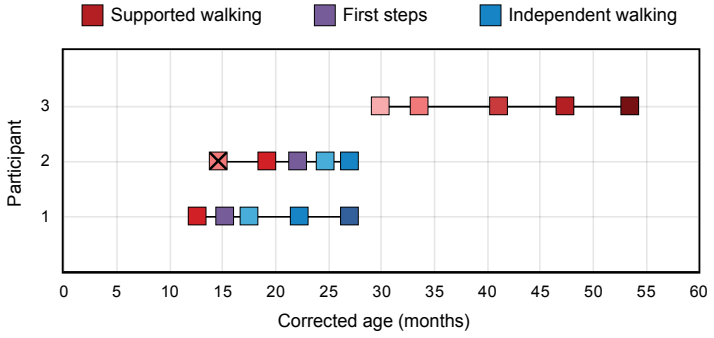


Figure 4.1 Experimental setup and gait analysis session overview. A) Marker locations are denoted by grey circles, and EMG electrode locations by black ovals. 5MT, head of fifth metatars-phalangeal joint; LM, lateral malleolus; LE, lateral femur epicondyle; GT, greater trochanter; IL, iliac crest; WRI, ulnar styloid; ELB, lateral humeral epicondyle; GH, gleno-humeral joint; C7, seventh cervical vertebra; EAR, ear; FHEAD, forehead; TA, tibialis anterior; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SOL, soleus; RF, rectus femoris; VM, vastus medialis; VL, vastus lateralis; BF, biceps femoris; TFL, tensor fascia latae; GLM, gluteus maximus; ES, erector spinae at L2 level. **B)** Schematic illustration of principal component (PC) analysis of elevation angles of lower limb segments (thigh: α_t, shank: α_s, and foot: α_f), and its corresponding gait loop plotted in 3D in one representative typically developing child (4 years old) during walking. A loop is obtained by plotting the thigh elevation angle vs. shank and foot elevation angles (after mean values subtraction). The shape and orientation of the gait loop reflect the coordination of limb segments as described by PC_1 (reflects the largest variance which corresponds to the length of the gait loop), and PC_2 (the second largest variance which corresponds to the width of the loop), here indicated with black arrows. Percentage of total variation explained by the three PCs (PV_1-PV_3) are indicated. Note in adult and older children walking, two principal components typically account for about 99% of the total variance (PV_3 = 0.9% in the example). **C)** Gait analyses over time (corrected age, in months) for P1-P3 showing supported walking (SW: shades of red), first steps (FS: purple), and independent walking (IW: shades of blue). Different color shades from light to dark indicate the change in age and/or independent walking experience. P3 did not start to walk independently. X means the child did not take any steps during that session.

Table 4.1 Participant characteristics

Partici-pant	Gender	Session	Walking ability	Age (mo)	CA (mo)	WA (mo)	Distri-bution	Subtype	GMFCS	Brain damage	BW (kg)	BL (cm)	N strides
P1	F	1	SW	12.7	12.7	-1.8	Bi	Spastic	HR	Infection	7.8	75	37
		2	FS	15.2	15.2	0.8	Bi	Spastic	HR		8.8	74	47
		3	IW	17.5	17.5	3.1	Bi	Spastic	I		9.4	80	62
		4	IW	22.3	22.3	7.9	Bi	Spastic	I		10.6	85	42
		5	IW	27.2	27.2	12.8	Bi	Undef	n/a		11.0	87	33
P2	M	1	SW	14.5	14.6	-6.6	Uni R	Spastic	HR	Media infarction	11.1	84	0
		2	SW	19.2	19.3	-1.4	Uni R	Spastic	HR		12.0	84	52
		3	FS	22.1	22.2	0.3	Uni R	Spastic	I		12.4	91	29
		4	IW	24.6	24.7	2.8	Uni R	Spastic	I		12.0	89	40
		5	IW	26.9	27	5.1	Uni R	Spastic	I		12.4	90	54
P3	F	1	SW	31.7	30.0	-	Bi	Spastic	III	PVL	12.7	85	60
		2	SW	35.4	33.7	-	Bi	Spastic	III		12.8	90	36
		3	SW	42.8	41.1	-	Bi	Spastic	III		14.0	95	59
		4	SW	49.1	47.4	-	Bi	Spastic	III		15.0	105	48
		5	SW	56.1	53.6	-	Bi	Spastic	III		12.6	105	51

Abbreviations: F, female; M, male; SW, supported walking; FS, first steps; IW, independent walking; mo, months; CA, corrected age; WA: walking age (expressed as time since onset of independent walking); Bi, bilateral; Uni, unilateral; R, right; undef, undefined; GMFCS, gross motor function classification system; n/a, not applicable; HR, high risk; asym, asymmetric; sym, symmetric; PVL, periventricular leukomalacia; BW, body weight; BL, body length; N, number.

Data acquisition

During every session, full-body kinematics were recorded bilaterally and sampled at 100 Hz using a VICON system (Oxford, UK) with 12 cameras surrounding the 10-meter-long walking area. Reflective markers (diameter 14 mm) were attached to the skin of the participants representative of anatomical reference points overlying the following bony landmarks on both sides of the body (Figure 4.1A): gleno-humeral joint (GH), seventh cervical vertebra (C7), ear (EAR), forehead (FHEAD), ulnar styloid (WRI), lateral humeral epicondyle (ELB), iliac spinal crest (IL), greater trochanter (GT), lateral femur epicondyle (LE), lateral malleolus (LM), head of fifth metatars-phalangeal joint (5MT). In addition, videos were recorded at 50 Hz using the VICON system using two cameras placed in the sagittal plane.

Muscle activity was assessed by means of surface electromyography (EMG) from 11 pairs of bilateral leg and trunk muscles, i.e., tibialis anterior (TA), gastrocnemius medialis (GM), gastrocnemius lateralis (GL), soleus (SOL), rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL), biceps femoris (BF), tensor fascia latae (TFL), gluteus maximus (GLM) and erector spinae at L2 level (ES). Mini-golden reusable surface EMG disc-electrode pairs (15 mm diameter electrodes, acquisition area of 4 mm^2) were placed at the approximate location of the muscle belly on the cleaned skin, with inter-electrode spacing of about 1.5 cm. EMG electrode placement was performed according to the Surface Electromyography for the Non-Invasive Assessment of Muscles protocol (Hermens et al., 2000) and the recommendations for minimizing cross-talk between adjacent muscles that was described in detail previously (Dominici et al., 2011; Ivanenko et al., 2004b, 2005a). Pre-amplified EMG sensor units were attached to the skin and fixed with elastic gauzes, to minimize movement artefacts (Figure 4.1A). Two Cometa Mini Wave wireless 16-channel EMG systems (Cometa, s.r.l, Italy) were used and EMG signal were sampled at 1 kHz. Electro-encephalography (EEG) recordings were made but not analyzed here. Sampling of kinematic, video and EMG signals was synchronized.

Data analysis

The gait patterns were described by: 1) kinematics: a) spatiotemporal gait parameters, including walking velocity, stride duration and double support duration; b) intersegmental coordination, estimated via principal component analysis (PCA) of the elevation angles of thigh, shank, and foot segments (Borghese et al., 1996), and 2) neuromuscular control: EMG activity and muscle synergies characteristics, described by the full-width at half-maximum (FWHM) of the muscle activity, temporal activation patterns of the muscles synergies and variability accounted for (VAF). Gait initiation and termination as well as jumps and turns were discarded from analysis. The lower body was modelled as an interconnected

chain of rigid segments: GT-LE for the thigh, LE-LM for the shank, and LM-5MT for the foot. All analyses were conducted in MATLAB (version 2020a, Mathworks Inc., Natick, USA).

Spatiotemporal gait parameters

Step events were manually defined based on the video recordings and later confirmed using the kinematic data. We defined a stride from foot strike to foot strike of the ipsilateral foot, stance duration as the percentage of the gait cycle from foot strike to foot off. Stride velocity was computed using the corresponding stride length (3D-displacement of the LM-marker) and duration of both legs. Data were time-interpolated over individual gait cycles to fit a normalized 201-point time base.

Intersegmental coordination

We constructed elevation angles of the thigh, shank, and foot segments (α_t, α_s, and α_f, respectively) to correspond with angles between the segment projected on the sagittal plane and the vertical, i.e. to be positive in the forward direction when distal markers fell anterior to the proximal one. In healthy adults and older children, these elevation angles describe a path (gait loop) that lies close to a plane that is defined by their eigenvectors (Figure 4.1B). To examine the development of intersegmental coordination (the gait loop and the corresponding plane) across sessions within subjects, PCA was applied to lower limb elevation angles of each gait cycle, for each session and participant separately (Bianchi et al., 1998; Borghese et al., 1996; Dominici et al., 2010; Ivanenko et al., 2007). Briefly, we computed the covariance matrix of the thigh-shank-foot elevation angels (after subtraction of the mean value of each angular coordinate) across the gait cycle. The three principal components (PC_1-PC_3) including the explained variance (PV_1-PV_3) and eigenvectors ($\mathbf{u_1}$-$\mathbf{u_3}$), were obtained (Figure 4.1B). In general, the first two eigenvectors lie on the best-fitting plane of angular covariation, with $\mathbf{u_2}$ orthogonal to $\mathbf{u_1}$, while the third eigenvector, $\mathbf{u_3}$ is the normal to the covariation plane and defines the plane orientation. For every eigenvector, the parameters u_{it}, u_{is}, and u_{if} correspond to the direction cosines with the positive semi-axis of the thigh, shank and foot angular coordinates, respectively. The percentage of variance accounted for by the second (PV_2) and third (PV_3) eigenvectors were assessed and compared between sessions, as well as the rotation of the normal to the plane by using the u_{3t} parameter (Bianchi et al., 1998; Cappellini et al., 2018; Dominici et al., 2007, 2010).

Muscle activity

EMG signals were notch and high-pass filtered (4th-order Butterworth 50 ± 0.01 Hz, and > 30 Hz, respectively), full-wave rectified, and subsequently low-pass filtered (4th-order Butterworth < 10 Hz) to obtain linear envelopes. For some sessions, EMG data from few muscles had to be discarded due to sustained artefacts after filtering. Possible contamination of the EMG recordings could be detected by the electrical cross-talk due to volume conduction of activity across adjacent muscles. This issue is particularly relevant when recording many muscles in young children due to their small body size and the resulting close spacing of nearby muscles. Nevertheless, the small size of the EMG electrodes used in our settings and the chosen inter-electrode distance should have minimized the pickup from adjacent muscles. Anyway, we attempt to quantify the potential electrical cross-talk by performing a cross-correlation analysis of selected pairs of adjacent flexor/extensor muscles. Cross-correlation was computed after notch (50 Hz) and high-pass (30 Hz) filtering the EMG data to remove any possible power line noise and movement artefacts. We identified the peak of the normalized cross-correlation > 0.3 between flexors and extensors as suspect cross-talk (d'Avella, Portone, & Lacquaniti, 2011; Dominici et al., 2011) in 0.1-9.9% of strides, though the values of the correlation coefficients were generally not very high (Appendix Table 4.S1). We verified that the removal of those strides potentially affected by cross-talk did not change any conclusion drawn from those analyses.

The data were time-normalized for each limb to $t = 201$ data points per gait cycle. The FWHM of the muscle activity was determined based on the minimum-subtracted envelopes of muscle activity patterns (Alves-Pinto, Blumenstein, Turova, & Lampe, 2016; Bach et al., 2021b; Cappellini et al., 2016, 2018), separately for every muscle, gait cycle, and session. To ease comparison of our experimental finding with literature, we also reported the FWHM of the mean muscle activity for each muscle, and session (see *Appendix* Figure 4.S1). In case of muscle activations with more than one peak, the peak with the largest maximum was chosen as the main peak. The FWHM quantifies the duration of the burst of activity as it equals the sum of the durations of the intervals in which the EMG signal exceeded half of their maximum. That is, the larger the FWHM the longer the muscle contraction is sustained.

Muscle synergies

Per session, EMG amplitudes of every muscle were normalized to the maximum of their mean value across strides plus its standard deviation (SD). Non-negative matrix factorization (NMF) was applied to the unilateral gait cycle averaged EMG envelopes to extract muscle synergies. NMF decomposes the EMG into $i = 1, ..., n$ temporal activation patterns P_i and corresponding synergy weights W_i by mean of the linear combination

$$EMG = \sum_{i=1}^{n} P_i W_i + \varepsilon \quad (4.1)$$

where the pre-processed EMG data ($m \times t$ matrix, where m is the number of muscles, here 11, and t is the number of time points, here 201) is a linear combination of the temporal activation patterns P ($n \times t$ matrix, where $n \leq m$ is a predetermined number of synergies, see below) and synergy weights W ($m \times n$ matrix), and ε denotes the residual error.

The reconstruction accuracy of the extracted synergies was determined by calculating the percent of variability accounted for (VAF), which is the ratio of the sum of squared errors to the total sum of squares computed with respect to the mean (Cappellini et al., 2016; Dominici et al., 2011). In order to compare the synergy patterns between sessions, we aimed to fix the number of synergies per participant across sessions, by varying the number of synergies from 1 to 8, and exploring the VAF slopes and synergy patterns across sessions. In addition, we investigated VAF by one synergy (VAF_1, Shuman et al., 2019; Steele et al., 2015), and compared this parameter between sessions.

To quantitatively characterize differences in the duration of synergy activation patterns, we also computed the corresponding FWHM (Bach et al., 2021b; Cappellini et al., 2016, 2018), in line with the approach of the muscle activation patterns (see above).

Statistical analysis

Statistical analyses were performed using SPSS (IBM SPSS Statistics for Mac, Version 26.0, IBM Corp., Armonk, NY, USA). All data are reported as mean (±SD). Normality was assessed using Q-Q plots, and homogeneity of variance was tested using a Levene's test. Development of spatiotemporal gait parameters, intersegmental coordination values, and FWHM per muscle across sessions within each participant was assessed using a linear mixed effects model (LMM, Molenberghs & Verbeke, 2000). LMM corrects for interdependency of repeated measures within one participant using random effects, and the number of observations per sessions can vary. We fitted an LMM with PV_2, PV_3, u_{3t}, stance duration, double support, velocity, and FWHM per muscle as outcome variables. *Session* was included as fixed and *trial* as random effect. To test whether development of the outcome variables differed between sides in P2, the interaction between session and side was included for P2. For all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

Medical history

Child P1 (female) was born at term after an uneventful pregnancy. Birth was complicated by umbilical cord entanglement and perinatal asphyxia with Apgar scores of 4 and 5, respectively, after 1 and 5 minutes, respectively. Clinically, she recovered quickly and there was no indication for therapeutic hypothermia. Because of a possible infection, she was treated with antibiotics for 7 days, but blood cultures remained negative. Twenty-two hours after birth, she developed severe neonatal convulsions, for which the child was treated with phenobarbitone, midazolam, lidocaine and levetiracetam. An MRI scan at the age of four days revealed extensive bilateral signal changes in the white matter of the frontal, temporal, parietal and occipital lobes, corticospinal tracts, internal capsule, and thalamus. A probable diagnosis of hypoxic-ischemic encephalopathy due to perinatal asphyxia was made. Neurological examination at the age of eight months showed a mild developmental delay and a mild paresis of the left arm. At follow-up, until the age of 3.5 years, development appeared normal and no further abnormalities were found via neurological examination, excluding a diagnosis of CP.

Child P2 (male) was also born at term after an uneventful pregnancy. The child developed normally in the first three months of life, although it was noted that the right arm appeared stiffer when parents changed clothes. When three months old, asymmetry in upper extremity motor function was established. At the age of five months, he developed West syndrome for which he was successfully treated with levetiracetam and vigabatrin. Neurological examination at the age of eight months showed spastic paresis of the right arm with hyperreflexia of the knee tendon reflex at the right side. An MRI scan of the brain at the age of eight months showed a congenital infarction of the medial cerebral artery at the left side. A diagnosis of unilateral spastic cerebral palsy, GMFCS I, was made.

Child P3 (female) was born preterm after 33 weeks and 1 day of pregnancy. At the age of 3 weeks she suffered a group B streptococcal infection. She showed a developmental delay, rolled over for the first time at the corrected age of 11 months and the tone of the lower extremities had been high since the first weeks. An MRI scan of the brain at the corrected age of 12 months, showed periventricular leukomalacia, related to prematurity. A diagnosis of bilateral spastic cerebral palsy, GMFCS III, was made.

Gait analysis session overview

For P1 and P2, the sessions covered a period of 14.5 and 12.4 months in between the first and the last session, and included one and two SW sessions, respectively, a FS session, and three and two IW sessions, respectively. For P3 the sessions covered

a period of 24 months and included five SW sessions (Figure 4.1C). P1 started to walk at a typical age, i.e. 14 months old, while P2 took its first independent steps around the corrected age of 22 months old. P3 did not walk independently yet at the corrected age of 53 months. During the first session of P2 (corrected age 14.6 months), the child did not take any steps, so for this participant only four sessions are reported in the following.

Spatiotemporal gait parameters

Video analysis revealed that P1 had a flat foot strike in session 1 and 2 (SW and FS, respectively), while both feet showed a heel strike from session 3 (IW) onwards. P2 had a flat foot strike of both feet from session 2 to 4 (SW, FS, and IW, respectively), and during session 5 (IW) only the least affected leg showed a heel strike. P3 had a toe landing and was crossing her legs in all sessions.

Walking velocity showed a significant main effect of session in all children (P1, $p < 0.001$; P2, $p < 0.001$; P3, $p < 0.01$). For P1, a significant increase was found from the SW session 1 to IW sessions 3-5 (Figure 4.2 and *Appendix* Table 4.S2). For P2, a significant increase was found from SW session 2 to IW sessions 4-5. For P3, a significant increase was found between SW session 1 and 4, but walking velocity decreased again in session 5.

The percentage of double support phase showed a significant main effect of session in all children (P1, $p < 0.001$; P2, $p < 0.01$; P3, $p < 0.001$). For P1, a significant decrease was found between SW session 1 and all sessions (Figure 4.2 and *Appendix* Table 4.S2). For P2, a significant decrease was found between SW session 2 and all sessions, in both the most and least affected side. For P3, a significant decrease was found between SW session 1 and SW sessions 3-4, but the percentage of double support phase increased again in session 5.

The percentage of relative stance duration showed a significant main effect of session in all children (P1, $p < 0.001$; most affected side P2, $p < 0.05$; least affected side P2, $p < 0.001$; P3, $p < 0.001$). For P1, a significant decrease was found between SW session 1 and all sessions (Figure 4.2 and *Appendix* Table 4.S2). For P2, a significant decrease was found between SW session 2 and all sessions in both the most affected and least affected side, although the developmental slope differed between sides, as shown by a significant interaction effect between session and side ($p < 0.01$). For P3, a significant decrease was found between SW session 1 and SW sessions 3-4, but the percentage of relative stance duration increased again in session 5.

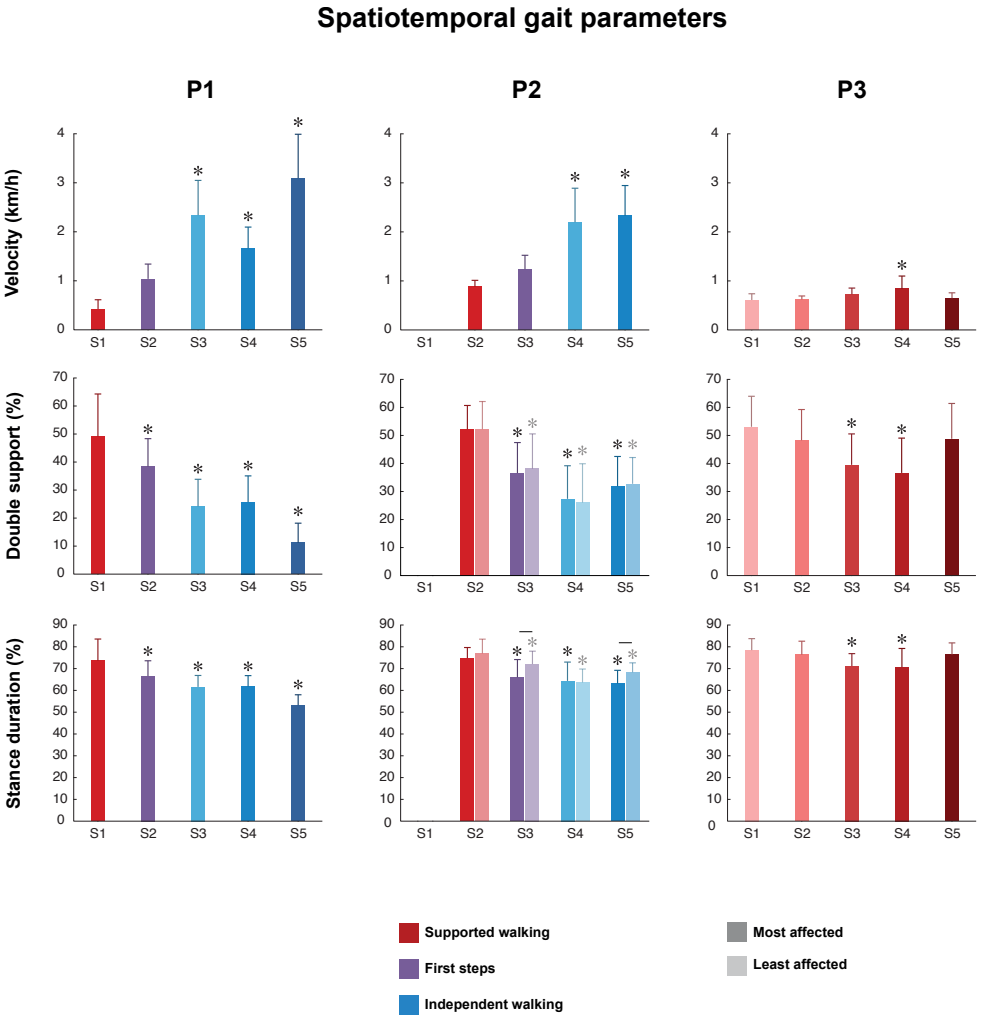


Figure 4.2 Spatiotemporal gait parameters. Velocity, percentage of double support and stance duration from S1-S5. Error bars indicate SDs. For the percentage of double support and stance duration of P2 the most affected side (dark color) and least affected side (light color) are shown, while for P1 and P3 one side is shown. Significant linear differences across sessions are denoted by asterisks, and significant interactions between session, and side are denoted by lines between the most and least affected side for P2 ($p < 0.05$).

