Mechanisms of Motor Impairment in Spastic Cerebral Palsy

by

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Abstract

This thesis discusses the pathophysiological mechanisms underlying neuromotor impairments in people with spastic cerebral palsy (CP). Ninety percent of people with CP have spastic CP and 60% have bilateral motor impairments. The first two chapters introduce CP and review the mechanisms known to contribute to motor impairments. Three original studies (Chapters 3-5) involving 17 adults with bilateral spastic CP and 15 neurologically intact (NI) controls were designed to advance this knowledge. All three studies focus on an ankle plantarflexor, the soleus muscle, which is frequently affected in bilateral spastic CP. They are followed by a general discussion.

The first chapter of this thesis provides a brief overview of the disorders grouped together as CP and of motor function in spastic CP including weakness and involuntary muscle activity. The second chapter is a novel review of mechanisms known, or theorized, to contribute to neuromotor impairments in spastic CP. Changes in the cortex, in white matter pathways and within spinal circuits are discussed.

The first study demonstrates abnormalities in the descending corticospinal motor pathways using transcranial magnetic stimulation (TMS) and diffusion tractography in the adults with bilateral spastic CP. New findings of this study include: decreased amplitude in motor evoked potentials (MEPs), an association between the MEP amplitudes and maximal voluntary muscle activity within the CP group and reduced facilitation of MEP amplitudes with voluntary activity. We also confirmed previous findings (often in younger participants and/or people with unilateral CP) of: changes in the motor representation to more lateral locations, higher thresholds for activation and reduced anatomic integrity of corticospinal pathways as suggested by diffusion tractography in the adults with bilateral spastic CP.

The second study demonstrates an association between reduced motor function and reduced activation of motoneuron inhibitory post-synaptic potentials (IPSPs) by sensory inputs in the adults with bilateral spastic CP. We measured the activation of IPSPs using single motor unit responses to sensory stimulation. While all NI adults had evidence of IPSP activation, almost half of the CP group lacked IPSP activation. Surface electromyography was used to quantify the depth of inhibition evoked by the sensory stimulation. In the CP group, this inhibition was correlated with reduced motor function as evaluated with the Gross Motor Functional Classification System and the Functional Mobility Scale.

The last original study presented is pilot work evaluating short-interval intracortical inhibition (SICI) within the motor cortex projecting to the soleus in 7 people from each of our CP and NI groups. As SICI, a paired-pulse TMS technique, is known to depend on the intensity of the initial, conditioning, stimulus we evaluated the recruitment profile in each individual. Reductions in cortical inhibitory circuits such as SICI have been theorized to contribute to involuntary muscle activity in people with spastic CP. However, we found that the participants with bilateral spastic CP had similar U-shaped recruitment profiles of SICI to our NI control group.

The final chapter provides some additional reflections on the three original studies. I provide arguments supporting the generalization of our findings involving the 17 adult individuals to the broader population of people with spastic CP. However, there are some technical limitations and those that have not been discussed previously and/or are common between two or more of our studies are discussed. The final subsection of this thesis proposes future work to further advance the study of mechanism of motor impairments in spastic CP.

Preface

This thesis is an original work by Elizabeth G. Condliffe. The research project, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, Project Name "Mechanisms of Spasticity in Cerebral Palsy", Pro00023530, October 18 2011 and Project Name "Motoneuron Excitability after CNS Trauma", Pro00001119, July 13 2009.

All neurophysiology experiments were performed in Dr. Monica Gorassini's laboratory and she was the supervising author on all manuscripts. Drs. Condliffe and Gorassini were responsible for study design, neurophysiological data collection and analysis and manuscript composition of the studies presented in Chapters 3-5. Some of the research conducted for this thesis results from a collaboration with radiologists, Dr. Dean Jeffery and Dr. Derek Emery and a collaboration with neuroimaging researchers, Dr. Sarah Treit and Dr. Christian Beaulieu. All imaging was performed at the Peter S. Allen MR Research Centre. Dr. Beaulieu generated the imaging protocols. Dr. Jeffery and Emery analyzed the clinical MRI data. Dr. Jeffery performed the diffusion tractography and Dr. Condliffe analyzed this data both with Drs. Treit and Beaulieu's supervision. All clinical assessments were performed by Dr. Condliffe with supervision from Dr. Lalith Satkunam. All contributors are at the University of Alberta.

Chapters 1 & 2 are original work done by Dr. Condliffe with Dr. Satkunam's and Gorassini's supervision. Chapter 2 of this thesis may be modified and submitted for publication.

Chapter 3 will be modified and submitted for publication. In addition to their roles described above, Dr.'s Jeffery, Emery, Treit and Beaulieu have contributed to the manuscript sections related to the imaging.

Chapter 4 has been published by the Journal of Physiology doi: 10.1113/JP271886. In addition to analyzing the clinical MRI data, Dr.'s Jeffery and Emery approved the final version submitted for publication.

Chapter 5 may be submitted for publication.

Chapter 6 is original work done by Dr. Condliffe with Dr. Gorassini's supervision.

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The work in this thesis represents only a few pieces toward the puzzle of understanding and ultimately improving recovery following damage to the nervous system. Many thanks to friends and my parents Nancy and Toby Condliffe, and to Keith Condliffe, Judy Suke and their families for always supporting and encouraging the fun I find in puzzles and to Brian Whatley for valuing my love of jigsaws.

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List of Abbreviations

AHP	afterhyperpolarization
aMT	active motor threshold
CM	cerebral malformation
CMR	cutaneomuscular reflex
CP	cerebral palsy
CST	corticospinal tract
CVA	cerebrovascular accident
DGMI	deep gray matter injury
DTI	diffusion tensor imaging
E1	first excitatory response
EMG	electromyography
EPSP	excitatory post-synaptic potential
FA	fractional anisotrophy
FLAIR	fluid-attenuated inversion recovery
fMRI	functional magnetic resonance imaging
FMS	Functional Mobility Scale
FMS_{TOTAL}	the combined FMS score at 5, 50, and 500 m
GABA	gamma-aminobutyric acid
GMFCS	Gross Motor Functional Classification System
I1	first inhibitory response
$I1_{ave}$	average EMG during the duration of the initial inhibitory response
$I1_{max}$