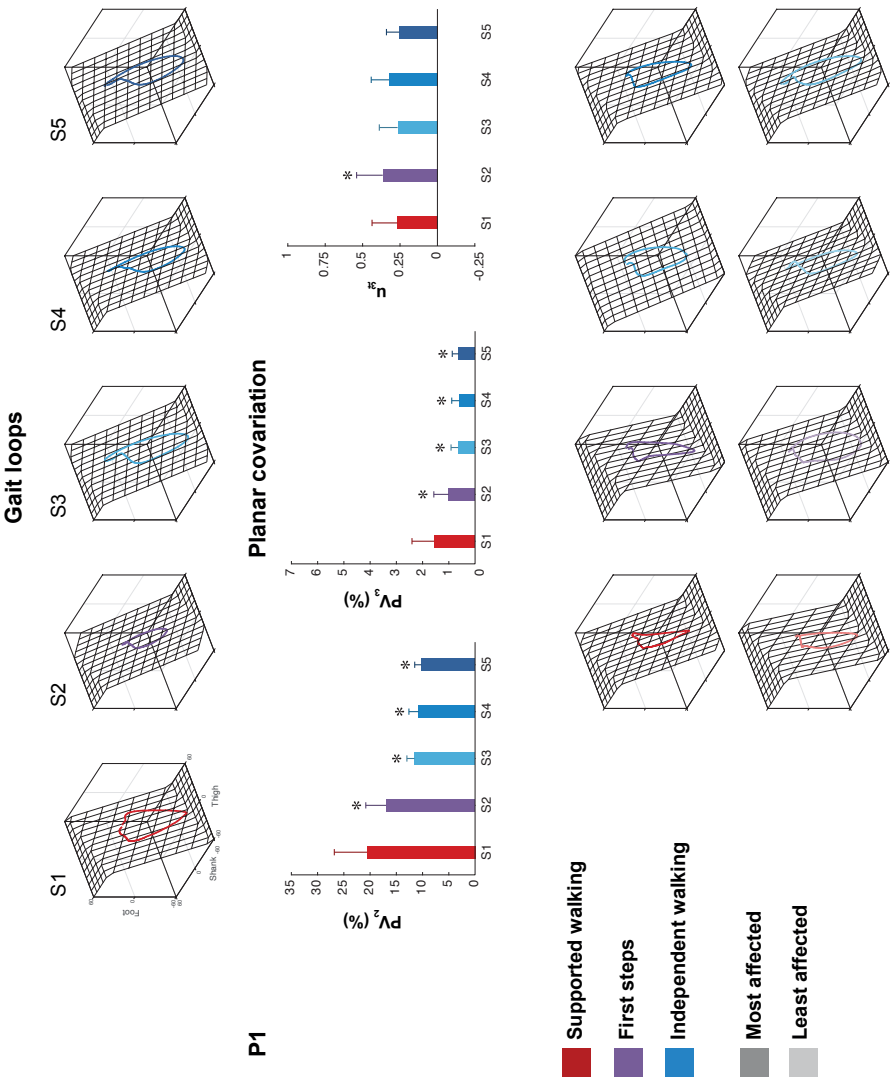
Intersegmental coordination

The intersegmental coordination of the thigh-shank-foot elevation angles was compared across sessions for the three participants and was evaluated using PCA. The planar covariation of the leg elevation angles is directly related to the dimensionality of the original data set, and the method is shown in Figure 4.1B. In summary, Figure 4.3 shows the mean gait loops for all sessions of each participant, and its corresponding values of planar covariation.

The percentage of variance accounted for by the second eigenvector (PV_2) showed a significant main effect of session in P1 ($p < 0.001$), the least affected side of P2 ($p < 0.01$), and P3 ($p < 0.001$), but not in the most affected side of P2 ($p = 0.11$). For P1 a significant decrease was found between SW session 1 and all sessions, indicating a reduction in the width of the gait loop from SW to IW, that is also visible in the changes of gait loops for the IW sessions compared to the SW session (Figure 4.3 and *Appendix* Table 4.S2). The least affected side of P2 showed a significant decrease between SW session 2 and IW session 5, but an increase during FS session 3. In contrast, P3 showed a significant increase between SW session 1 and all sessions, but the gait loops showed similar shapes in all the sessions.

The percentage of variance accounted for by the third eigenvector (PV_3), that quantified the planarity of the gait loop, showed a significant main effect of session in P1 ($p < 0.001$), the most and least affected side of P2 ($p < 0.001$ and $p < 0.01$, resp.), and P3 ($p < 0.01$). For P1, a significant decrease was found between SW session 1 and all sessions, indicating a reduction in the deviation from planarity (Figure 4.3 and *Appendix* Table 4.S2). For P2, a significant decrease was found between SW session 2 and IW sessions 4-5 in both the most and least affected side. In contrast, P3 showed a significant increase in PV_3 between SW session 1 and session 5.

The orientation of the covariance plane (u_{3t}) showed a significant main effect of session in P1 ($p < 0.001$), the most and least affected side of P2 ($p < 0.001$ and $p < 0.01$, resp.), and P3 ($p < 0.01$). For P1, a significant increase was found between SW session and FS session 2, while the most affected side of P2 showed a significant decrease between SW session 1 and IW sessions 4-5 (Figure 4.3 and *Appendix* Table 4.S2). In both the least affected side of P2 and in P3, there was no significant difference between session 1 and the other sessions. The analysis showed a significant session interaction effect between session and side for PV_2, PV_3 and u_{3t} ($p < 0.001$ for all) in P2, indicating that these parameters developed differently over time for the most and least affected side.


Gait loops
S1
S2
S3
S4
S5
Foot
Shank
Thigh
Planar covariation
P1
PV$_z$ (%)
PV$_3$ (%)
u$_{3x}$
Supported walking
First steps
Independent walking
Most affected
Least affected

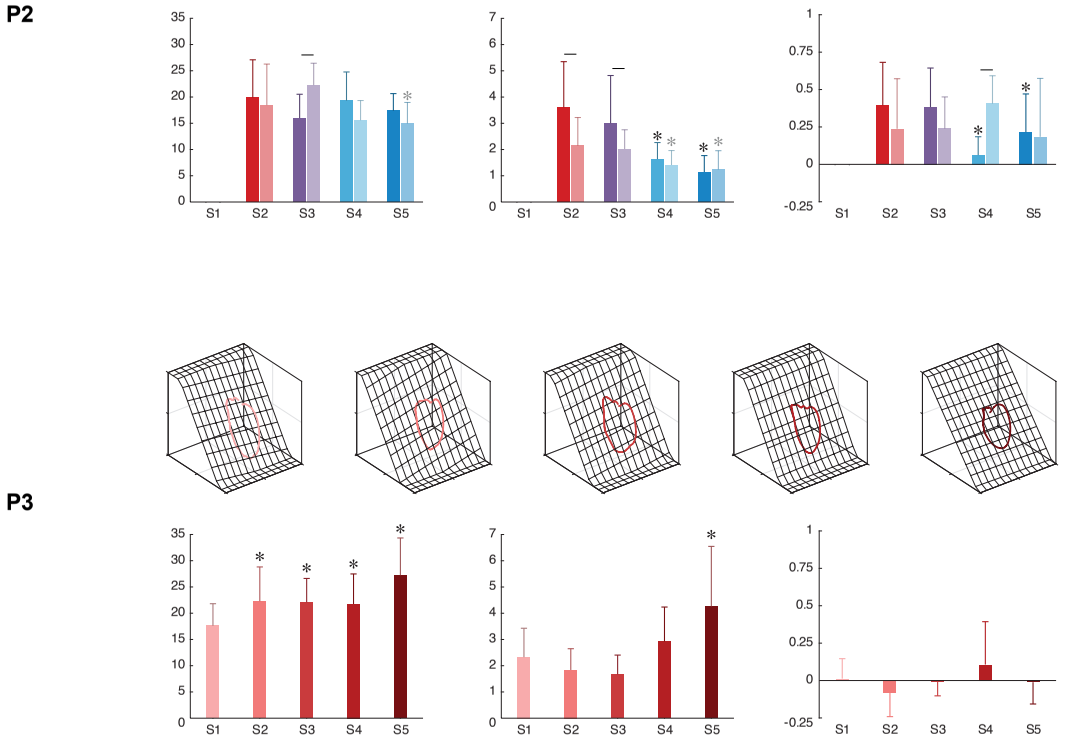
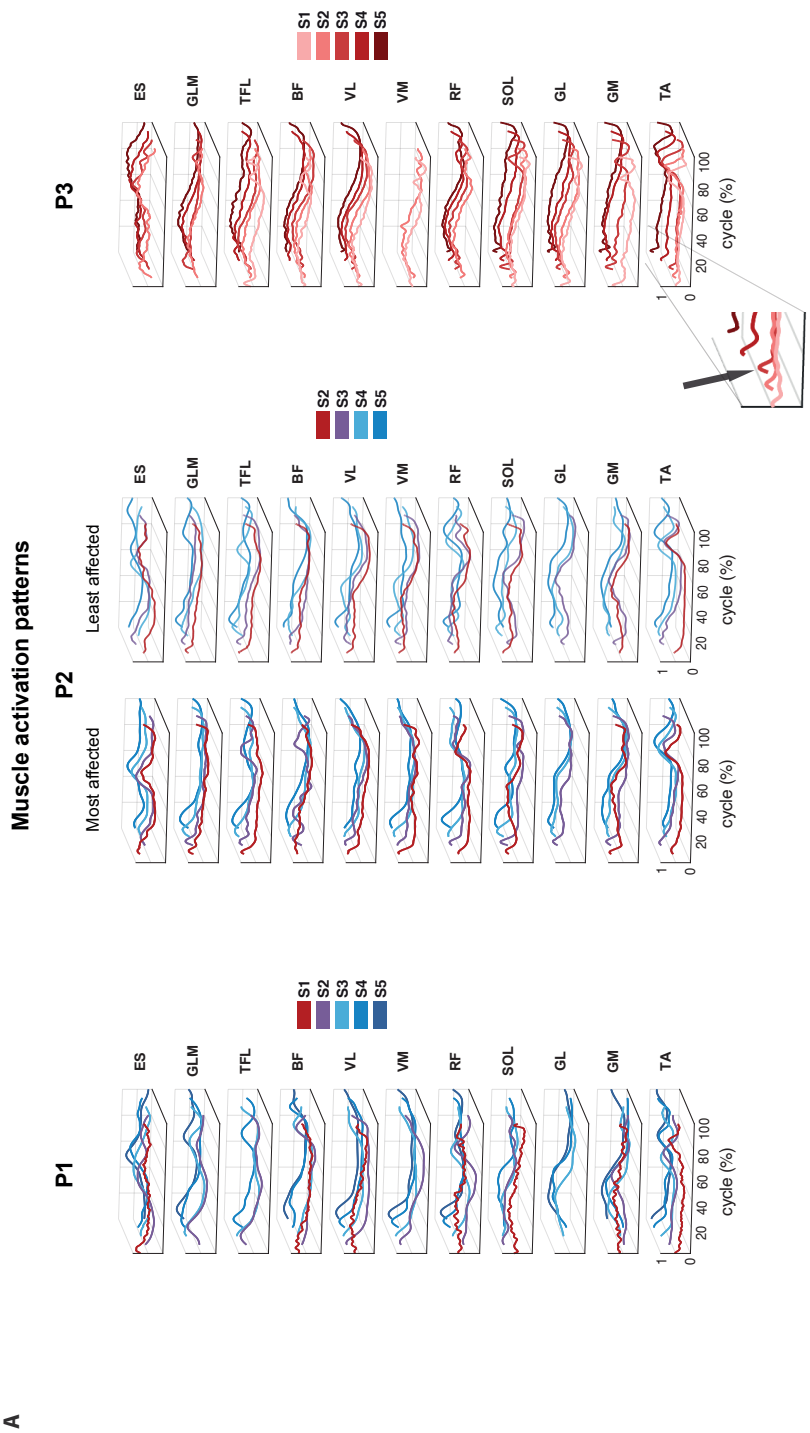


Figure 4.3 Intersegmental coordination. Gait loops plus the corresponding mean percentage of total variance explained by the 2nd and 3rd eigenvectors (PV_2 and PV_3), and the mean orientation of the normal to the plane (u_{3t}) are shown from S1-S5 for each participant. Error bars indicate SDs across strides. For P2, the most affected side (dark color) and the least affected side (light color) are shown, while for P1 and P3 one side is shown. Significant differences with session 1 are marked by asterisks, and significant interactions between session and side are denoted by lines between the most and least affected side for P2 ($p < 0.05$).


A
Muscle activation patterns
P1
P2
P3
Most affected
Least affected
ES
GLM
TFL
BF
VL
VM
RF
SOL
GL
GM
TA
S1
S2
S3
S4
S5
cycle (%)
0
20
40
60
80
100
1

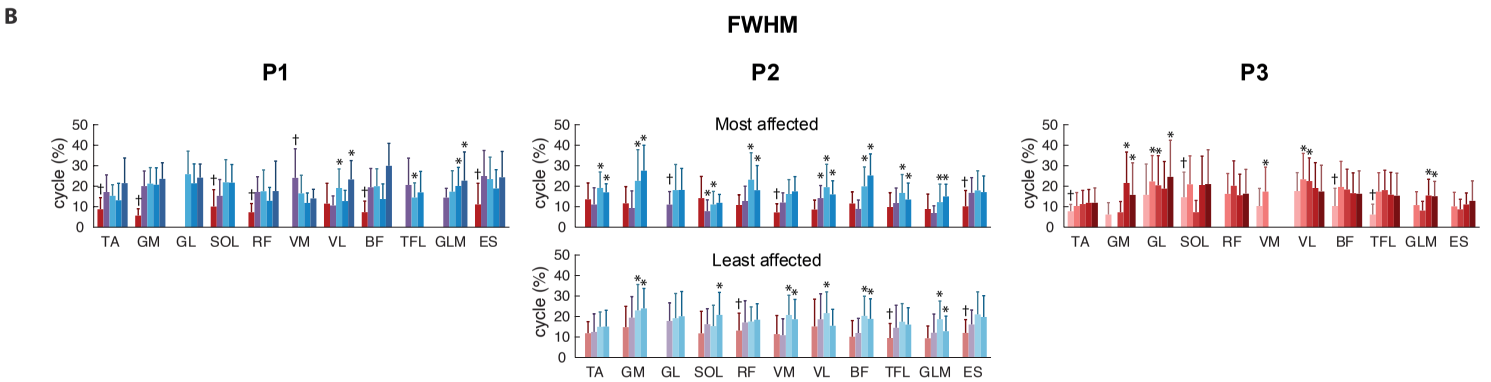


Figure 4.4 EMG activity. A) Muscle activation patterns averaged across strides per session for P1-P3, for the most and least affected leg in P2. The peak around foot strike is emphasized in the zoom-in view of the TA muscle of P3. **B)** Full-Width of the Half Maximum (FWHM) per muscle per session in P1-P3. Abbreviations: TA, tibialis anterior; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SOL, soleus; RF, rectus femoris; VM, vastus medialis; VL, vastus lateralis; BF, biceps femoris; TFL, tensor fascia latae; GLM, gluteus maximus; ES, erector spinae at L2 level. * indicates a significant difference compared to the first recorded session of this muscle, † indicates that all sessions are significantly different from the first recorded session.

Muscle activity

Despite the large variability in EMG activity between strides per muscle, we could observe a clear modification between different sessions in P1 and P2. All activation patterns became smoother and displayed increasingly distinct peaks (Figure 4.4A). A short burst of activity was present around foot strike in the TA and calf muscles (GM, GL and SOL) of P3 during all sessions (see zoom-in view of P3 Figure 4.4A), in the SW and FS sessions of P2, in particular in the most affected leg. In P1 this activity was absent.

For P1, FWHM showed a significant main effect of session for all muscles except GL ($p = 0.072$). FWHM of TA, GM, SOL, RF, VL, BF, and ES significantly increased from SW session 1 to IW sessions 3-5, and of GLM from FS session 2 to IW sessions 4-5. In contrast, FWHM significantly decreased from FS session 2 to IW session 3 for TFL, and to session 3-4 for VM (Figure 4.4B and *Appendix* Table 4.S3).

For P2, FWHM showed a significant main effect of session for all muscles in the most affected side, and for all muscles except TA ($p = 0.061$) and GL ($p = 0.682$) in the least affected side. In the most affected side, FWHM of TA, GM, GL, RF, BF, and TFL was significantly higher during IW session 4-5 compared to SW session 2, while FWHM of SOL, VM, VL, and ES already increased during FS session 3. In the least affected side of P2, FWHM of GM, VM, BF, and GLM significantly increased during IW session 4-5 compared to SW session 2, while FWHM of RF, TFL, and ES already increased during FS session 3. FWHM of SOL was only significantly larger during IW session 5 compared to SW session 2 (Figure 4.4B and *Appendix* Table 4.S3). An interaction effect between session and side was found for GM, SOL, RF, BF, and GLM ($p < 0.01$), indicating that FWHM of these muscles developed differently in the most compared to the least affected side.

For P3, FWHM showed a significant main effect of session for all muscles ($p < 0.05$), except RF ($p = 0.120$). FWHM of TA, SOL, VM, BF and TFL was significantly smaller in session 1 compared to all other sessions. FWHM of GM and GLM was significantly larger during sessions 4-5, and of GL during sessions 2, 3, and 5, compared to the first recorded session (Figure 4.4B and *Appendix* Table 4.S3). In all participants variability in FWHM between strides was large.

The results of the FWHM computed on the average muscle activity can be found in *Appendix* Figure 4.S1. Similar to the results showed in Figure 4.4B, this analysis revealed clear changes between different sessions in P1 and P2. In contrast, P3 showed similar duration of the main bursts of the mean activity of almost all the muscles between sessions, and on average wider EMG bursts with respect to P1 and P2.

Muscle synergies

P1 showed an increase in VAF_1 after FS. The most affected side of P2 showed an increase in VAF_1 from SW to IW where it stayed constant, while the least affected leg showed a decreasing trend in VAF_1 from FS to IW. VAF_1 was higher for the most compared to the least affected leg during the FS and IW sessions, but vice versa for the SW session. For P3, VAF_1 slightly differed between sessions, but there was no clear trend (Figure 4.5A). Based on the VAF slopes and exploration of the synergy patterns, P1 and P2 generally recruited four synergies across sessions, while P3 recruited two synergies. To compare activations patterns across sessions, we fixed the number of synergies of P1 and P2 to four, and of P3 to two.

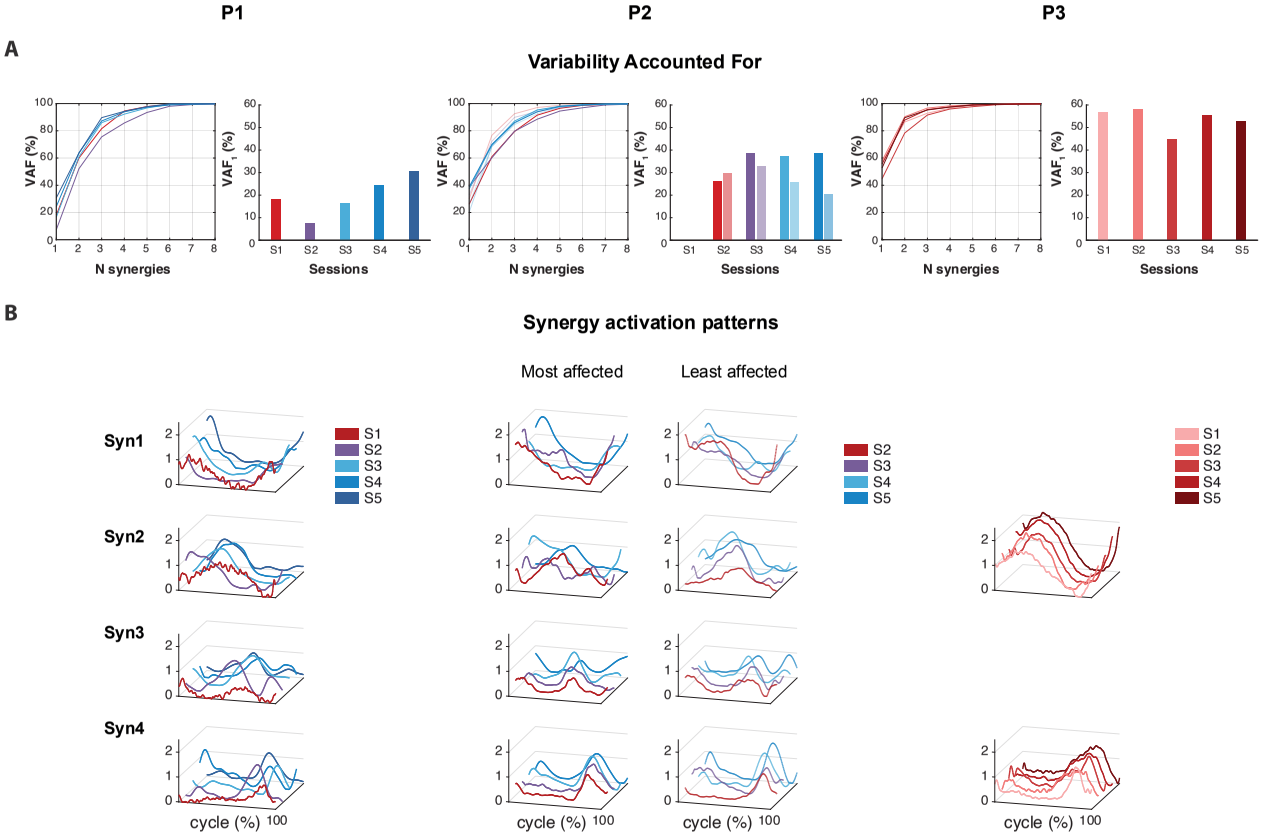
Synergies were ordered based on the highest correlations between the synergy weights per session (Figure 4.5B/C). For P1, FWHM of synergy pattern 1 (Syn1) and 2 (Syn2) decreased, i.e. bursts become narrower. By contrast, FWHM of Syn3 increased towards session 4, but decreased again in session 5. Syn4 did not change in FWHM. Despite the changes of synergy patterns of P2, FWHM appeared quite variable between sessions, and we failed to identify any (qualitative) trend. P3 showed an increase in FWHM of Syn4 over time.

Despite the development in synergy activation patterns in P1 and P2, the synergy weights appeared similar across sessions (Figure 4.5D). In P1 and P2, mainly RF, VM, VL, and BF were active in Syn1, and GM, GL, and SOL in Syn2. ES, and TA to a lesser extent, dominated Syn3, and TA and RF in Syn4. P3 showed simultaneous activations of GM, GL, SOL, RF, VM, VL, TFL, and GLM in Syn2, while TA and ES were present in Syn4.

DISCUSSION

Our longitudinal study captured the early development of walking in three young children at high risk of CP via comprehensive instrumented gait assessments. The exploratory findings illustrate how the combination of kinematic and electromyographic measures can contribute to our understanding of walking maturation in children with early brain lesions.

We followed three children with divergent development trajectories. Two of them made the transition from supported to independent walking, while the third child had not reached independent walking at 4.5 years of age. Spatiotemporal gait parameters, intersegmental coordination and neuromuscular control changed from supported to independent walking in the child that did not develop CP and the child with unilateral CP. Maturation of gait patterns was delayed in the child with unilateral CP and differed between sides. Walking development appeared entirely absent in the more severely affected child with bilateral CP.


P1
P2
P3
A
Variability Accounted For
VAF (%)
VAF$_1$ (%)
N synergies
Sessions
S1
S2
S3
S4
S5
B
Synergy activation patterns
Most affected
Least affected
Syn1
Syn2
Syn3
Syn4
cycle (%) 100

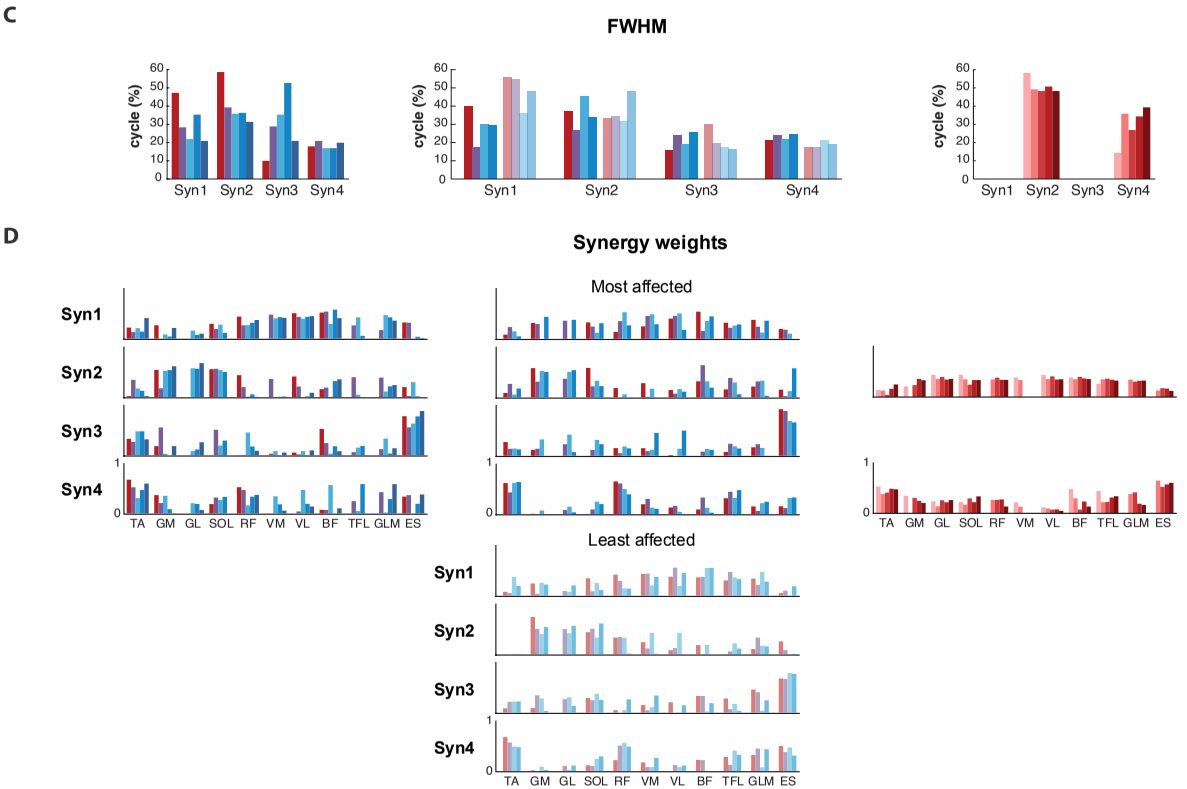


Figure 4.5 Muscle synergies. A) Variability accounted for (VAF) for by 1-8 synergies, and for the first synergy (VAF_1). **B)** Synergy activation patterns for a fixed number of synergies: four for P1 and P2, and two for P3. **C)** Full-Width of the Half Maximum (FWHM) per muscle synergy. **D)** Synergy weights: contribution of each muscle to each synergy across sessions. Abbreviations: N, number; Syn, synergy; TA, tibialis anterior; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SOL, soleus; RF, rectus femoris; VM, vastus medialis; VL, vastus lateralis; BF, biceps femoris; TFL, tensor fascia latae; GLM, gluteus maximus; ES, erector spinae at L2 level.

Spatiotemporal gait parameters and intersegmental coordination changed over time from supported to independent walking in the child without CP, and the child with unilateral CP, although to a lesser extent, whereas there was no development in the child with bilateral CP. This was expected in view of previous research reporting developmental trajectories in children with CP that differ from those in TD children (Berger, 1998; Berger et al., 1984; Leonard et al., 1991). The rapid maturation of planar covariation in the first months after the first independent steps was present whenever children developed independent walking, irrespective of their age at their first steps. The most affected side of the child with unilateral CP deviated more from planarity than the least affected side during SW and FS, although it was comparable between sides during IW. These results suggest that the intersegmental coordination matured side-specific from just before independent walking onset during the consecutive six months. This period might be devoted to postural gait requirements, while in the following years gait coordination is (merely) refined (Breniére & Bril, 1998; Bril & Brenière, 1992).

In contrast to the rapid maturation of intersegmental coordination after the onset of independent walking, muscle activity and its major burst duration appeared quite variable between strides in the first 6-12 months after the first independent steps. This agrees with previous findings from, e.g., Chang, Kubo, Buzzi, & Ulrich, 2006. Probably, toddlers slowly discover how to optimize their muscular activity. In our study, the duration of EMG bursts increased from supported to independent walking. Cappellini et al. (2016) reported a decrease in FWHM from TD toddlers (1-3 years old) to older TD children while this reduction was not visible in the TD toddler group. The latter falls in the (corrected) age range in our study. Moreover, Cappellini et al. (2016) reported wider EMG bursts in children with CP (2-11 years old), arguably similar to muscle activation patterns TD toddlers. Similar results were reported by Prosser et al. (2010) in children with CP (2-9 years old) when compared with TD children with similar walking experience (average of 28 months of walking experience). Despite the presence of young children, the age range of the children with CP included in these cross-sectional studies is quite large. In our study, we did not have a TD control group, but we did observe wider average EMG bursts in the more severely affected child with bilateral CP with respect to the children who developed independent walking. Here we would like to note that gait patterns and their corresponding muscle activity are highly variable in toddlers. It is well possible that a reduction in EMG burst duration occurs later on during development, while children refine their motor pattern (see above). We did notice short bursts of EMG activity immediately after foot strike in the shank and calf muscles of the child with bilateral CP and the calf muscles of mainly the

most affected leg of the child with unilateral CP, which might have been caused by spasticity, i.e. hyperreflexive reactions upon muscle stretch following foot strike (Forssberg, 1985).

The control modules that account for muscle activity during walking seemed to develop in the children who developed independent walking, while there were no major changes in the more severely affected child with bilateral CP. The two children walking independently recruited four synergies, whereas the child without the ability to walk independently recruited only two synergies. While gait kinematics improved during independent walking in the child with unilateral CP, the modulation of groups of muscles to efficiently perform this motor action may have lagged behind. This finds support by previous research reporting the absence of a direct relation between gait kinematics and muscle activations (Buurke et al., 2008) or muscle synergies (Booth et al., 2019). In fact, – if true – this implies that gait kinematics and neuromuscular control may follow a different developmental path. The muscle synergy patterns of the child with bilateral CP resemble that of the 'primitive' neonate stepping, recruiting two wider synergies with a lot of co-contraction in antagonist muscles (Dominici et al., 2011). Neonate stepping reflects the immature walking pattern that lacks a heel strike and with the tendency to walk on the toes (Forssberg, 1985). Put differently, the child with bilateral CP might still depend mainly on spinal input, while supraspinal influence is lacking.

The children were small, especially in the early sessions, yielding limited space for EMG electrodes, and as a consequence, a possible contamination of the EMG recordings due to electrical cross-talk between adjacent muscles that could have affected the data quality. However, the small size of the EMG electrodes used in our experiments and the chosen interelectrode distance should have minimized the pickup from nearby muscles. While it is not possible to dissociate co-activation from cross-talk in adjacent muscles, muscle synergy analysis can identify whether a muscle is activated independent from an adjacent muscle even in the presence of cross-talk. It has been recognized that, if cross-talk did exist, it would likely have affected only the synergy weights and not the number of muscle synergies or the temporal activation patterns (Chvatal & Ting, 2013; Ivanenko et al., 2004b). In addition, the child with bilateral CP was already 2.5 years old at the time of the first session, while it would have certainly been interesting to investigate its walking pattern below that age. Presumably it would have been a very similar pattern given the little change observed between the age of 2.5 and 4.5 years old for this child.

We identified changes in gait kinematics and neuromuscular control underlying the development of walking in three cases at high risk of CP with divergent developmental trajectories. We showed that such analyses are feasible in very young children. Future longitudinal research with a larger sample of children

at high risk of CP could and should provide more insight into the underlying mechanisms of the development of walking. Yet, to establish whether muscle synergies are encoded in the cortex (Zandvoort et al., 2019) or whether they originate solely from spinal cord (Cappellini, Ivanenko, Dominici, Poppele, & Lacquaniti, 2010; Ivanenko, Cappellini, Poppele, & Lacquaniti, 2008) or brainstem (Schepens & Drew, 2004) remains a topic of debate (Bizzi & Cheung, 2013). To solve this puzzle, supplementing our approach by, e.g., synchronous EEG recordings, appears a valid option. Following this idea might be an important step towards the design of early interventions targeting the neural pathways. Combined with the use of novel technologies, such as wearable sensors (Airaksinen et al., 2020; Redd, Barber, Boyd, Varnfield, & Karunanithi, 2019; Xu, Jayaraman, & Rogers, 2019), and treatments like feedback training (Booth et al., 2019), and electrical stimulation of muscles, tendons (Sommerfelt et al., 2001; Stackhouse et al., 2007; Wright et al., 2012), or spinal cord (Solopova et al., 2017), during the critical period of walking development, we might become able to improve early identification of motor deficits in children with early brain lesions and identify targets for early intervention to effectively improve walking function in CP.

APPENDIX

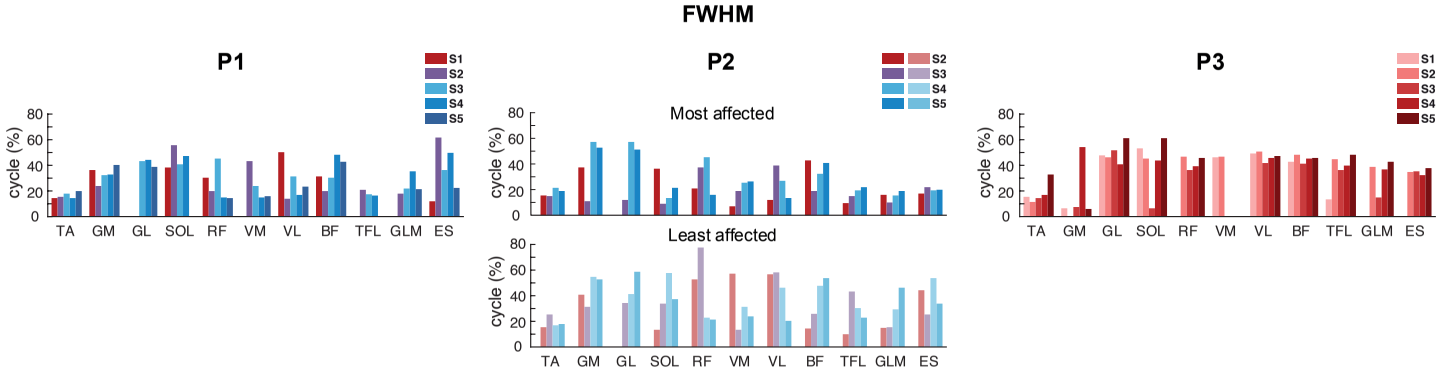


Figure 4.S1 Full-Width of the Half Maximum (FWHM) of the mean muscle activity for each muscle, and session in P1-P3. Abbreviations: TA, tibialis anterior; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SOL, soleus; RF, rectus femoris; VM, vastus medialis; VL, vastus lateralis; BF, biceps femoris; TFL, tensor fascia latae; GLM, gluteus maximus; ES, erector spinae at L2 level.

Table 4.S1 The percentage of strides with coefficients of correlation (r) higher than the predefined threshold (> 0.3) between raw EMG signals (30 Hz cut-off high-pass) of indicated pairs of muscles. The mean value of r (found over the samples r > 0.3 of data) is reported in brackets.

	TA-SOL	TA-GM	RF-BF	RF-TFL	GLM-TFL
Right Leg	0.9% (0.36)	0%	7.3% (0.49)	0.9% (0.41)	9.9% (0.49)
Left Leg	7.0% (0.47)	0.1% (0.31)	9.2% (0.43)	0.1% (0.30)	5.5% (0.43)

Table 4.S2 LMM results gait kinematics: estimates of fixed effects session

Participant	Parameter	Session	Estimate (mean±SD)	df	t	p-value	95% CI	
							Lower bound	Upper bound
P1	Velocity	1	.4±0.3	15.9	1.5	-	-.163	1.012
		2	.6±0.4	15.6	1.6	.134	-.212	1.445
		3	1.9±0.3	16.0	5.9	.000*	1.249	2.641
		4	1.2±0.3	16.1	3.6	.002*	.5146	1.957
		5	2.6±0.3	16.3	7.7	.000*	1.914	3.361
	DS	1	47.9±2.8	10.2	17.2	-	41.738	54.135
		2	-9.6±3.8	9.3	-2.5	.032*	-18.200	-1.005
		3	-24.0±3.4	11.1	-7.1	.000*	-31.339	-16.581
		4	-22.1±3.5	11.9	-6.3	.000*	-29.813	-14.439
		5	-36.5±3.7	13.8	-10.0	.000*	-44.338	-28.593
	STdur	1	72.9±1.5	8.9	49.1	-	69.564	76.287
		2	-6.4±2.0	8.1	-3.2	.013*	-11.038	-1.770
		3	-11.4±1.8	10.2	-6.3	.000*	-15.365	-7.359
		4	-11.1±1.9	11.3	-5.8	.000*	-15.286	-6.931
		5	-19.7±2.0	13.7	-9.8	.000*	-23.981	-15.361
	PV_2	1	20.6±0.7	145	28.9	-	19.174	21.985
		2	-3.6±0.9	145	-4.1	.000*	-5.392	-1.869
		3	-9.0±0.8	145	-10.8	.000*	-10.693	-7.376
		4	-9.8±0.9	145	-10.6	.000*	-11.659	-8.005
		5	-10.8±1.2	145	-8.8	.000*	-13.219	-8.350
	PV_3	1	1.6±0.1	18.4	13.8	-	1.320	1.793
		2	-.6±0.1	14.3	-3.8	.002*	-.852	-.238
		3	-.9±0.1	19.4	-6.9	.000*	-1.186	-.633
		4	-1.0±0.1	23.9	.6.7	.000*	-1.261	-.664
		5	-.9±0.2	44.3	-4.9	.000*	-1.305	-.549
	u_{3t}	1	.3±0.03	145	9.3	-	.206	.316
		2	.1±0.04	145	2.7	.000*	.026	.165
		3	.002±0.03	145	.05	.958	-.063	.067
		4	.06±0.04	145	1.6	.110	-.013	.13
		5	-.02±0.05	145	-.4	.711	-.114	.078
P2 MA	DS	2	52.2±3.8	9.9	13.6	-	43.672	60.744
		3	-15.7±4.9	11.9	-3.2	.008*	-26.379	-5.001
		4	-24.3±5.2	12.2	-4.7	.001*	-35.625	-13.009
		5	-21.0±4.5	11.1	-4.7	.001*	-30.796	-11.179
	STdur	2	74.7±2.6	10.8	28.4	-	68.919	80.526
		3	-8.7±3.3	12.6	-2.6	.022*	-15.989	-1.482
		4	-11.3±3.5	12.9	-3.2	.007*	-19.021	-3.678
		5	-11.9±3.1	11.9	-3.9	.002*	-18.575	-5.251
	PV_3	2	3.6±2.7	110	13.5	-	3.076	4.130
		3	-.6±0.4	110	-1.6	.103	-1.331	.125
		4	-2.0±0.4	110	-5.3	.000*	-2.706	-1.233
		5	-2.5±0.3	110	-7.9	.000*	-3.079	-1.840

(Continued)

Table 4.S2 Continued

Participant	Parameter	Session	Estimate (mean±SD)	df	t	p-value	95% CI	
							Lower bound	Upper bound
	u_{3t}	2	4±0.1	111	7.3	-	.268	.466
		3	-.006±0.07	111	-.1	.927	-.141	.129
		4	-.3±0.07	111	-4.4	.000*	-.445	-.168
		5	-.2±0.06	111	-2.7	.008*	-.275	-.043
P2 LA	DS	2	52.2±3.7	9.1	14.0	-	43.805	60.688
		3	-14.3±4.8	11.2	-3.0	.012*	-24.933	-3.755
		4	-25.5±5.1	11.5	-5.0	.000*	-36.655	-14.259
		5	-20.2±4.4	10.5	-4.6	.001*	-29.902	-10.456
	STdur	2	77.0±1.1	3.0	71.0	-	73.572	80.460
		3	-5.0±1.6	6.1	-3.2	.019*	-8.825	-1.134
		4	-13.4±1.7	6.4	-8.0	.000*	-17.509	-9.376
		5	-8.8±1.4	4.9	-6.3	.002*	-12.425	-5.251
	PV_2	2	18.3±1.1	5.1	16.6	-	15.525	21.161
		3	3.9±1.8	13.9	2.1	.05	-.008	7.863
		4	-2.7±1.7	9.2	-1.6	.146	-6.538	1.135
		5	-3.4±1.4	7.3	-2.5	.039*	-6.655	-.238
	PV_3	2	2.2±0.2	10.1	9.9	-	1.726	2.725
		3	0.2±0.3	17.5	-.7	.519	-.927	.485
		4	-.8±0.3	14.1	-2.5	.023*	-1.518	-.131
		5	-1.0±0.3	12.2	-3.8	.003*	-1.585	-.422
	u_{3t}	2	.2±0.1	112	3.7	-	.102	.341
		3	.01±0.1	112	.1	.906	-.202	.227
		4	.2±0.1	112	1.8	.076	-.019	.369
		5	-.1±0.08	1123	-1.7	.102	-.281	.026
P2 both	Velocity	2	.9±0.2	13.8	3.9	-	.394	1.368
		3	.4±0.3	14.5	1.3	.220	-.241	.9619
		4	1.2±0.3	15.0	3.8	.002*	.514	1.790
		5	1.5±0.3	14.2	5.8	.000*	.956	2.065
P3	Velocity	1	.6±0.05	12.161	11.8	-	.498	.724
		2	.01±0.07	13.598	.2	.834	-.127	.155
		3	.1±0.07	12.170	1.5	.158	-.049	.270
		4	.2±0.06	13.131	3.8	.002*	.100	.370
		5	.05±0.08	14.159	.7	.501	-.111	.216
	DS	1	53.1±2.1	8.131	24.9	-	48.196	57.990
		2	-4.5±3.1	14.938	-1.5	.166	-11.041	2.086
		3	-13.4±3.0	8.132	-4.4	.002*	-20.327	-6.472
		4	-16.5±2.8	13.081	-5.8	.000*	-22.611	-10.334
		5	-5.1±3.5	14.161	-1.5	.161	-12.548	2.300
	STdur	1	78.5±1.3	6.209	62.5	-	75.424	81.522
		2	-1.9±1.8	10.882	-1.1	.303	-5.874	2.005
		3	-7.2±1.8	6.211	-4.1	.006*	-11.521	-2.897
		4	-8.1±1.7	9.566	-4.9	.001*	-11.822	-4.396
		5	-2.4±2.0	10.293	-1.2	.258	-6.887	2.058

(Continued)

Table 4.S2 Continued

Participant	Parameter	Session	Estimate (mean±SD)	df	t	p-value	95% CI	
							Lower bound	Upper bound
	PV_2	1	17.6±1.7	112	10.4	-	14.238	20.968
		2	4.7±2.0	112	2.3	.022*	.685	8.669
		3	4.5±2.0	112	2.3	.025*	.571	8.373
		4	4.1±2.0	112	2.05	.043*	.135	8.164
		5	10.4±2.0	112	4.7	.000*	6.039	14.782
	PV_3	1	2.3±0.4	112	6.6	-	1.625	3.025
		2	-.5±0.4	112	-1.2	.248	-1.317	.343
		3	-.7±0.4	112	-1.6	.113	-1.466	.157
		4	.6±0.4	112	1.4	.158	-.236	1.434
		5	3.4±0.5	112	7.4	.000*	2.493	4.311
	u_{3t}	1	.01±0.05	112	.3	-	-.083	.107
		2	-.1±0.06	112	-1.8	.081	-.213	.013
		3	-.02±0.6	112	-.4	.710	-.131	.090
		4	.08±0.06	112	1.3	.187	-.038	.190
		5	-.01±0.06	112	-.2	.882	-.133	.114

Abbreviations: PV_2, percentage of total variation explained by the 2nd principal component; PV_3, percentage of total variation explained by the 3rd principal component; u_{3t}, orientation of the normal to the plane; Stdur, stance duration; DS, double support; MA, most affected side; LA, least affected side; LMM, linear mixed effects model; SD, standard deviation; CI, confidence interval.

Table 4.S3 LMM results FWHM muscle activity: estimates of fixed effects session

Participant	Parameter	Session	Estimate (mean±SD)	df	t	p-value	95% CI	
							Lower bound	Upper bound
P1	TA	1	8.5±1.3	222	6.7	-	6.013	11.033
		2	8.6±1.7	222	5.0	.000*	5.260	12.011
		3	6.8±1.6	222	4.2	.000*	3.608	9.983
		4	4.6±1.7	222	3.6	.009*	1.178	8.031
		5	13.0±1.9	222	6.8	.000*	9.221	16.711
	GM	1	5.7±1.2	222	4.9	-	3.365	7.947
		2	14.4±1.6	222	9.2	.000*	11.312	17.473
		3	15.6±1.5	222	10.6	.000*	12.699	18.518
		4	15.1±1.6	222	9.5	.000*	11.954	18.209
		5	17.9±1.7	222	10.3	.000*	14.518	21.354
	SOL	1	10.07±1.5	191	7.6	-	7.118	13.018
		2	5.2±2.0	191	2.6	.010*	1.271	9.206
		3	11.8±1.9	191	6.2	.000*	8.084	15.577
		4	11.7±2.0	191	5.7	.000*	7.648	15.703
		5	-	-	-	-	-	-
	RF	1	7.3±1.5	222	5.0	-	4.397	10.161
		2	9.9±2.0	222	5.0	.000*	6.035	13.788
		3	10.2±1.9	222	5.5	.000*	6.585	13.907
		4	5.6±2.0	222	2.8	.005*	1.664	9.534
		5	10.3±2.2	222	4.7	.000*	6.042	14.642

(Continued)

Table 4.S3 Continued

Participant	Parameter	Session	Estimate (mean±SD)	df	t	p-value	95% CI	
							Lower bound	Upper bound
	VM	1	-	-	-	-	-	-
		2	24.1±1.3	184	17.9	-	21.430	26.734
		3	-7.6±1.8	184	-4.3	.000*	-11.100	-4.067
		4	-12.2±1.9	184	-6.3	.000*	-16.045	-8.418
		5	-10.1±2.1	184	-4.7	.000*	-14.261	-5.849
	VL	1	11.5±1.3	222	9.1	-	8.992	13.998
		2	-0.9±1.7	222	-.05	.599	-4.265	2.467
		3	7.6±1.6	222	4.7	.000*	4.464	10.822
		4	1.2±1.8	222	0.7	.472	-2.169	4.665
		5	11.8±1.9	222	6.2	.000*	8.073	15.542
	BF	1	7.3±1.3	222	5.3	-	4.663	9.974
		2	12.2±1.8	222	6.7	.000*	8.588	15.729
		3	12.7±1.7	222	7.4	.000*	9.298	16.042
		4	6.4±1.8	222	3.5	.001*	2.795	10.044
		5	22.6±2.0	222	11.3	.000*	18.683	26.606
	TFL	1	-	-	-	-	-	-
		2	20.6±1.5	153	14.0	-	17.720	23.521
		3	-6.1±1.9	153	-3.1	.002*	-9.982	-2.291
		4	-3.6±2.1	153	-1.7	.087	-7.808	.534
		5	-	-	-	-	-	-
	GLM	1	-	-	-	-	-	-
		2	14.4±1.4	184	10.4	-	11.697	17.180
		3	2.9±1.8	1884	1.6	.117	-.733	6.537
		4	5.7±2.0	184	2.9	.005*	1.757	9.642
		5	8.3±2.2	184	3.8	.000*	3.922	12.619
	ES	1	11.1±1.8	221	6.2	-	7.567	14.646
		2	13.9±2.4	221	5.8	.000*	9.133	18.596
		3	12.4±2.3	221	5.4	.000*	7.892	16.837
		4	7.8±2.4	221	3.2	.002*	2.986	12.590
		5	13.2±2.7	221	5.0	.000*	7.998	18.481
P2 MA	TA	2	13.6±1.0	173	13.9	-	11.662	15.522
		3	-2.5±1.6	173	-1.6	.119	-5.712	.659
		4	5.6±1.5	173	3.8	.000*	2.680	8.470
		5	3.4±1.4	173	2.5	.013*	.746	6.103
	GM	2	11.6±1.7	170	7.0	-	8.344	14.923
		3	-2.3±2.7	170	-.8	.402	-7.592	3.059
		4	11.0±2.5	170	4.5	.000*	6.139	15.843
		5	16.0±2.3	170	7.0	.000*	11.452	20.450
	GL	2	-	-	-	-	-	-
		3	11.0±1.9	123	5.8	-	7.240	14.822
		4	7.1±2.5	123	2.8	.006*	2.074	12.033
		5	7.2±2.4	123	3.0	.003*	2.455	11.856

(Continued)

Table 4.S3 Continued

Participant	Parameter	Session	Estimate (mean±SD)	df	t	p-value	95% CI	
							Lower bound	Upper bound
	SOL	2	14.2±1.0	171	14.0	-	12.180	16.178
		3	-6.4±1.7	171	-3.7	.000*	-9.742	-2.991
		4	-3.1±1.5	171	-2.0	.043*	-6.095	-0.099
		5	-2.3±1.4	171	-1.6	.107	-5.049	.498
	RF	2	10.8±1.4	175	7.9	-	3.134	13.545
		3	2.0±2.3	175	.9	.394	-2.563	6.479
		4	12.3±2.1	175	5.9	.000*	8.241	16.447
		5	7.2±1.9	175	3.8	.000*	3.418	11.000
	VM	2	7.2±0.8	173	8.5	-	5.547	8.881
		3	4.8±1.4	173	3.4	.001*	2.060	7.564
		4	9.0±1.3	173	7.1	.000*	6.492	11.493
		5	10.3±1.2	173	8.8	.000*	7.950	12.577
	VL	2	8.6±1.1	164	8.2	-	6.525	10.695
		3	5.6±1.7	164	3.2	.002*	2.129	9.045
		4	10.9±1.6	164	6.9	.000*	7.794	14.015
		5	7.4±1.5	164	4.9	.000*	4.404	10.365
	BF	2	11.6±1.1	167	10.1	-	9.297	13.819
		3	-2.6±2.1	167	-1.2	.220	-6.841	1.589
		4	8.4±1.7	167	4.8	.000*	4.926	11.784
		5	13.7±1.6	167	8.5	.000*	10.510	16.845
	TFL	2	9.8±1.1	171	8.8	-	7.617	12.055
		3	1.9±1.8	171	1.1	.289	-1.666	5.565
		4	6.9±1.7	171	4.1	.000*	3.601	10.184
		5	3.7±1.5	171	2.4	.019*	.621	6.720
	GLM	2	8.0±1.0	170	9.0	-	6.935	10.806
		3	-2.0±1.6	170	-1.2	.219	-5.091	1.177
		4	3.4±1.4	170	2.3	.022*	.501	6.211
		5	6.1±1.3	170	4.5	.000*	3.450	8.794
	ES	2	10.2±1.1	175	9.1	-	7.966	12.393
		3	6.6±1.9	175	3.5	.001*	2.882	10.280
		4	7.8±1.7	175	4.6	.000*	4.411	11.125
		5	6.9±1.6	175	4.4	.000*	3.837	10.039
P2 LA	GM	2	14.8±1.5	171	10.1	-	11.896	17.896
		3	4.6±2.5	171	1.9	.066	-.305	9.602
		4	8.2±2.2	171	3.7	.000*	3.777	12.560
		5	9.2±2.1	171	4.4	.000*	5.061	13.251
	SOL	2	11.8±1.4	173	8.4	-	8.997	14.539
		3	4.5±2.3	173	1.9	.059	-.170	9.092
		4	3.5±2.1	173	1.7	.099	-.672	7.733
		5	9.0±2.0	173	4.5	.000*	5.113	12.950
	RF	2	13.1±1.2	173	11.3	-	10.843	15.392
		3	4.0±1.9	173	2.1	.041*	.169	7.771
		4	4.4±1.7	173	2.5	.012*	.971	7.870
		5	5.3±1.6	173	3.3	.001*	2.112	8.546

(Continued)

Table 4.S3 Continued

Participant	Parameter	Session	Estimate (mean±SD)	df	t	p-value	95% CI	
							Lower bound	Upper bound
	VM	2	11.4±1.3	173	9.0	-	8.897	13.893
		3	-.5±2.1	173	-.3	.800	-4.710	3.639
		4	9.4±1.9	173	4.9	.000*	5.588	13.165
		5	7.3±1.8	173	4.1	.000*	3.739	10.804
	VL	2	15.1±1.5	173	9.9	-	12.140	18.151
		3	3.5±2.5	173	1.4	.170	-1.520	8.525
		4	6.5±2.3	173	2.8	.005*	1.939	11.054
		5	.3±2.1	173	.2	.873	-3.906	4.595
	BF	2	10.1±1.2	168	8.4	-	7.746	12.518
		3	1.8±2.1	168	.9	.390	-2.359	6.015
		4	10.2±1.8	168	5.5	.000*	6.545	13.834
		5	8.7±1.7	168	5.1	.000*	5.352	12.100
	TFL	2	9.5±1.2	173	8.1	-	7.189	11.831
		3	5.0±2.0	173	2.5	.012*	1.107	8.865
		4	7.9±1.8	173	4.4	.000*	4.420	11.460
		5	6.6±1.7	173	3.9	.000*	3.271	9.836
	GLM	2	9.4±1.1	172	8.9	-	7.278	11.436
		3	2.7±1.8	172	1.5	.128	-.789	6.240
		4	9.3±1.6	172	5.8	.000*	6.171	12.478
		5	3.5±1.5	172	2.3	.022*	.513	6.394
	ES	2	12.0±1.2	173	9.7	-	9.556	14.439
		3	4.1±2.1	173	2.0	.048*	.031	8.191
		4	9.0±1.9	173	4.8	.000*	5.320	12.725
		5	7.8±1.7	173	4.4	.000*	4.326	11.231
P3	TA	1	7.8±.8	254	9.9	-	6.220	9.302
		2	2.6±1.3	254	2.0	.046*	.048	5.216
		3	3.5±1.1	254	3.2	.002*	1.345	5.703
		4	4.1±1.2	254	3.5	.000*	1.840	6.437
		5	4.2±1.2	254	3.6	.000*	1.873	6.445
	GM	1	6.2±1.4	218	4.3	-	3.345	9.000
		2	-	-	-	-	-	-
		3	1.1±2.0	218	.5	.587	-2.883	5.082
		4	15.4±2.1	218	7.2	.000*	11.215	19.612
		5	9.6±2.1	218	4.5	.000*	5.423	13.774
	GL	1	15.8±1.9	254	8.3	-	12.042	19.534
		2	6.5±3.1	254	2.1	.038*	.372	12.715
		3	4.6±2.7	254	1.7	.086	-.654	9.941
		4	3.0±2.8	254	1.0	.287	-2.561	8.614
		5	8.8±2.8	254	3.1	.002*	3.223	14.336
	SOL	1	14.6±1.6	254	9.0	-	11.396	17.825
		2	6.3±2.7	254	2.3	.020*	1.004	11.595
		3	-7.3±2.3	254	-3.2	.002*	-11.851	-2.759
		4	6.0±2.4	254	2.5	.015*	1.187	10.775
		5	6.4±2.4	254	2.7	.008*	1.667	11.202

(Continued)

Table 4.S3 Continued

Participant	Parameter	Session	Estimate (mean±SD)	df	t	p-value	95% CI	
							Lower bound	Upper bound
	VM	1	10.3±1.3	95	8.1	-	7.800	12.847
		2	7.0±2.1	95	3.4	.001*	2.889	11.204
		3	-	-	-	-	-	-
		4	-	-	-	-	-	-
		5	-	-	-	-	-	-
	VL	1	17.6±1.5	254	12.0	-	14.725	20.532
		2	5.7±2.4	254	2.4	.019*	.957	10.524
		3	4.9±2.1	254	2.3	.020*	.778	8.990
		4	1.5±2.2	254	.7	.496	-2.830	5.831
		5	-.2±2.1	254	-.09	.925	-4.512	4.101
	BF	1	10.3±1.3	252	7.9	-	7.738	12.908
		2	9.3±2.2	252	4.3	.000*	5.077	13.594
		3	8.1±1.9	252	4.4	.000*	4.420	11.732
		4	6.3±2.0	252	3.2	.002*	2.412	10.123
		5	6.0±2.0	252	3.0	.003*	2.124	9.879
	TFL	1	6.2±1.2	254	5.2	-	3.819	8.502
		2	11.2±2.0	254	5.7	.000*	7.337	15.053
		3	11.9±1.7	254	7.1	.000*	8.620	15.244
		4	9.7±1.8	254	5.5	.000*	6.206	13.191
		5	9.2±2.8	254	5.2	.000*	5.749	12.695
	GLM	1	-	-	-	-	-	-
		2	10.8±1.1	194	9.7	-	8.601	12.949
		3	-2.7±1.4	194	-1.9	.055	-5.409	.062
		4	4.7±1.4	194	3.2	.001*	1.842	7.535
		5	4.3±1.4	194	3.0	.003*	1.505	7.174
	ES	1	-	-	-	-	-	-
		2	10.1±1.2	194	8.7	-	7.854	12.444
		3	-1.5±1.5	194	-1.0	.323	-4.339	1.437
		4	.9±1.5	194	.6	.534	-2.056	3.953
		5	2.7±1.5	194	1.8	.076	-.286	5.699

Abbreviations: TA, tibialis anterior; GM, gastrocnemius medialis; GL, gastrocnemius lateralis; SOL, soleus; RF, rectus femoris; VM, vastus medialis; VL, vastus lateralis; BF, biceps femoris; TFL, tensor fascia latae; GLM, gluteus maximus; ES, erector spinae at L2 level; MA, most affected side; LA, least affected side; SD, standard deviation; CI, confidence interval.

5

Muscle synergies and coherence networks reflect different modes of coordination during walking

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ABSTRACT

Background When walking speed is increased, the frequency ratio between the arm and leg swing switches spontaneously from 2:1 to 1:1. We examined whether these switches are accompanied by changes in functional connectivity between multiple muscles.

Methods Subjects walked on a treadmill with their arms swinging along their body while kinematics and surface electromyography (EMG) of 26 bilateral muscles across the body were recorded. Walking speed was varied from very slow to normal. We decomposed EMG envelopes and intermuscular coherence spectra using non-negative matrix factorization (NMF), and the resulting modes were combined into multiplex networks and analyzed for their community structure.

Results We found five relevant muscle synergies that significantly differed in activation patterns between 1:1 and 2:1 arm-leg coordination and the transition period between them. The corresponding multiplex network contained a single module indicating pronounced muscle co-activation patterns across the whole body during a gait cycle. NMF of the coherence spectra distinguished three EMG frequency bands: 4-8 Hz, 8-22 Hz, and 22-60 Hz. The community structure of the multiplex network revealed four modules, which clustered functional and anatomical linked muscles across modes of coordination. Intermuscular coherence at 4-22 Hz between upper and lower body and within the legs was particularly pronounced for 1:1 arm-leg coordination and was diminished when switching between modes of coordination.

Conclusion These findings suggest that the stability of arm-leg coordination is associated with modulations in long-distant neuromuscular connectivity.

INTRODUCTION

Human locomotion requires a well-organized activation of multiple muscles to coordinate movements of upper and lower limbs. The degree of interlimb coordination can be characterized by the strength of frequency and phase locking between limbs. To understand the emergence of coordination patterns and, by this, the way muscle activity is orchestrated, one typically challenges the stability of phase locking by altering a control parameter. For example, if speed is increased from loaf (very slow) to normal walking, one can observe a switch in frequency locking from a 2:1 to a 1:1 ratio between the arm and leg swing (Craik, Herman, & Finley, 1976; Schöner, Jiang, & Kelso, 1990; Van Emmerik, 1992; van Emmerik & Wagenaar, 1996): At very low speeds, the arm swing is phase locked to the step cycle, while at fast speeds it locks to the stride cycle. This switch is accompanied by a change in the phase relationship between the arms from in-phase to antiphase phase locking (Wagenaar & Van Emmerik, 2000), and in the immediate vicinity of the transition the variability of frequency (phase) locking drastically increases[1]. The methodological benefit of investigating such changes in coordination is that they arguably share characteristics of classic phase transitions, in the sense of non-equilibrium thermostatistics (Beek, Peper, & Daffertshofer, 2002; Kelso, 1995). In the vicinity of a phase transition, one may expect the dynamics' dimensionality to be drastically reduced and muscle activity patterns to stay on low-dimensional manifolds.

Interestingly, the switch in coordination during walking depends on whether the walking speed is increased or decreased (Schöner et al., 1990; van Emmerik & Wagenaar, 1996). This suggests that the underlying mechanisms are not purely mechanical or energetic, as has been conjectured in other cases of altered interlimb coordination (Hoyt & Taylor, 1981; Owaki & Ishiguro, 2017). Our working hypothesis is that the central nervous system substantially contributes to the stability of coordination patterns. As such, we sought to identify (low-dimensional) neural contributions to transitions in upper and lower limb coordination. Well-designed mechanical manipulations may already hint at the relevance and location of such neural contributions. For instance, Bondi, Zeilig, Bloch, Fasano, and Plotnik (2017) reported how changes of swing of one arm can affect both the swing of the other arm as well as lower limb coordination during walking. The same effects have also been shown in neonates (La Scaleia et al., 2018), children with hemiplegic cerebral

1 In particular the increase in phase variability in the immediate vicinity of the behavioural switch in interlimb coordination resembles so-called critical fluctuations which implies the presence of a likewise critical slowing down, i.e. drastic increase of response time after (mechanical) perturbation.

palsy (Meyns et al., 2012b), and are known for long for stroke survivors where they can be strongly elevated (Stephenson, Lamontagne, & De Serres, 2009). By the same token, the arm swing can have little to no influence on leg movement after spinal cord injury (Tester, Barbeau, Howland, Cantrell, & Behrman, 2012). These findings suggest that a partial interruption of the spinal cord may suffice to limit the interaction between spinal motor neurons such that switches in interlimb coordination no longer emerge.

Targeting neural dynamics more directly during motor coordination is not new (Matsuyama et al., 2004). Several groups studied modulations of muscle activity of upper and lower extremities during locomotor tasks via electromyography (EMG) – a proxy of neural activity in the spinal cord (Boonstra, Farmer, & Breakspear, 2016; Ferris, Huang, & Kao, 2006; Zehr et al., 2016). Muscle activity of different muscles is found to couple at several time or frequency scales. Coherence at low frequencies (0-5 Hz) seems associated with common modulation of motor unit mean firing rate and muscle force generation and, hence, likely reflects co-modulation of muscle activities (Boonstra et al., 2008; De Luca & Erim, 1994; Mochizuki, Semmler, Ivanova, & Garland, 2006) and the modulation of EMG envelopes (Hansen, Hansen, Christensen, Petersen, & Nielsen, 2001). Common modulations of EMG envelopes of groups of muscles are considered as muscle synergies (Tresch, Cheung, & d'Avella, 2006) that reveal how movements are manifested through synchronized muscle co-activation (Cappellini et al., 2006; Cheung, d'Avella, Tresch, & Bizzi, 2005; Dominici et al., 2011; Ivanenko et al., 2004b, 2005a). In a recent review, Bruton and O'Dwyer (2018) outlined numerous studies suggesting that muscle synergies are vital motor control modules. Obviously, muscle synergies change with altered coordination, but what are the origins of these changes? An answer to this may lie in the higher frequencies of the EMG signal, as they may provide the spectral 'fingerprints' of distinct neural pathways involved in the control of muscles (Boonstra et al., 2009a; Boonstra et al., 2016; Danna-Dos-Santos et al., 2014; Farmer, 1998). For example, intermuscular coherence at higher frequency components may reflect supra-spinal drives (Grosse, Cassidy, & Brown, 2002) that modulate the activation of multiple muscles by means of a common input (Danna-Dos-Santos et al., 2014).

Here, we studied the dynamics of muscle activation during changes in interlimb coordination using the experimental design of Wagenaar and Van Emmerik (2000). Rather than focusing on isolated muscles, we employed synergy analysis and constructed functional muscle networks (Boonstra et al., 2015). We determined the minimal (i.e. low-dimensional) set of muscle synergies and combined them into a network with multiple synergy-specific layers. In a similar spirit, we used intermuscular coherences to construct networks with multiple frequency-specific

layers (Kerkman, Daffertshofer, Gollo, Breakspear, & Boonstra, 2018). Both types of networks were constructed under the proviso that they could be based on a low-dimensional representation[2], i.e. a small number of relevant muscle synergies vis-à-vis a small number of frequency components with pronounced coherence determined through conventional mode decomposition of multivariate time series. Network analysis offers new possibilities to assess synchronization between motor units across a large number of muscles. It hence allows for an encompassing study of functional changes in muscle activity during a transition in physiological coupling (Bartsch & Ivanov, 2014; Bashan, Bartsch, Kantelhardt, Havlin, & Ivanov, 2012). In particular, modulations of the network can highlight modifications in the neuromuscular system related to changes in functional behavior during walking.

For the individual synergies, we expected the switch in interlimb coordination to be accompanied by rapid changes in temporal activation patterns, in line with Yokoyama et al. (2016). For the corresponding low-frequency muscle networks, we expected a strong resemblance of anatomical and biomechanical constraints (Bruton & O'Dwyer, 2018; Kutch & Valero-Cuevas, 2012) and switches in coordination to result in concomitant changes in network topology. Given that the higher EMG frequency components are thought to represent supra-spinal input to multiple muscles (Kerkman et al., 2018), we expected these frequency components to discern neural pathways involved in the stability of arm-leg coordination patterns and the switches between them.

MATERIALS AND METHODS

Subjects

Sixteen healthy subjects (five males and eleven females, mean age of 25.3 ± 2.4 years) without any neurological or motor disorder were included in this study. The study was approved by the Ethics Committee Human Movement Sciences of the Vrije Universiteit Amsterdam (VCWE-2017-132). All subjects were informed about the procedure of the study and provided, in accordance with the Declaration of Helsinki, written informed consent prior to participation.

Procedure

Subjects were instructed to walk on a treadmill (Motek Medical BV, Amsterdam, the Netherlands) with their arms swinging along their body while full-body kinematics, ground reaction forces and muscle activities were recorded. Subjects walked at controlled speeds between 1.0 and 4.0 km/h with increments of 0.5 km/h. The

2 As said, we investigated the dynamics in the vicinity of a phase transition.

ordering of speeds was randomized between subjects and trials. Subjects walked for at least fifteen strides at each speed.

Data acquisition

Ground reactions forces (Motek Medical BV, Amsterdam, the Netherlands) and full-body 3D-kinematics (Optotrak, Northern Digital, Waterloo ON, Canada), using five cluster markers (heel, lower and upper leg, and upper and lower arm) and three cameras (left and right backside and one at the front), were measured to define the fifth metatarsophalangeal joint, heel, ankle, knee, hip trochanter, shoulder, elbow and wrist. Kinetic and kinematic data were sampled at 70 Hz. Surface EMG of 26 bilateral muscles (Table 5.1) distributed across the body was recorded (two Mini Wave Wireless 16-channel EMG system, Cometa s.r.l, Italy) and sampled at 2 kHz after online band-pass filtering between 10-1000 Hz. Electrodes were placed according to the SENIAM recommendations (Hermens et al., 1999). Kinematic, ground reaction force and EMG data were synchronized online.

Data analysis

Kinematics

Gait cycles were defined based on the right heel strikes obtained from the force plate data. The heel strike was defined as the moment when the vertical ground reaction force exceeded 8% of the average ground reaction force during the trial. This kinetic criterion was verified by comparison with foot strike measured from the kinematic data (Borghese et al., 1996; Roerdink, Lamoth, & Beek, 2008). We determined the mode of interlimb coordination via the maximum spectral overlap after rescaling the frequency axis (Daffertshofer, Peper, Frank, & Beek, 2000) and the circular variance of the generalized relative phase of the kinematics of the arms and legs for every walking speed and subject (cf. Table 5.2). We focused on the frequency locking between arms and legs at 2:1 (~ very low speed) and 1:1 (~ normal), and the transition (T) between these modes of coordination. The 2:1 and the 1:1 condition were dominated by spectral overlap at a 2:1 or 1:1 frequency ratio, respectively, and almost constant corresponding generalized relative phases. The transition was characterized by spectral overlap at both frequency ratios of 2:1 and 1:1, and a changing generalized relative phase (Figure 5.1).

Table 5.1 Muscles included in the recordings

Muscle	Abbreviation
tibialis anterior	TA
gastrocnemius medialis	GM
tensor fascia latae	TFL
rectus femoris	RF
vastus medialis	VM
adductor longus	AL
biceps femoris	BF
gluteus maximus	GMA
erector spinae	ES
latissimus dorsi	LD
trapezius	TZ
deltoid	D
triceps brachii	TRB

EMG pre-processing

Independent component analysis was used to reduce heart beat contamination in the EMG signals (Willigenburg, Daffertshofer, Kingma, & Van Dieën, 2012). Subsequently, EMG signals were high-pass filtered (2nd order, bi-directional Butterworth, cut-off at 30 Hz) and rectified using the modulus of the analytic signal. Here we would like to note that rectification can re-introduce low-frequency amplitude modulations (Boonstra & Breakspear, 2012; Myers et al., 2003).

Muscle synergies

EMG envelopes were determined by low-pass filtering the rectified EMGs (2nd order, bi-directional Butterworth filter, cut-off at 10 Hz). Subsequently, these envelopes were time normalized such that every stride had an equal number of samples ($N = 200$ samples). For every subject we further normalized the amplitudes to the average activity during the fastest walking speed (4.0 km/h)[3]. Next, EMG data for every subject were averaged over all strides per mode of coordination yielding $EMGs \times subjects \times conditions$ time series containing one average stride each. Finally, time series were concatenated along subjects and conditions yielding 26 (number of muscles) discrete time series containing $subjects \times conditions\ (SC)$ strides each[4].

3 Here we would like to note that we verified that the amplitude normalization had little to no effect on the temporal and spatial representation of the muscle synergies.

4 Estimating muscle synergies per condition had only minor effects on both weightings and wave forms; details can be found in the *Appendix*.

We denote the data by X_{ij} where i indexes the time point and j the muscle, that is, $i = 1, \ldots, SC \cdot N$ spans the SC time-normalized strides with N samples each and $j = 1, \ldots, 26$ are all muscles. These data entered our synergy analysis, namely non-negative matrix factorization (NMF). NMF is a linear mode decomposition $X \longmapsto W^{(m)} A^{(m)}$ that includes the constraint that both extracted wave forms $A^{(m)}$ and weights $W^{(m)}$ are positive semi-definite, and that $W^{(m)}$ and $A^{(m)}$ have rank m; we used a multiplicative update algorithm to solve the corresponding minimization of the Frobenius norm $||X - W^{(m)} A^{(m)}||_{\mathrm{F}}^2$ (Lee & Seung, 1999).

To fix the number of relevant synergies, i.e. the rank m of $W^{(m)}$, we determined the quality of data reconstruction as

$$\lambda^{(m)} = \left(1 - \frac{\left\|X - W^{(m)} A^{(m)}\right\|_F^2}{\|X\|_F^2}\right) \times 100\% \tag{5.1}$$

and required $\lambda^{(m)} \geq \lambda_{\mathrm{cutoff}} = 80\%$ (Zandvoort et al., 2019) and, additionally, $\lambda^{(m)} - \lambda^{(m-1)} \geq \Delta\lambda_{\mathrm{cutoff}} = 1.5\%$. This notion let us also define the contribution of every synergy to the representation of $W^{(m)} A^{(m)}$ by realizing that $W^{(m)} = [w_1, \ldots, w_m]$ and $A^{(m)} = [a_1, \ldots, a_m]$. That is, the contribution of an individual synergy s could be given as

$$\lambda_s = \left(1 - \frac{\|X - w_s a_s\|_F^2}{\|X\|_F^2}\right) \times 100\% \tag{5.2}$$

Note that by combining the signals as described above, we obtained different wave forms between and common muscle weights across conditions and subjects, i.e. fixed muscle groups over conditions with varying activation patterns. For the sake of legibility, in the following we denote these outcomes as $X \longmapsto W^{(syn)} A^{(syn)}$.

Intermuscular coherence

The rectified EMGs were down-sampled to 256 Hz to reduce computational load. Data of the same condition were mean-centered and concatenated. Intermuscular coherence was determined between all $26 \times 25/2 = 325$ muscle pairs per subject and condition. The power spectral densities P_x and P_y of signal pairs (x, y) and the complex-valued cross-power spectral density P_{xy} were estimated using Welch's periodogram method (Hamming taper of 200 ms length and about 50% overlap). With this we computed the squared coherence $C_{xy}^2 = (P_{xy} P_{xy}^*)/(P_{xx} P_{yy})$; here $(\cdot)^*$ denotes the conjugate complex.

We corrected the coherence estimates for the bias due to differences in data length. We employed a bootstrapping approach (100 surrogates) of the complex-valued cross-spectral density through phase randomization (Hurtado, Rubchinsky, & Sigvardt, 2004; Kantz & Schreiber, 2004). In brief, phase randomization destroys coherence implying that the resulting bootstrap distribution is zero-centered. However, due to finite-size estimation the distribution may have a finite, frequency-dependent variance even for infinitely many surrogates. This variance yields a null distribution indicating the absence of coherence, which served as normalization factor for the coherence estimates. Since the latter is the modulus of the normalized cross-spectral density, the resulting distribution of squared coherences is a Chi-squared distribution with two degrees of freedom for which we considered squared coherences below $\alpha = 0.05$ not distinguishable from chance. Accordingly, these values were set to zero.

In line with the synergy analysis, we concatenated the data, i.e. now the corrected coherence spectra across the frequencies (f, 4-60 Hz), over subjects and conditions (SC) and 325 muscle pairs. This yielded a $f \times (SC \times 325)$ matrix, and we applied NMF to obtain $C \mapsto W^{(coh)} A^{(coh)}$. This NMF yielded m modes, $W^{(coh)} = [w_1, \dots, w_m]$ with $w_{j = 1, \dots, m}$, containing $SC \times 325$ coherence weights each, and $A^{(coh)} = [a_1, \dots, a_m]$ with $a_{j = 1, \dots, m}$, with defining the modes for all subjects, conditions and muscle pairs. To anticipate, these modes separated distinct frequency ranges. From hereon we therefore refer to these modes as frequency components. The number of these components was fixed using Eq. (5.1) with adjusted cut-off values: $\lambda_{\text{cutoff}} = 55\%$ and $\Delta\lambda_{\text{cutoff}} = 4\%$.

Muscle networks

We constructed muscle synergy and coherence networks with muscles as nodes and their functional connectivity as edges between them. The synergy-NMF yielded $w_{j = 1, \dots, m}$ that contained 26 muscle activity weights each for every synergy. We used the outer product $w_j \cdot w_j$ to define the connectivity matrix $C_j^{(syn)}$ of synergies $j = 1, \dots, m$ to create a one mode projection of a bipartite network (Murphy et al., 2018) with m layers (Horvát & Zweig, 2012). In this synergy network, every element of the connectivity matrices represented the weighted appearance of two muscles in the same synergy. To include the contribution of the synergies by means of the amplitude of the wave forms, the connectivity matrices were weighted for the sum of the integrals of the wave forms of the three modes of coordination.

The intermuscular coherence weights of the m frequency modes (NMF modes) served to define the edges of the coherence network. We thus obtained $m \times SC$ different 26×26 connectivity matrices $C_j^{(coh)}$ that we averaged over subjects and combined into an $m \times 3$-conditions multiplex network. The community structures across layers of both the synergy and coherence networks were determined by the Louvain algorithm (Jeub, Bazzi, Jutla, & Mucha, 2019).

To compare topological characteristics of the coherence networks between modes of coordination, we determined the global connectivity, clustering of muscles and strength of connections in the network by means of global efficiency, transitivity, and average strength across nodes (Bullmore & Sporns, 2009; Rubinov & Sporns, 2010) for all layers. Before doing so, the corrected coherence networks were thresholded to construct a minimally-connected network across the layers of the network, i.e., every node (muscle) was connected to at least one other node in one of the layers and the number of edges within the layers was constant across the layers.

Additionally, we time normalized the EMG data and estimated coherence again, but now with a Hamming taper of 5 s over the 0.6-4 Hz frequency range to directly compare synergy and (very) low-frequency coherence networks. Details of this analysis can be found as *Appendix*.

Statistical analysis

Statistical differences between conditions were assessed over subjects who exhibited both conditions (either 2:1 and transition, 2:1 and 1:1, or transition and 1:1).

Changes in the synergy wave forms were compared in two ways. First, we compared the amplitude during the gait cycle between modes of coordination. Subsequently, the amplitudes were normalized to the maximum of the wave form and we compared the amplitude-normalized wave forms between modes of coordination. We determined the samples of the time series which were significantly different in either amplitude or wave form between the conditions using statistical parametric mapping including paired t-tests (Pataky, Vanrenterghem, & Robinson, 2015; see also www.spm1d.org). Significance was identified based on an alpha threshold value corrected for multiple comparisons in three conditions and five synergies, i.e. $\alpha = 0.05/(3 \times 5) = 1/300$.

Differences between the network metrics of the layers of the coherence networks, i.e. modes of coordination and frequency components, were compared with a univariate ANOVA with subject as random factor ($\alpha = 0.05$). *Post-hoc* tests were performed to examine differences between conditions per frequency component ($\alpha = 0.005$).

RESULTS

Behavior

The kinematic assessment of the modes of coordination revealed that only seven subjects showed both modes of coordination and the transition between the two. The 2:1, transition, and the 1:1 mode of coordination appeared in nine, fourteen, and sixteen subjects, respectively (Table 5.2).

Figure 5.1 represents a typical example (subject 1) of the movement of the right arm and ipsilateral leg in the sagittal plane, the corresponding spectral power and overlap, and the relative phase for the 2:1, transition and 1:1 condition.

Table 5.2 Overview of modes of coordination per subject per walking speed

Speed \ Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1.0 km/h	2:1	T	T	2:1	2:1	2:1	T	2:1	2:1	T	T	N/A	2:1	T	2:1	T
1.5 km/h	2:1	T	1:1	T	T	1:1	1:1	T	1:1	1:1	2:1	T	T	1:1	2:1	T
2.0 km/h	2:1	1:1	1:1	T	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	T	1:1	T	1:1
2.5 km/h	T	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
3.0 km/h	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
3.5 km/h	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
4.0 km/h	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1

2:1 represents a double arm swing of both arms during one gait cycle, 1:1 represents a 1:1 coordination pattern with one arm swing of both arms during one gait cycle, and T represents the transition between the 2:1 and 1:1 mode of coordination in which both patterns were observed.

Muscle activity

Differences between modes of coordination were clearly visible in both the amplitudes and wave forms of the EMG envelopes (*Appendix* Figure 5.S1). EMG amplitudes particularly differed around the heel strike event in the ipsilateral leg and contralateral back and arm muscles in the 1:1 mode of coordination. The peak activity in the arm muscles around the contralateral heel strike shifted to earlier in the gait cycle when the coordination pattern switched towards a 1:1 mode of coordination between arms and legs.

Muscle synergies

Five muscle synergies accumulated 80% to the Frobenius norm of the original concatenated EMG envelopes and a sixth synergy added very little, which let us fix $m^{(syn)} = \text{rank}\,[W^{(syn)}] = 5$ (Figure 5.2). We found $\lambda_{1,\ldots,5}^{(syn)} = [17,13,16,19,15]\%$ on average across conditions.

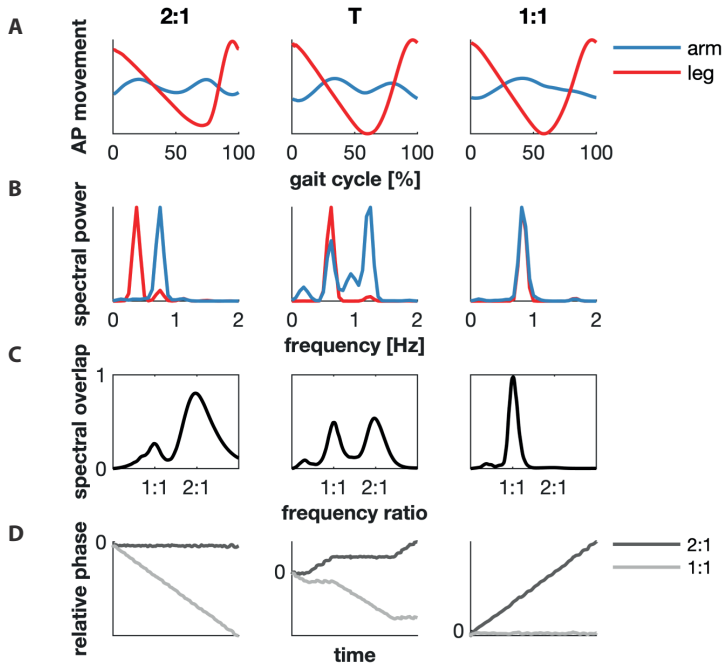

Figure 5.1 Example of the determination of the modes of coordination based on kinematics of subject 1. A) The average arm (blue) and ipsilateral leg (red) movement in the anterior-posterior (AP) direction as a function of the gait cycle in the 2:1 (1.0 km/h), transition (T, 2.5 km/h) and 1:1 (4.0 km/h) mode of coordination. **B)** The spectral power. **C)** The spectral overlap between the power spectra of the arm and leg is maximal for a 2:1 or 1:1 coupling between the arm and leg movement in the 2:1 and 1:1 mode of coordination, respectively. The transition contained peaks at both 2:1 and 1:1 coupling. **D)** The relative phase between arm and leg. A generalized relative phase of zero slope implies that arm and leg move at a fixed frequency ratio (2:1, black and 1:1, grey).

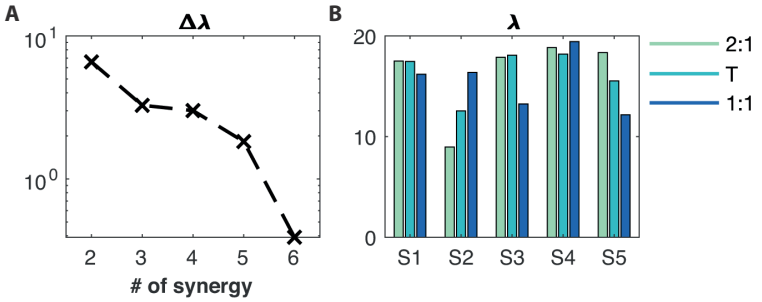

Figure 5.2 Reconstruction quality of the muscle synergies. A) Additional value of an extra synergy ($\Delta\lambda$) to the total contribution of the synergies to the Frobenius norm, **B)** the contribution of every synergy (S1 to S5) to the Frobenius norm (λ). The order of synergies S1 to S5 is shown in Figure 5.3. Green, cyan and blue bar plots represent the 2:1, transition (T) and 1:1 mode of coordination, respectively.

Synergies were ordered based on the relative timing of the main peak in the activation patterns (Figure 5.3A). S1 and S4 were active during the heel strike and weight acceptance response of the right and left leg, while S3 and S5 were active mainly in the calf muscle during the stance phase of the right and left leg, respectively. The muscle weights of S1 and S4 showed activity in both the leg and the contralateral trunk and arm muscles; bilateral calf and contralateral shank muscles were dominant in S3 and S5. S2 was active during the stance and swing phases with primarily activity of muscles around the pelvis (Figure 5.3B). The contribution $\lambda_2^{(syn)}$ of S2 increased from 2:1 to 1:1, while $\lambda_3^{(syn)}$ and $\lambda_5^{(syn)}$ decreased.

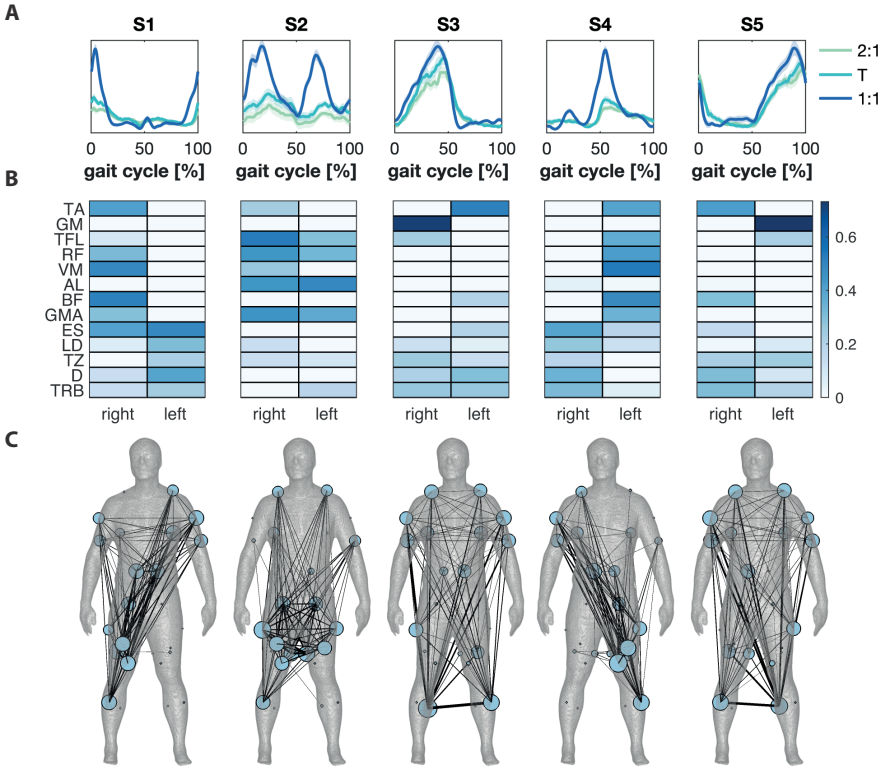

Figure 5.3 Muscle synergies across modes of coordination. A) The synergies' temporal activation patterns as a function of the gait cycle derived from average muscle activity patterns for the different modes of coordination. Green, cyan and blue represent the 2:1, transition (T) and 1:1 mode of coordination. Error patches represent the standard error of the mean across subjects. **B)** Synergies' weightings across conditions and subjects in color scale. **C)** Muscle synergy network plotted separately for each synergy on the body mesh (Makarov, Noetscher, & Nazarian, 2015). A minimally-connected network was created for visualization. Node size represent the degree of the muscle and edge thickness represent weighted appearance of both muscles in the synergy.

Significant differences were found between the synergies' wave forms between the 2:1 and the 1:1 and between the transition and the 1:1 mode of coordination (Figure 5.4). The amplitude of S1 increased in 1:1 compared to 2:1 and the transition around the right heel strike and the activity decreased quicker with an increase in walking speed. Similar results were found for S4 at the corresponding left heel strike. Changes in the amplitude were also visible in S2 between 2:1 and 1:1 and between the transition and 1:1 during the stance and swing phases of both legs. The activation pattern of S3 revealed some minor differences between the transition and 1:1 in the amplitude halfway the stance phase of the right leg and after the left heel strike, while no significant changes were found for S5.

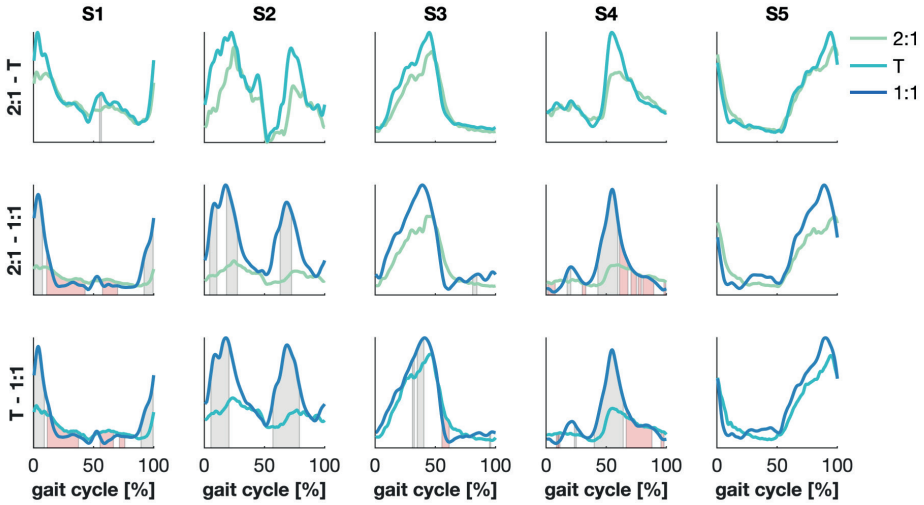

Figure 5.4 Significant differences between synergies' wave forms between modes of coordination. Green, cyan and blue represent 2:1, transition (T) and 1:1, respectively. Patches represent significant differences in time between the amplitude (grey) and the temporal patterns (red) of the synergies' wave forms. $\alpha = 1/300$.

Intermuscular coherence

The coherence spectra were decomposed in three modes, i.e., $m^{(coh)} = \text{rank}\,[W^{(coh)}] = 3$. These modes reflected distinct frequency bands, 4-8 Hz, 8-22 Hz and 22-60 Hz, in line with our previous findings (Boonstra et al., 2015; Kerkman et al., 2018). The frequency components contained in total 57% of the Frobenius norm of the coherence spectra; $\lambda^{(coh)}_{1,\ldots,3}$ = [19,19,19]%.

We extracted two frequency components (λ_{cutoff} = 19%) from the low-frequency coherence (0.6-4 Hz) showing peaks at 1.5 or 2.5 and 3.5 Hz; $\lambda^{(coh)}_{1,2}$ = [9,10]%.

Muscle networks

Both the muscle synergies and coherence spectra were represented as multiplex networks to facilitate quantitative comparison. For the muscle synergies, each synergy was represented as a layer of the multiplex network (Figure 5.3C). We subsequently estimated the community structure across all five layers (Figure 5.5A). As the connectivity in the layers of the synergy network did not overlap substantially, the community structure across layers yielded a single module and the synergy network contained several contralateral connections between arms and legs. These long-distance edges were distinctive for the layers of the synergies active around heel strike (S1 and S4). S3 and S5 also showed symmetries between left and right, but represented a more comprehensive network in which the whole human body was involved. S2 mainly showed connectivity around the pelvis and between the pelvis and the shoulder muscles (trapezius, Figure 5.3C).

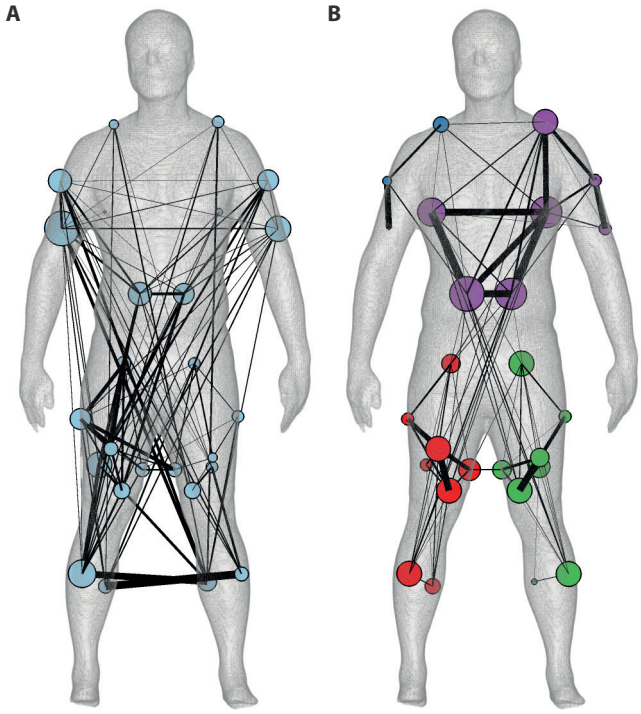

Figure 5.5 The community structure of the multiplex A) muscle synergy B) and coherence networks based on the synergy and coherence spectra muscle weightings. Community structure is visualized by color-coded nodes and the average degree across layers of every muscle is displayed as node size on the body mesh (Makarov et al., 2015). The edge width is based on the average connectivity across layers between the muscles in either the minimally-connected synergy or coherence network.

In contrast, the community structure of the multiplex coherence network divided the body in modules of both legs separate, the trunk with the left arm and the right arm (Figure 5.5B). The average modularity per frequency component was 0.14, 0.30, and 0.32, respectively. By constructing minimally-connected multiplex networks, we removed on average 293 significant edges (threshold was 0.0970) with weights of 0.0015 ± 0.0011 (mean ± standard deviation), 0.0018 ± 0.0011 and 0.0055 ± 0.0039 for 2:1, transition and 1:1, respectively. The preserved edges had weights of 0.0114 ± 0.0077, 0.0114 ± 0.0067 and 0.0184 ± 0.0069. In contrast to the synergy network, the community structure of the coherence network was not affected by this thresholding (see *Appendix*).

The community structure of the coherence network over 0.6-4 Hz was very similar to the community structure of the coherence network over the frequency range of 4-60 Hz: the Rand and adjusted Rand indices were 0.85 and 0.63, $p < 0.001$, respectively. Yet, individual layers of the coherence network revealed similarities with the layers of the synergy network; cf. *Appendix* for more details.

Changes in coherence networks

The topology of the coherence network was reorganized when the coordination pattern changed to the 1:1 mode of coordination (Figure 5.6). The network metrics, i.e. global efficiency, transitivity and average strength, were significantly different between conditions ($F(2,21) = 56.0$, $F(2,21) = 12.1$, and $F(2,21) = 38.7$, respectively, $p < 0.001$). The 1:1 mode in the 4-8 Hz frequency component contained several long-distance connections between the leg and the contralateral arm with high connection strengths corresponding to a high global efficiency (Figure 5.6C). In contrast, both the 2:1 and the transition showed mainly connections within and between upper body and arms. At 8-22 Hz, 1:1 coordination again deviated from 2:1 and the transition, and was associated with a relatively high global efficiency, transitivity and strength. Some long-distance connections were found in 1:1 between the legs and the lower back, and high within-module connectivity appeared within the legs. For the 22-60 Hz frequency component, the connectivity was high within the trunk in 2:1 and the transition, while this connectivity was lower in 1:1. In the latter condition, the connectivity was higher between arm muscles. The highest frequency component was without connections between the upper and lower body in all conditions.

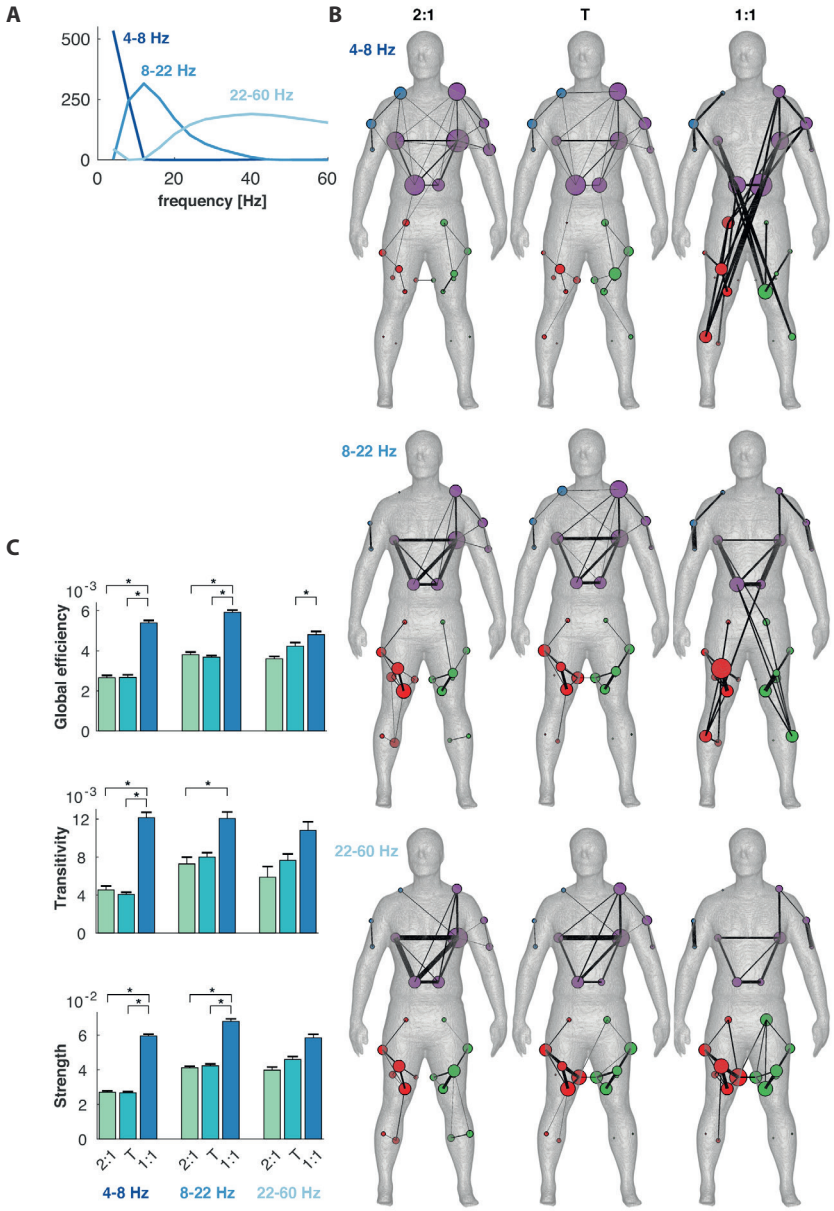

Figure 5.6 Changes in connectivity between conditions and frequency components in the minimally-connected multiplex coherence network. A) Frequency components 4-8, 8-22 and 22-60 Hz, obtained with non-negative matrix factorization. **B)** Coherence networks in the 2:1, transition (T) and 1:1 mode of coordination (columns) and the frequency components (rows). Colors in the networks depict different modules and node size and edge width represent degree and connectivity strength between muscles, respectively. **C)** Global efficiency, transitivity and average strength of the coherence networks per frequency component and condition. Error bars indicate standard errors of the mean and asterisks significant differences between conditions ($\alpha < 0.005$).

DISCUSSION

The aim of this study was to identify neural correlates of spontaneous switches in interlimb coordination during walking, i.e. transitions in frequency locking ratios between the arms and legs when walking speed changes. We applied more conventional synergy analysis and extended this to multiplex networks in line with the more recently introduced coherence-based muscle networks (Kerkman et al., 2018). As expected, we found changes between task conditions in the activation patterns of specific muscle synergies and in the network metrics of specific frequency layers of the coherence networks. In particular, we found increased activation of the synergies active around right and left heel strike (S1 & S4, respectively) during 1:1 phase locking compared to the other two coordination modes. Likewise, synergy S2 involved the muscles around the pelvis and also showed increased activation during 1:1 locking; note that this synergy appeared left/right symmetric. In contrast, synergies S3 & S5, involved in the initiation of the swing of the left and right leg, respectively, remained largely unchanged across modes of coordination. Similar to the muscle synergies, 1:1 coordination revealed increased connectivity between upper and lower limbs in two (lower) frequency components (4-8 and 8-22 Hz) compared to the other two modes of coordination. The increase in long-distance connectivity was associated with a corresponding increase in global efficiency, transitivity and average strength. We found four modules grouping either left and right leg muscles or left and right arm muscles, though, the module containing the left arm also included all the recorded trunk muscles. These findings indicate that the transition to a 1:1 coordination pattern is associated with a reorganization in the muscle activation patterns.

Arm-leg coordination switched from 2:1 to 1:1 frequency locking mode when walking speed was increased. During the transition period both coordination patterns could be observed supporting the notion of multi-stability (van Emmerik & Wagenaar, 1996). However, this was not observed in all subjects, in line with earlier studies reporting that the incidence of the 2:1 coordination pattern is reduced in treadmill compared to over-ground walking (Carpinella, Crenna, Rabuffetti, & Ferrarin, 2010). Future studies may focus on even lower treadmill speeds to pinpoint neurophysiological changes possibly underlying the transition in coordination. Yet, we identified statistically significant differences between the coordination modes in individual muscle activation patterns. We are confident that these findings underwrite earlier documented importance of arm muscle activity during walking (Craik et al., 1976; Goudriaan, Jonkers, van Dieen, & Bruijn, 2014; Meyns, Bruijn, & Duysens, 2013). They also revealed phase-specific modulations of arm muscle activity associated with the kinematic switches in interlimb coordination (see Figure 5.S1 *Appendix*). Last but not least, the modulations of EMG activity were reflected in the reorganization of the muscle synergies.

Speed-induced adaptations in muscle synergy strength and timing have been reported earlier (Ivanenko et al., 2004b; Yokoyama et al., 2016), which led Den Otter, Geurts, Mulder, and Duysens (2004) to speculate that modulations of muscle synergies are a mere by-product of a change in stance and swing time. We found that the synergy active during the stance and swing phases (S2) became stronger accounting for an increase in upper leg activity which may serve to control the relative movement between the trunk and the legs when walking faster. We found left/right-mirrored synergies for both S1 and S4 and S3 and S5; the muscles in S3 and S5 appeared important in preserving the upright body position, while synergies S1 and S4 induced the forward propulsion of the body. Synergies that were active during heel strike were also affected in both the strength and the wave form when switching to another mode of coordination, which was in accordance with the changes in relative timing of the arm swing. The synergy analysis revealed a fairly strong contribution of arm and shoulder muscles in the heel strike synergies (S1 and S4) and the switches between the modes of coordination were marked by a decrease in the involvement of arm muscles when the arm swing was in-phase with the leg swing. These phase-specific modulations could hence be directly related to the changes in kinematic behavior. Moreover, not all synergies were affected. Taken together, we rather support the notion of modular motor control, in which synergies can be modulated depending on the task while other synergies are robust across conditions (Nazarpour, Barnard, & Jackson, 2012).

We used one-mode projections, commonly employed in bipartite networks (Murphy et al., 2018), of the muscle synergy weights to construct multiplex networks (Horvát & Zweig, 2012), with each layer reflecting a synergy. These synergy networks can reveal functional connections between multiple muscles in line with functional modules related to the biomechanical constraints of walking (Neptune, Clark, & Kautz, 2009). For example, next to the coordination-related coupling between contralateral arms and legs, we also found ipsilateral connections between arms and legs specific for the 2:1 locking mode. The networks of synergies S3 and S5 were dominated by activities important for push-off (GM) and foot raise (contralateral TA), but this modulation did not depend on the mode of coordination. When collapsing the multiplex network across layers, the synergy network only reflected the biomechanical characteristics of walking that kept the mechanisms underlying synergy formation opaque (Tresch & Jarc, 2009). Yet, the muscle synergy network approach supports the idea of functionally organized synergies that are modulated by changes in interlimb coordination.

The topology of the muscle synergy network showed clear similarities with the network derived from intermuscular coherence at lower frequencies (0.6-4 Hz, see *Appendix*). Coherence at very low frequencies likely captures the co-variation of EMG envelopes which underpins the synergy analysis. Hence, both synergy and coherence networks may yield equivalent results, though, very low-frequency coherence might be difficult to estimate reliably due to the brevity of the gait cycles. At higher frequencies, the agreement between both types of networks was largely absent, as we did not observe a modular structure in the multiplex synergy network. This suggests that synergy and coherence analyses are complementary and potentially capture different aspects of motor control. As expected, the community structure of the coherence networks was closely related to the anatomical relationships of the muscles (Kerkman et al., 2018).

Higher frequency components of intermuscular coherence may indicate different functional pathways in the neuromuscular system, which were affected by the coordination between limbs. We found major changes in the 1:1 mode of coordination compared to the 2:1 mode and the transition, indicating a reorganization in the structure of common input during 1:1 coordination. The connectivity between 4 and 8 Hz was strongly increased between the arm and contralateral leg muscles in the 1:1 mode, indicative for altered afferent input (Bourguignon, Jousmäki, Dalal, Jerbi, & De Tiège, 2019) and seemingly relevant for maintaining forward propulsion (cf. above). Connectivity in the frequency range of 8-22 Hz covers both alpha and low beta frequency ranges and have frequently been observed in intermuscular (Boonstra et al., 2015; Kerkman et al., 2018) and cortico-muscular coherence (Boonstra, van Wijk, Praamstra, & Daffertshofer, 2009b; Conway et al., 1995; de Vries, Daffertshofer, Stegeman, & Boonstra, 2016; Petersen, Willerslev-Olsen, Conway, & Nielsen, 2012; Roeder, Boonstra, Smith, & Kerr, 2018). Although cortico-muscular connectivity was not assessed in our study, we are tempted to interpret these frequency ranges as different neural pathways, possibly reflecting afferent and efferent inputs to spinal motor neurons, respectively (Bourguignon et al., 2019; McAuley & Marsden, 2000; Rathelot & Strick, 2009). The connectivity at 8-22 Hz was only affected when the legs and arms were in antiphase, i.e. in the 1:1 mode of coordination, with stronger long-distance connections between both lower back and leg muscles. First and foremost, the overall connectivity changed instead of a reorganization in connectivity patterns. That is, the conjunction between the upper and lower body muscles gained importance arguably because of an increasing demand of upper relative to lower body movements when walking faster. Finally, the connectivity in the frequency component of 22-60 Hz was less affected by changes in interlimb coordination.

The absence of neural connectivity during the 2:1 mode of coordination is in contrast to the kinematic coupling between the limbs. The increase in long-distance connectivity between the upper and lower limbs when switching to 1:1 coordination may indicate additional demands when switching to antiphase coordination. The absence of interlimb coupling in the EMG envelopes might indicate a largely passive contribution of the arm swing at slow walking speeds, while at higher speeds muscle activity is needed to actively establish interlimb coordination and possibly reduce the cost of walking (Collins, Adamczyk, & Kuo, 2009). The active contribution of arm muscle activity in the 1:1 mode of coordination seemingly underlies the reorganization of muscle synergies. In our study, this reorganization was associated with increased functional connectivity between the arms and legs specifically at 4-22 Hz, which again implies increased common input to both arm and leg muscles (Boonstra et al., 2016). Muscle networks showed an abrupt change in network topology with increased long-distance connections when switching to a 1:1 mode of coordination. The increase in connectivity between arm and leg muscles is also reflected in the layers of synergy network corresponding to synergies S1 and S4, while muscle networks during quiet standing were mainly dominated by local connectivity (Boonstra et al., 2015; Kerkman et al., 2018). The switches in interlimb coordination were hence associated with distinct changes in the functional connectivity in the neuromuscular system reflecting common input to multiple muscles.

Admittedly, our results do not provide undeviating evidence for possible neural causes of synergy formation or stability of interlimb coordination. A promising future step could be to infer the dynamic coupling functions between muscle activation profiles that, in principle, do contain all information about the functional mechanisms underlying the interactions and prescribe the physical rule specifying how an interaction occurs (cf. Stankovski, Pereira, McClintock, & Stefanovska, 2017). We also have to admit that we did not directly assess the contribution of the supra-spinal inputs and it might be a 'natural' step to evaluate these inputs using measures like partial directed coherence (e.g., Boonstra et al., 2015) or other directed information theoretic measures (e.g., Boonstra, Faes, Kerkman, & Marinazzo, 2019). While evidence about the functional role of intermuscular coherence is rapidly accumulating (Boonstra et al., 2015, 2019; De Marchis, Severini, Castronovo, Schmid, & Conforto, 2015; Farmer, Bremner, Halliday, Rosenberg, & Stephens, 1993), research on possible cortical contributions during whole-body movements comes with challenges (Gwin, Gramann, Makeig, & Ferris, 2010). Several studies already revealed the phasic modulation of cortico-muscular coherence (Gwin & Ferris, 2012; Gwin, Gramann, Makeig, & Ferris, 2011; Roeder et al., 2018) and their importance of stabilizing modes of coordination (Bruijn, Van

Dieën, & Daffertshofer, 2015). Interestingly, a recent experiment by Zandvoort et al. (2019) successfully identified cortical contributions to synergy formation by combining electro-encephalography with EMG-based synergy analysis. Future work may adopt this approach to substantiate our suggestions about high-frequency, long-distant neural activation in the context of interlimb coordination and their sources in the central nervous system.

Conclusion

The reorganization in muscle synergies and the concomitant alterations in coherence modulations of common neural input to multiple muscles highlight that switches in interlimb coordination are associated with changes in neuromuscular control. Network analysis of connectivity between all muscle pairs showed that the modularity of the neuromuscular system couples anatomical and functional linked muscles. The speed-induced transition to a 1:1 arm-leg frequency locking is accompanied by strong intermuscular coherence between upper and lower body muscles. This functional connectivity is particularly pronounced at higher frequencies indicating a significant long-distance neural interaction that accompanies the formation of muscle synergies.

APPENDIX

Muscle activity

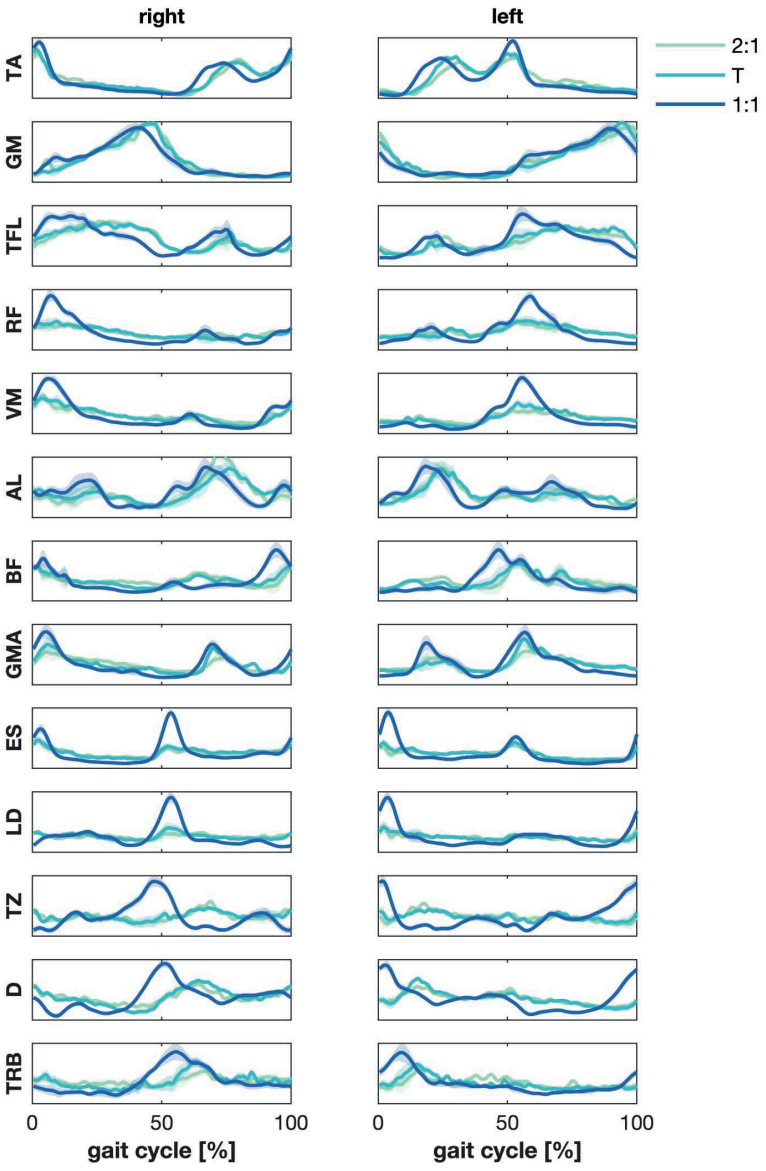

Figure 5.S1 Grand average muscle activity patterns for all muscles in the different modes of coordination during the gait cycle. 0 and 100% indicate the right heel strike. Green, cyan and blue temporal patterns represent the 2:1, transition (T) and 1:1 mode of coordination, respectively. Error patches represent the standard error of the mean across subjects.

Muscle synergies per condition

We obtained both varying wave forms and weightings when muscle synergies were estimated per condition, i.e. the EMG data was not concatenated. Nevertheless, the wave forms and weightings were similar to the synergies estimated over the concatenated data.

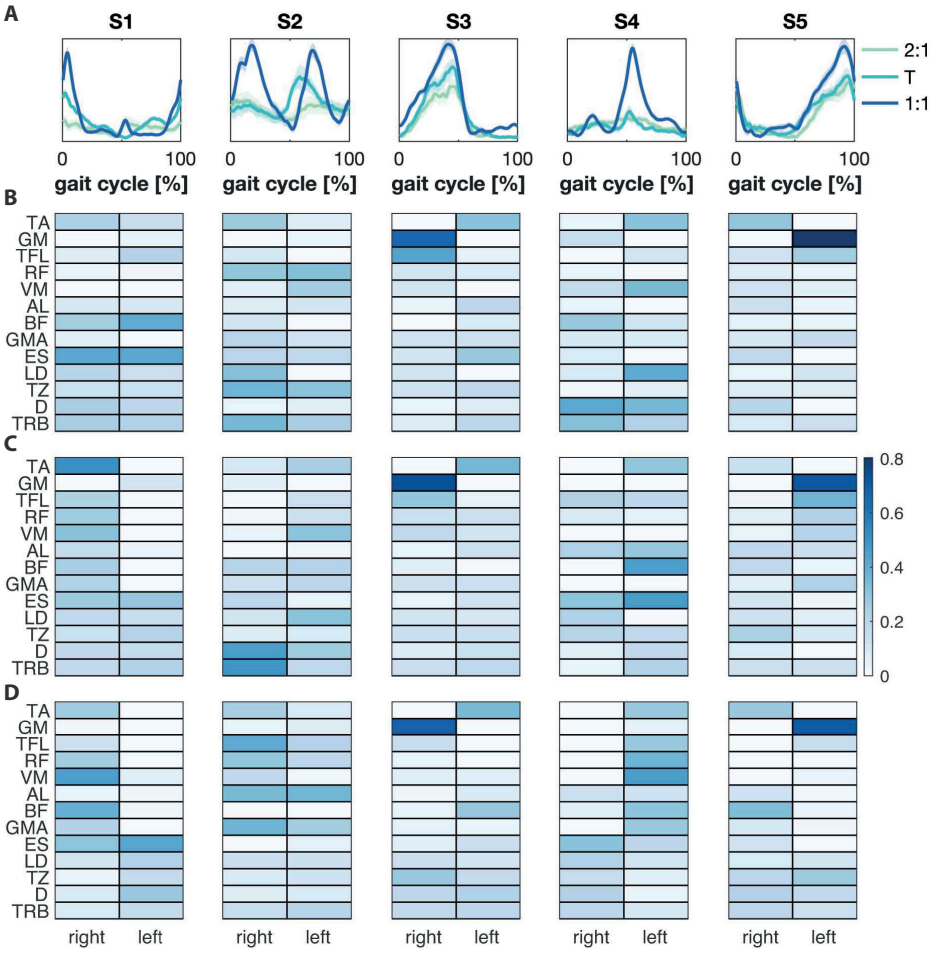

Figure 5.S2 Muscle synergies estimated per mode of coordination. A) Muscle synergy wave forms. Green, cyan and blue represent the 2:1, transition (T) and 1:1 mode of coordination, respectively. **B)** Muscle synergy weightings of the 2:1, **C)** transition, **D)** 1:1 mode of coordination.

Comparison low frequency coherence and synergy networks

Muscle synergies reveal slow-temporal dynamics of muscle activity while intermuscular coherence is mainly focused on high-frequency connectivity between muscles. Both provide information about the function of the same neuromuscular system in different modes of coordination. Low-frequency intermuscular coherence is expected to display similarities with muscle synergies. To show this, we here discarded condition specific frequency information (e.g. stride time) by time normalization of the stride. Subsequently, we estimated intermuscular coherence between all muscle pairs in the frequency range of 0.6-4 Hz in which 1 Hz represents the stride duration. We used the same procedure as described in the method section *Intermuscular coherence* but used a window of 5 s instead of 200 ms, which allowed to focus on coherence at low frequencies with a frequency resolution of 0.2 Hz. We applied non-negative matrix factorization over the coherence spectra and again used Eq. (5.1) to select the number of frequency components and estimated the community structure across frequency components and conditions. We examined the similarity of the community structure of the low and high frequency coherence networks by permutation testing (number of iterations = 10.000) of the Rand index and the adjusted Rand index. The Rand index is the sum of the edges present within the same and in different modules of both networks divided by the total number of edges in the networks. A Rand index of 1 implies that all edges are placed in the same module in both networks. The adjusted Rand index additionally accounts for grouping of the edges by chance (Fortunato, 2010; Qannari, Courcoux, & Faye, 2014).

Two modes were used to decompose the coherence spectra; λ_c = 19%. and $\lambda_{1,2}^{(coh)}$ = [9,10]%. One frequency component showed a peak at 1.5 Hz, while intermuscular coherence was high in the other frequency component at 2.5 and 3.5 Hz (Figure 5.S3A). The community structure of the low-frequency coherence was very similar to the community structure of the coherence networks over the frequency range of 4-60 Hz: The Rand and adjusted Rand index were 0.85 and 0.63, $p < 0.001$, respectively. The legs were again mainly divided into two modules, though, the muscles at the medial and posterior side (AD and BF) of the right upper leg were part of the left leg module, and the trunk and arms formed one module. The coherence networks of 1:1 revealed the clearest similarities with the synergy networks (Figure 5.S3B). The frequency component of 1.5 Hz showed high within leg connectivity and was very similar to the heel strike synergies (S1 and S4), while the connectivity in this network was also high within the trunk and arms. The latter was also shown in all synergies except of S2. Connectivity at 2.5 and 3.5 Hz was even stronger within and between the trunk and arms. This network also showed the high interlimb connectivity around the pelvis which was typical for S2. Other

notable connectivity in the low-frequency coherence networks was found in the 2:1 condition with long-distance connectivity between the leg and the ipsilateral arm which was also shown S3 and S5 and related to the in-phase movement of the arm and leg.

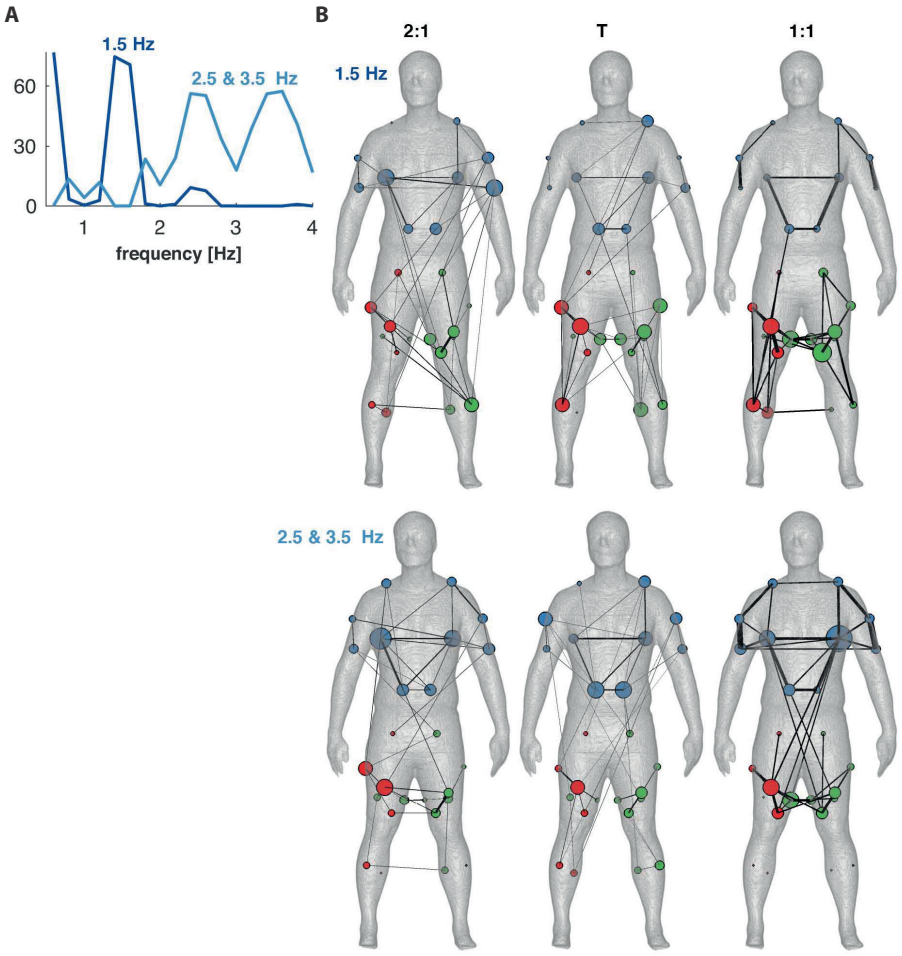

Figure 5.S3 Minimally-connected multiplex coherence network for frequency range of 0-4 Hz. A) Frequency components and **B)** the corresponding coherence networks for the 2:1, transition (T) and 1:1 mode of coordination. The community structure is color-coded and the node size and edge width represent degree and connectivity strength between muscles, respectively.

Community structure minimally-connected muscle networks

Thresholding of the edges is a common procedure in the analysis of networks. The removal of meaningful edges can induce ambiguities in the interpretation of differences between networks. Here we used a community structure algorithm to detect modules based on all the significant weighted edges in the networks. Another option to determine the community structure is to construct a minimally-connected binary network in which only the highest edges are considered for the determination of the clustering in the network (Didier, Brun, & Baudot, 2015).

We found that thresholding the synergy and coherence networks to construct a minimally-connected multiplex network barely affected the community structures of the networks (Figure 5.S4). The synergy network consisted of two modules, one mainly at the lower legs, while the other one covered the pelvis and the upper body, but the division of the two modules does not seem to represent any clear anatomical or functional constraint and hence seemed not to be meaningful. The community structure of the coherence network, in contrast, was the same as the one of the unthresholded and weighted network and did resemble the anatomical and task constraints. Thresholding of the edges in this data set seemed not to affect the clustering of the network. The clustering in the network is probably driven by the edges with high connectivity.

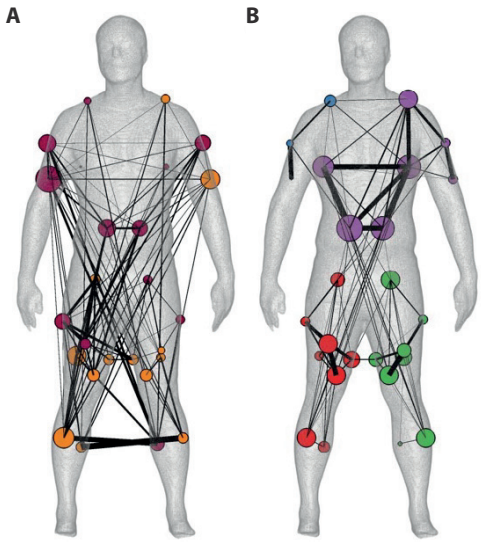

Figure 5.S4 The community structure of the minimally-connected multiplex A) muscle synergy B) and coherence networks based on the synergy and coherence spectra muscle weightings. Community structure is visualized by color-coded nodes and the average degree across layers of every muscle is displayed as node size on the body mesh (Makarov et al., 2015). The edge width is based on the average connectivity across layers between the muscles in either the synergy or coherence network.

6

General discussion

SUMMARY OF FINDINGS

My overarching aim was to identify mechanisms that accompany impaired walking development in children who are at high risk of developing cerebral palsy (CP). The literature review in **Chapter 2** confirmed the general hypothesis that older children with CP recruit fewer synergies than typically developing (TD) children. Both the spatial and the temporal synergy structure seem to differ between these groups rendering muscle synergy analysis a promising method to quantify altered neuromuscular control and to predict outcomes of clinical interventions.

While the vast majority of studies in this review focused on walking in school-age children with CP, one can expect the window before the onset of independent walking to be critical for improving the walking function in CP. In **Chapter 3** I therefore investigated neuromuscular control in young children before and just after the onset of independent walking. I compared the corresponding differences between children at high risk of CP and TD children. As expected, children at high risk of CP recruited fewer synergies than TD children already during this early phase of motor development, suggesting that early brain lesions in CP express as early modifications of neuromuscular control.

In view of the high within-subject variability in children with CP, I adopted a longitudinal design in **Chapter 4**. I explored different gait kinematic and EMG measures in three children at high risk of CP with divergent developmental trajectories. Two of them started to walk independently, whereas the third child did not reach independent walking at 4.5 years of age. The first two displayed gradual changes in spatiotemporal gait parameters, intersegmental coordination, muscle activation patterns and muscle synergy structure, from supported to independent walking. In contrast, the child who did not develop independent walking, did not show any significant changes across gait parameters over the course of two years. To generalize conclusions, however, a much larger sample (n » 3) will be required.

To obtain a broader view on mechanisms that hamper the development of walking in children with CP in terms of altered synergies, their origin should be clarified in a more principle sense. Do synergies emerge at a spinal level or is there a 'higher' level of control in the central nervous system (CNS)? The higher frequency components of the EMG signals may help to provide an answer to this profound question. In **Chapter 5** I investigated both muscle synergies and intermuscular coherence during tempo-induced changes of interlimb coordination during walking in adults. These changes seemingly caused a reorganization of muscle synergies together with alterations in coherence modulations at higher frequencies between muscles of the upper and the lower body and between bilateral leg muscles. Put differently, long-distant neuromuscular connections accompany the formation of muscle synergies, rendering contribution of higher-

level centers in the CNS to the formation of muscle synergies likely. Whether or not this also applies to children with CP remains to be seen, but if so, these findings appear promising tackling the searched-for mechanisms that accompany impaired walking development.

DEVELOPMENT OF NEUROMUSCULAR CONTROL

The studies in this thesis addressed the development of neuromuscular control, as evaluated by muscle synergy analysis, following the hypothesis that muscle synergies reflect neuromuscular control from the CNS. Different approaches where applied, using a cross-sectional (**Chapter 2** and **Chapter 3**) and a longitudinal (**Chapter 4**) design, in order to answer the following question:

How is the development of neuromuscular control affected by early brain lesions in very young children with or at high risk of CP?

Quantification of muscle synergies

In TD children, the number of synergies increases from two during neonate stepping, to four in toddlers who start to walk independently (Dominici et al., 2011). Previous studies revealed that school-age children with CP, who walk independently, recruit fewer synergies compared to TD children (**Chapter 2**). Yet, it was unknown how the number of synergies developed from supported to independent walking in children with CP. In the study described in **Chapter 3**, I found that children with early brain lesions at high risk of CP recruit fewer synergies than TD children already before the onset of independent walking in the supported walking phase and also in the first years of independent walking. It seems that even before the emergence of independent walking, the dimensionality of neuromuscular control is reduced. Children with asymmetric CP, who walked independently, recruited fewer synergies that accounted for more muscle activity in the most compared to the least affected side. This agrees with previous research in post-stroke individuals with unilateral lesions (Coscia et al., 2015), and children with unilateral CP (Cappellini et al., 2016). This difference between sides in the asymmetric CP group appeared to be absent in the supported walking phase. Likewise, the child with unilateral CP described in **Chapter 4** showed a more pronounced, principal synergy in the most compared to the least affected side during the first steps and independent walking sessions, while this was not visible during supported walking before the first independent steps. Admittedly, this might have been influenced by the support the children received as this could have minimized differences between the legs. However, it may also mean that this disparity between sides occurs later in motor development,

after the onset of independent walking. The latter seems more compelling, but it remains to be seen whether this interpretation holds true.

Varying levels of functional mobility in children with CP may impact the quantification of synergies. Previous studies found a relationship between the number of synergies and severity of CP, where children with higher GMFCS levels recruited fewer synergies (Steele et al., 2015; Tang et al., 2015). The child with bilateral CP GMFCS III described in **Chapter 4** recruited only two synergies during supported walking at the corrected age of 30 months, and this stayed constant longitudinally in the four sessions recorded afterwards until the corrected age of 54 months. During this period, this child did not start to walk independently. The lower number of synergies in children with CP may result from a lack of fractionation of muscle synergies, i.e. splitting of synergies into more, during development (Clark et al., 2010; Hashiguchi et al., 2016). If true, this may imply that children with CP do have access to four synergies, but fail to manage to recruit all of them independently.

Structure of muscle synergies

Spatial structure (muscle weights) and temporal structure (timing and duration of peaks of the temporal activation patterns) of muscle synergies are typically compared given a fixed number of synergies. Although previous studies found differences in both spatial and temporal structure of synergies between children with CP and TD children (**Chapter 2**; Cappellini et al., 2016, 2018; Kim et al., 2018b; Steele et al., 2015; Torricelli et al., 2014; Yu et al., 2019), no major differences in synergy structure were found in **Chapter 3**. This was the case for both supported and independent walking between children with or at high risk of CP and TD children. One may explain this by the relatively high functional mobility levels of the children in the CP group, who were previously found to have synergy structures comparable to TD children (Cappellini et al., 2016; Yu et al., 2019). In children walking with support, spatial structure was similar for synergy 2 and synergy 4, but not for synergies 1 and 3. It might be that – in general – synergies 2 and 4 are similar in this young group as they are already present during neonate stepping (Dominici et al., 2011), whereas synergies 1 and 3 are still maturing. In my experiment, this may have caused a larger variability between children in both the CP and TD group.

Interestingly, the spatial synergy structure appears to be more robust than the temporal synergy structure. The two children at high risk of CP described in **Chapter 4**, who developed independent walking, did not show a change in spatial synergy structure during the transition from supported to independent walking. However, the temporal synergy structure did change in shape and burst width

over time. Shuman et al. (2019) reported that the spatial structure of synergies remained unaltered after treatment, while the temporal structure did change. This change was related to improved gait. Apparently, the spatial structure of synergies is less adaptable than its temporal counterpart, much in line with a reduction in neural plasticity in children with CP. These children may be able to activate the same groups of muscles together as TD children, but the timing and duration of the activation of these muscles may differ. Previously found similarities in the synergy activation patterns between older children with CP and TD toddlers at their first steps (1-1.2 years old) suggest that the development of the temporal structure of synergies lags behind in children with CP compared to TD children (Cappellini et al., 2016). This agrees with previous research showing similarities in the walking pattern in older children with CP and young TD children (Berger et al., 1982, 1984; Leonard et al., 1991).

DEVELOPMENT OF GAIT KINEMATICS

The relation between the development of gait kinematics and neuromuscular control is important to understand the underlying mechanisms of walking development. An important question about the development of treatments for children with CP remains: Can we improve gait kinematics by adapting muscle synergies or vice versa?

What is the relation between the maturation of gait kinematics and neuromuscular control in children with or at high risk of CP?

Irrespective of the age of the children, planar covariation of limb elevation angles matured rapidly in the first months after the first independent steps (**Chapter 4**), which agrees with previous research (Cheron et al., 2001; Ivanenko et al., 2004a, 2007). During supported walking and first steps the most affected side of the child with unilateral CP deviated more from planarity than the least affected side, while we could not establish differences between sides during independent walking. This contrasts the findings on the first synergy's contribution (VAF_1) in the same child and in children with asymmetric CP in **Chapter 3**, which differed between sides during independent walking only. It seems that gait kinematics and neuromuscular control do not develop simultaneously. Previous research supports this idea, reporting no association between gait kinematics and muscle activations (Buurke et al., 2008) or muscle synergies (Booth et al., 2019). More recently, a simulation-based study indicated muscle-tendon properties to be more predictive for the impaired crouch gait pattern than for impaired neuromuscular

control (Falisse et al., 2020).

Although we showed that gait kinematics improved during the transition from supported to independent walking, the modulation of muscle groups to effectively conduct this motor behavior did not coincide. Presumably both neural and kinematic factors underlie the development of walking (Dewolf et al., 2020), but apparently their developmental paths towards mature walking differ. Gaining more insight into the time frame in which gait kinematic and neuromuscular development occur, and unravelling the sensitive period for maturation of these features will be important for the design of novel diagnostics and therapies.

METHODOLOGICAL CONSIDERATIONS

As indicated in **Chapter 2**, several methodological choices are needed when determining muscle synergies. As such I sought to address the following question:

How do methodological choices impact the investigation of neuromuscular control?

Muscle synergy analysis

EMG signal processing techniques, i.e., filtering and amplitude scaling, are known to affect muscle synergy outcomes. The chosen frequency range of interest influences the number of synergies considered relevant (Santuz et al., 2017; Shuman et al., 2017; van der Krogt et al., 2016), while filter type and order seem to have minor effects (Devaprakash et al., 2016; Santuz et al., 2017). The majority of studies investigating muscle synergies in children with CP use a low-pass filter with a cut-off frequency of 10 Hz when distilling EMG envelopes (**Chapter 2**), a choice that has been adopted here (**Chapters 3, 4 and 5**). The (type of) amplitude scaling can also impact the weighting of synergies per muscle. Unfortunately, a commonly accepted way of scaling the amplitude is few and far between. Yet, in this thesis this important pre-processing step has been applied consistently, namely EMG amplitudes of every muscle were normalized to the maximum of its mean value plus its standard deviation.

Decomposing EMG signals in muscle synergies require recording of a sufficiently large number of muscles over a sufficiently long period (i.e. number of strides). Many previous studies that employed retrospective data from clinical measurements suffered from a limited number of muscles as few as four or five muscles, which may readily lead to an over-estimation of synergy contributions and, hence, misinterpretation (Damiano, 2015; Steele et al., 2013; Zelik et al., 2014). In the studies of **Chapters 3, 4,** and **5**, I sought to record a number of 11 muscles per side, which resulted in sufficiently accurate estimates of muscle synergies. A

minimum of 20 strides was recorded to minimize the effect of variability between strides (Oliveira et al., 2014).

Defining the number of relevant synergies by their contribution to the total data variance remains a topic of debate. In the literature values easily span a range from 80-95% rendering comparison between studies difficult, if at all possible. Not only is there no consensus on the optimum amount of variance that ought to be covered, the very fact of judging the relevance of a mode decomposition by a measure (variance) that is not optimized by the method used for the decomposition renders this approach questionable. Non-negative matrix factorization (NMF) defines modes by minimizing the Frobenius (or matrix) norm. Hence, in **Chapter 3** and **Chapter 5** the synergies' contribution to this norm was determined. For the sake of comparability with other studies, however, the 'traditional' variance-accounted-for (VAF) was also presented in **Chapter 3 and 4**.

Conventional approaches to reduce dimensionality are principal component analysis (PCA), independent component analysis (ICA), and NMF. The latter is mainly used for the decomposition of EMG data, as muscle activity are considered to superimpose constructively; in fact it has been suggested to be the method for identifying muscle synergies in dynamic tasks (Rabbi et al., 2020). The NMF approach comes in various algorithmic implementations. Like most studies on muscle activation modularity in children with CP, we used the so-called synchronous synergy model (time-invariant approach) and it might be interesting to see to what extent alternatives like the time-varying synergy model (d'Avella & Tresch, 2002) or the space-by-time model (Delis et al., 2014) yield different results.

Muscle network analysis

Muscle network analysis provides new opportunities for the evaluation of the synchronization between motor units across a large number of muscles, in addition to the conventional synergy analysis. As shown in **Chapter 5**, muscle network modulations can illustrate modifications in the neuromuscular system related to changes in functional behavior during walking. At lower frequencies, synergy and network analysis yielded equivalent results, while at higher frequencies there was no obvious match between the two. Strong intermuscular coherence between upper and lower body muscles was especially prominent at higher frequencies, suggesting long-distant neural communication to coincide with the formation of muscle synergies. Network analysis should be considered complementary to synergy analysis, as both appear to capture distinct aspects of neuromuscular control. Our findings in adults are promising for future research investigating neuromuscular control in children with CP.

LIMITATIONS

Experimental constraints like number of muscles and strides, methodological factors such as EMG (pre-)processing, and the choice of synergy model, may impact muscle synergy outcomes. This may cause variability in results between different studies investigating muscle synergies during walking, limiting the ability to compare the outcomes in this thesis with other studies in this research field. The participants under study provided further limitations. The body size of the children recorded in the studies in this thesis was small, particularly in the early sessions before the onset of independent walking during supported walking, leaving limited space for mounting EMG electrodes. This might have led to cross-talk in the EMGs of adjacent muscles, affecting data quality and causing spurious covariates. To minimize this potential problem, we used particularly small-size electrodes. Moreover, the possibility of improper electrode positioning and also improper marker placement or skin movement artefacts in the 3D motion capture is unavoidable in movement analysis, but might even be higher in small children. And, it is a challenge to record 'normal' walking in young children who are eager to play rather to walk on a boring treadmill, leading to variability in the walking pattern between trials of one child and between children. Also in young TD children some strides can be classified as abnormal, when compared to a group averaged curve, due to stride-to-stride variability (Oudenhoven, Booth, Buizer, Harlaar, & van der Krogt, 2019a).

The heterogeneity of the group of children with CP, including a wide diversity of walking ability, caused large variations in the number and structure of muscle synergies. This is particularly true as in my thesis I aimed to cover a wide variety of children with CP who would develop independent walking, including children with functional mobility levels ranging from GMFCS I-III. Due to a limited number of subjects, it was impossible to distinguish separate groups to examine the diversity in the group of children with CP. **Chapter 4** revealed that development of gait kinematics and neuromuscular control in a child with severe CP (GMFCS III) who did not develop independent walking differed from the child with less severe CP (GMFCS I). Thus, we cannot take the whole group of children with CP as one, and in follow-up studies it is advised to examine the diversity of this group by including separate functional mobility level groups with sufficient sample sizes.

Recording a large amount of muscles in very young children was considered impossible up until recently. Using portable EMG system and small, wireless electrodes, the work in this thesis shows that it is feasible to measure high-quality muscle activation data in very small children, even before the onset of independent walking. True, data acquisition is laborious, making it difficult to include in the clinic and in large cohorts. Without a doubt, further development of these new

techniques will facilitate the use of muscle synergy analysis in young children with early brain lesions, at high risk of CP, in larger sample sizes for clinical purposes.

Investigating young children at high risk of CP is important. The crucial time for early detection and interventions may be well before the age of two years, when the brain is most plastic and the corticospinal tract is still maturing (Friel et al., 2014; Hadders-Algra, 2014; Morgan et al., 2021; Novak et al., 2017; Yang et al., 2013). The practical problem of including children at high risk of CP without diagnosis of CP, yet, is that they may not develop CP. We are not yet able to deviate children who do develop CP from children who do not, making it even more important to devote more studies on the investigation of this early period of development in children with early brain lesions.

FUTURE DIRECTIONS

The work in this thesis was an exploratory investigation of the underlying mechanisms of walking development in children with CP or at high risk of CP. The longitudinal case series (**Chapter 4**) investigating walking development in three children at high risk of CP with divergent development trajectories, thereby minimizing inter-subject variability, shows that it is possible and promising to use a longitudinal design in larger samples. This could offer new insights into developmental changes in young children with CP, which may reveal new paradigms for early interventions in CP.

Crawling is an important developmental milestone, which is used in clinic to identify delayed motor development on a regular basis. Recent research showed that children with developmental delay and high risk of developing CP used fewer muscle synergies (decomposed from four leg and four arm muscles) and increased co-activation of muscles during hand-and-knees crawling compared to age-matched TD children (Gao et al., 2018; Xiong et al., 2018). Up to now, movement abnormalities in very young children before the onset of independent walking are often assessed through visual inspection of the quality of movement. The assessment of general movements has been shown a sensitive tool for early identification of children at high risk of CP (Hadders-Algra, 2004, 2014; Herskind, Greisen, & Nielsen, 2015; Kanemaru et al., 2014). In addition, the investigation of neuromuscular control during crawling, in combination with supported walking as proposed in this thesis, might serve as a promising method to reveal impaired motor control prior to the onset of independent walking.

Whether or not muscle synergies have a neural origin is still a controversial topic (Bizzi & Cheung, 2013). Some argue muscle synergies are encoded in the cortex (Zandvoort et al., 2019), whereas others claim they arise in the spinal cord

(Cappellini et al., 2010; Ivanenko et al., 2008), or in the brain stem (Schepens & Drew, 2004). The origin of muscle synergies may even depend on the phase of development, as the spinal cord and brainstem were shown to reflect muscle synergies in neonates, while the integration of supraspinal and sensory motor control increased during development (Dominici et al., 2011). To solve this issue, future research should add direct assessments of cortical activity, i.e. synchronous EEG recordings (Short et al., 2020). Likewise promising appears to be the study of spinal maps (Ivanenko et al., 2013) when combined to the approaches outlined in this thesis. Moreover, muscle network analyses, as shown in **Chapter 5,** are ready to be implemented and – by now – are known for their additive value when searching for the origin of muscle synergies.

CLINICAL IMPLICATIONS

Following the idea of a combination of muscle synergy-, muscle network-, spinal activity-, and EEG-analysis may be a significant step in the design of early interventions that can target neural pathways. Together with the development of wearable sensors (Airaksinen et al., 2020; Redd et al., 2019; Xu et al., 2019), biofeedback (Booth et al., 2019; He et al., 2019), movement analysis without markers using machine learning models (Kidziński et al., 2020), and electrical stimulation of muscle, tendons (Sommerfelt et al., 2001; Stackhouse et al., 2007; Wright et al., 2012), or spinal cord (Solopova et al., 2017), we might become able to enhance early detection of impaired motor development in children with early brain lesions. We may identify targets for early interventions to promote walking improvement in children with CP.

Muscle synergy analysis may also be used as a method to predict treatment outcomes. Several studies discussed in **Chapter 2** reported a relation between the effect of treatment and muscle synergies before treatment, and a recent simulation-based study confirmed these findings (Pitto et al., 2020). It seems that knowledge about neuromuscular control could potentially assist in treatment planning. However, we need to be careful when linking neuromuscular control to gait kinematics directly: gait kinematics may follow a different developmental path than muscle synergies (**Chapter 4**). The overall findings of my thesis do provide further insight in the underlying mechanisms of walking development in CP and the results are promising.

The clinical use of EMG and muscle synergies to assess impaired neuromuscular control in CP remains limited. Although the findings in this thesis do not directly imply that the calculation of muscle synergies would help clinicians to make clinical decisions, they may lay a foundation for further research. To bridge the

gap between researchers and clinicians, a constant dialogue and interdisciplinary collaboration is necessary (Cappellini et al., 2020a). This may indeed increase the impact of muscle synergy analysis for rehabilitation of children with CP. Together with other researchers in the field we must join forces to improve motor function in children with CP.

CLOSING REMARKS

The work in my thesis should be considered a small step towards a better understanding of the underlying mechanisms of impaired walking development in children with CP. Children with early brain lesions, at high risk of CP, recruit fewer synergies compared to TD children with the same walking ability already in the early phase of motor development. Apparently, brain lesions in CP express as early modifications of neuromuscular control. The proper way of assessing this are longitudinal studies over the course of walking development as they help to minimize inter-subject variability in the heterogenous CP group. My studies provide evidence that muscle synergy analysis is a promising objective method for the detection of impaired neuromuscular control in young children, and that muscle network analysis may add valuable information in future research. Confidently, these findings encourage researchers to continue investigating the underlying mechanisms of walking development to support the design of early interventions to improve walking ability in children with CP.

B

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S

Summary

Malformation or lesions of an immature brain may cause cerebral palsy (CP). Children with CP typically start to walk independently later than typically developing (TD) children, and their walking patterns were previously found to retain some characteristics of immature toddler walking patterns. These walking problems can be explained by limited selective motor control, at least to some part. At young age, the brain is highly plastic and the corticospinal tract still maturing. Hence, early interventions that target in particular neuromuscular mechanisms appear excellent candidates for improving functional mobility in children with CP. A possibility to unravel neuromuscular mechanisms is muscle synergy analysis. Walking requires refined coordination, and the central nervous system arguably simplifies neuromuscular control during walking by the recruitment of groups of muscles, i.e. muscle synergies. Capitalizing on a wealth of earlier findings on muscle synergies, I used this concept to identify mechanisms that underlie impaired walking development in young children with or at high risk of CP.

While my introductory **Chapter 1** provides a more detailed sketch of this conceptual framework and a brief explanation of the definition and classification of CP, for **Chapter 2** I systematically reviewed the literature for the current status of research on muscle synergies during walking in children with CP compared to TD children. This resultant overview includes an assessment of muscle synergy analysis as a means to quantify and predict treatment outcomes in children with CP. Twelve out of 16 included studies revealed that children with CP recruited fewer muscle synergies and eight studies showed that either the spatial or the temporal structure differed between children with CP and TD. Overall, the variability was larger in children with CP, yet, it appears that children with CP use a 'simpler' motor control strategy compared to TD children. Importantly, three studies indicated that muscle synergy characteristics before treatment may serve to predict treatment outcomes.

The time before the onset of independent walking may be a critical period for early interventions to improve motor functions including walking. In **Chapter 3** I hence assessed whether neuromuscular control in young children with CP differs from that of TD children with the same walking ability in the early phase of walking development. Twenty children with (high risk of) CP and twenty TD children (age 6.5-52.4 months) were grouped based on their walking ability, supported or independent walking. Muscle synergies were extracted from electromyographic signals of bilateral leg muscles. Conform earlier findings in, e.g., Chapter 2, the children with (high risk of) CP were found to recruit fewer synergies than TD children in both the supported and independent walking groups. And, the most affected side in children with asymmetric CP walking independently showed fewer synergies than the least affected side. This suggests that early brain lesions result

in early alterations of neuromuscular control specific for the most affected side in children with asymmetric CP.

The study summarized in Chapter 3 had a cross-sectional design, rendering inferences about causes of (altered) development limited. In **Chapter 4** I therefore adopted a longitudinal design, employing a combination of kinematic and electrophysiological measures. Three children at high risk of CP with different developmental trajectories were followed during five consecutive sessions covering a period of one to two years. The assessments had started prior to the onset of independent walking and included a session during the first independent steps, when present. One child did not develop CP, one was diagnosed with unilateral and one with bilateral CP. The child without CP and the one with unilateral CP started to walk independently with spatiotemporal gait parameters, intersegmental coordination, muscle activation patterns and muscle synergy structure that altered when switching from supported to independent walking. In the child with unilateral CP, especially for the most affected leg, changes were less pronounced. The child with bilateral CP did not develop independent walking and did not show any changes over time in the aforementioned parameters. While these findings did indicate differences in maturation of locomotor patterns between children with divergent developmental trajectories, a clearly larger sample should be assessed to verify the current results. Only then one may further speculate about the mechanisms that may underlie impaired walking development in very young children at high risk of CP, let alone use this approach to improve prognosis and to pinpoint possible targets for early intervention.

Muscle synergy analysis is a by now classic approach to assess neuromuscular control in children with CP. Yet, there are alternatives. Muscle network analysis builds on intermuscular coherence and hence evaluates the synchronization between motor units across a large number of muscles. **Chapter 5** is a further contribution to this methodological advance. Both muscle synergy and muscle network analysis were used to examine whether switches in interlimb coordination in healthy adults are accompanied by changes in functional activity between multiple muscles. Speed changes during treadmill walking served to induce switches in the frequency and phase relationships between arm- and leg-swing. This rapid transition coincided with changes in both muscle synergies and intermuscular coherence. While the coherence network changes at low frequencies largely resembled the dynamic pattern of the synergies (with the latter being clearer), at higher frequencies other coherence patterns came to the fore. In a nutshell, the change in stability of arm/leg coordination could be associated with modulations in long-distant neuromuscular connectivity between arms and legs. Muscle synergy and muscle network analysis should hence be considered complementary as they potentially capture different

aspects of neuromuscular control. These findings in adults are promising for future research investigating neuromuscular control in children with CP.

Chapter 6 reflects on the findings in this thesis. Particular focus is put on their implications in the quest on the mechanisms that underlie impaired walking development in CP. Brain lesions in CP express as early modifications of neuromuscular control. Muscle synergy analysis, possibly in conjunction with muscle network analysis, is a promising objective method for the detection of impaired neuromuscular control. In the future, this may help in designing early interventions to improve walking ability in children with CP.

S

Samenvatting

Hersenbeschadiging in een onvolgroeid brein kan de diagnose cerebrale parese (CP) als gevolg hebben. Kinderen met CP beginnen doorgaans later zelfstandig te lopen dan zich normaal ontwikkelende (TD) kinderen en uit eerder onderzoek bleek dat hun looppatronen enkele eigenschappen van onvolwassen looppatronen behouden. Deze loopproblemen kunnen tot op zekere hoogte verklaard worden door een beperkte selectieve motorische aansturing. Bij jonge kinderen is het brein nog zeer plastisch en zijn de corticospinale banen nog in ontwikkeling. Daarom zouden vroege interventies, die zich richten op de neuromusculaire aansturing tijdens het lopen, belangrijk kunnen zijn voor het verbeteren van functionele mobiliteit van kinderen met CP. Een mogelijkheid om de neuromusculaire mechanismen van loopontwikkeling te ontrafelen is spiersynergie-analyse. Verfijnde aansturing van een groot aantal spieren is vereist tijdens het lopen en naar alle waarschijnlijkheid vereenvoudigt het centrale zenuwstelsel dit proces door het aansturen van groepen spieren, zogenaamde spiersynergieën. Voortbouwend op eerdere bevindingen van spiersynergieën heb ik dit concept gebruikt om de mechanismen te identificeren die ten grondslag liggen aan de verstoorde loopontwikkeling van jonge kinderen met, of met een hoog risico op, CP.

Terwijl mijn inleidende **Hoofdstuk 1** een meer gedetailleerde schets geeft van dit conceptueel kader, inclusief een korte uitleg van de definitie en classificatie van CP, heb ik in **Hoofdstuk 2** de literatuur systematisch geïnspecteerd om de huidige staat van onderzoek naar spiersynergieën tijdens lopen in kinderen met CP ten opzichte van TD-kinderen weer te geven. Dit resulterende literatuuroverzicht bevat een beoordeling van spiersynergie-analyse als middel om behandelresultaten bij kinderen met CP te kwantificeren en te voorspellen. Twaalf van de 16 geïncludeerde studies lieten zien dat kinderen met CP minder spiersynergieën gebruikten in vergelijking met TD-kinderen en acht studies toonden dat de spatiële en temporele structuur van spiersynergieën verschilden tussen kinderen met CP en TD. In het algemeen was de variabiliteit bij kinderen met CP groter, echter lijken kinderen met CP een 'simpelere' motorische aansturingsstrategie te gebruiken dan TD-kinderen. Daarnaast gaven drie studies aan dat spiersynergie-eigenschappen gemeten voorafgaand aan de behandeling mogelijk behandelingsuitkomsten kunnen voorspellen.

De periode voor aanvang van zelfstandig lopen is mogelijk cruciaal voor vroege interventies om motorische functies, inclusief lopen, te verbeteren. In **Hoofdstuk 3** heb ik daarom onderzocht of neuromusculaire aansturing bij jonge kinderen met CP verschilt van die van TD-kinderen met dezelfde loopvaardigheid, met behulp van spiersynergie-analyse. Twintig kinderen met (een hoog risico op) CP en twintig TD-kinderen (6.5-52.4 maanden oud) werden gegroepeerd op

basis van hun loopvaardigheid, ondersteund of zelfstandig lopen. Spiersynergieën werden onttrokken uit de spieractiviteit-data van bilaterale beenspieren. In lijn met eerdere bevindingen, bijvoorbeeld Hoofdstuk 2, gebruikten kinderen met (een hoog risico op) CP minder synergieën dan TD-kinderen in zowel de ondersteund als zelfstandig lopen groep. Daarnaast liet de meest aangedane kant van kinderen met asymmetrische CP die zelfstandig lopen minder synergieën zien dan de minder aangedane kant. Deze bevindingen suggereren dat vroege hersenbeschadiging resulteert in vroege afwijkingen in de neuromusculaire aansturing, specifiek voor de meest aangedane kant bij kinderen met asymmetrische CP.

De studie samengevat in Hoofdstuk 3 had een cross-sectioneel design, waardoor conclusies over de oorzaken van een (veranderde) ontwikkeling beperkt worden. Om die reden heb ik in **Hoofdstuk 4** een longitudinaal design aangenomen, waarbij ik gebruik maakte van een combinatie van kinematische en elektrofysiologische metingen. Drie kinderen met een hoog risico op CP, met uiteenlopende ontwikkelingstrajecten, werden gevolgd gedurende vijf opeenvolgende sessies over een periode van één tot twee jaar. Het onderzoek begon voor de aanvang van zelfstandig lopen en omvatte een sessie met de eerste zelfstandige stapjes. Eén kind ontwikkelde geen CP, één was gediagnostiseerd met unilaterale CP, en één met bilaterale CP. Het kind zonder CP en het kind met unilaterale CP gingen zelfstandig lopen en tijdens de switch van ondersteund naar zelfstandig lopen lieten zij veranderingen zien in spatiotemporele loopparameters, intersegmentele coördinatie, spieractivatiepatronen en spiersynergie-structuur. Bij het kind met unilaterale CP waren deze veranderingen minder prominent in de meest aangedane kant. Het kind met bilaterale CP ging niet zelfstandig lopen en liet geen veranderingen zien in de eerdergenoemde parameters over de tijd. Hoewel deze bevindingen verschillen laten zien in de ontwikkeling van bewegingspatronen tussen kinderen met uiteenlopende ontwikkelingstrajecten zijn er grotere steekproefsamples nodig om de huidige resultaten te verifiëren. Alleen dan kunnen we verder speculeren over mechanismen die mogelijk ten grondslag liggen aan de verstoorde loopontwikkeling bij zeer jonge kinderen met een hoog risico op CP, laat staan over het gebruik van deze aanpak als middel om prognoses te verbeteren en om mogelijke doelen voor vroege interventie vast te stellen.

Spiersynergie-analyse is een inmiddels conventionele manier om neuromusculaire aansturing bij kinderen met CP te onderzoeken. Er zijn echter alternatieven. Spiernetwerkanalyse bouwt voort op intermusculaire coherentie en evalueert de synchronisatie tussen motorische eenheden van een groot aantal spieren. **Hoofdstuk 5** is een verdere bijdrage aan deze methodologische voorspiegeling. Zowel spiersynergie- als spiernetwerkanalyse zijn gebruikt om te onderzoeken

of de overgangen in de coördinatie tussen ledematen bij gezonde volwassenen samengaan met veranderingen in de functionele activiteit tussen meerdere spieren. Verschillende loopsnelheden werden opgelegd tijdens het lopen op een loopband om veranderingen in de frequentie- en fase-verhoudingen tussen de arm- en beenzwaai te induceren. Deze snelle overgang ging samen met veranderingen in zowel spiersynergieën als intermusculaire coherentie. Terwijl het coherentienetwerk bij lage frequenties grotendeels leek op het dynamische patroon van spiersynergieën (waarbij de laatste duidelijker was), kwamen bij hogere frequenties andere coherentiepatronen naar voren. In een notendop, de verandering in stabiliteit van arm/been coördinatie kan geassocieerd worden met modulatie in neuromusculaire connectiviteit over een lange afstand tussen de armen en benen. Spiersynergie- en spiernetwerkanalyse zouden daarom als complementair beschouwd moeten worden, omdat ze mogelijk verschillende aspecten van neuromusculaire aansturing omhelzen.

Hoofdstuk 6 reflecteert op de bevindingen in dit proefschrift. Bijzondere nadruk is gelegd op de implicaties in de zoektocht naar de mechanismen die ten grondslag liggen aan de verstoorde loopontwikkeling bij CP. Hersenbeschadiging bij CP uit zich in vroege wijzigingen van neuromusculaire aansturing. Spiersynergie-analyse, eventueel in combinatie met spiernetwerkanalyse, is een veelbelovende objectieve methode voor de detectie van verstoorde neuromusculaire aansturing. In de toekomst zal dit mogelijk helpen bij de ontwikkeling van vroege interventies om de loopkwaliteit van kinderen met de CP te verbeteren.

P

List of publications

Publications as part of this thesis

Articles published in peer-reviewed journals

Bekius, A., Bach, M. M., van de Pol, L. A., Harlaar, J., Daffertshofer A., Dominici, N., & Buizer, A. I. (2021). Early development of locomotor patterns and motor control in very young children at high risk of cerebral palsy, a longitudinal case series. *Frontiers in Human Neuroscience, 15,* 232.

Bekius, A., Zandvoort, C. S., Kerkman, J. N., van de Pol, L. A., Vermeulen, R. J., Harlaar, J., Daffertshofer, A., Buizer, A. I., & Dominici, N. (2021). Neuromuscular control before and after independent walking onset in children with cerebral palsy. *Sensors*, *21*(8), 2714.

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Other publications

Articles published in peer-reviewed journals

Bekius, A., Cope, T. E., & Grube, M. (2016). The beat to read: a cross-lingual link between rhythmic regularity perception and reading skill. *Frontiers in Human Neuroscience*, *10*, 425.

Conference proceedings

Abstracts published in journals

Bekius, A., Kerkman, J. N., Zandvoort, C. S., Daffertshofer, A., Harlaar, J., Buizer, A. I., & Dominici, N. (2019). Muscle synergy complexity in children with cerebral palsy during the development of walking. *Gait & Posture, 73*, 141.

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Abstracts not published

Bekius, A., Kerkman, J. N., Zandvoort, C. S., Daffertshofer, A., Harlaar, J., Buizer, A. I., & Dominici, N. Muscle synergy complexity in children with cerebral palsy during the development of walking. PMCXII conference, *invited oral presentation,* Amsterdam, The Netherlands (2019).

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Bekius, A., Kerkman, J. N., Zandvoort, C. S., Daffertshofer, A., Buizer, A. I., Harlaar, J., & Dominici, N. Muscle synergies during walking in very young children with cerebral palsy. Annual PhD meeting of the Vereniging voor Bewegingswetenschappen Nederland*, poster presentation,* Amsterdam, The Netherlands (2017).

Bekius, A., Kerkman, J. N., Daffertshofer, A., & Dominici, N. Muscle synergies change with arm-swing transition. Autumn School Berlin, *poster presentation,* Berlin, Germany (2016).

Bekius, A., Cope, T. E., & Grube, M. Systematic invesitgation of the 'feeling of the beat': auditory regularity processing in behaviour and EEG. Rhythm Production and Perception Workshop, *poster presentation*, Amsterdam, The Netherlands (2015).

A

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BabyGaitLab team

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VUmc Revalidatiegeneeskunde/Kinderneurologie

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Maastricht UMC+

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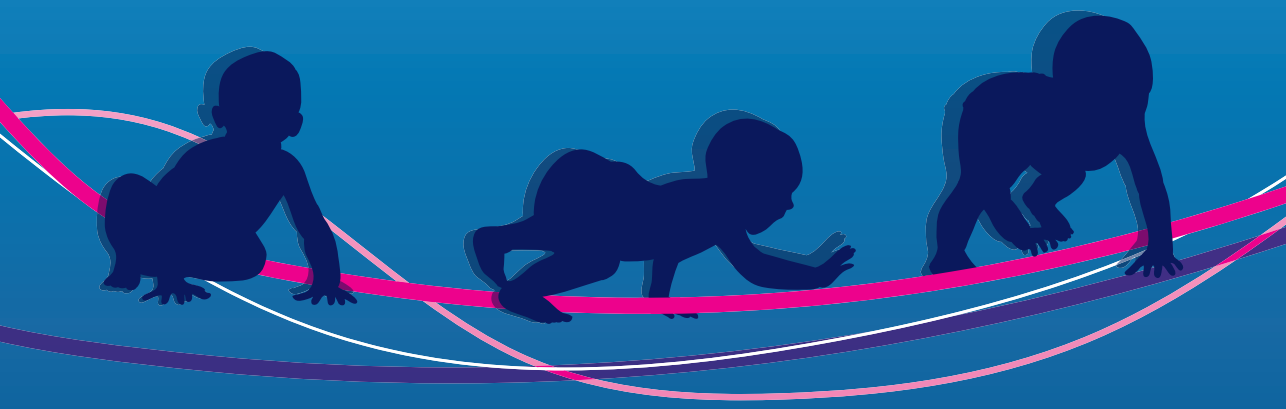
Abstract

Early brain lesions can give rise to cerebral palsy (CP), which may affect the development of walking. Given the highly plastic brain and still maturing corticospinal tract of young children, early interventions targeting underlying mechanisms of walking impairment may be important to improve functional mobility in children with CP. The overarching aim of this thesis is to identify the underlying mechanisms of impaired walking development in children at high risk of CP. The findings in this thesis suggest that early brain lesions in CP express as modifications of neuromuscular control, already in the early phase of motor development. Muscle synergy analysis, possibly in conjunction with muscle network analysis, is a promising objective method for the detection of impaired neuromuscular control. This may support the design of early interventions to improve walking ability in children with CP in the future.



Amsterdam Movement Sciences

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Amsterdam Movement Sciences conducts scientific research to optimize physical performance in health and disease based on a fundamental understanding of human movement in order to contribute to the fulfillment of a meaningful life.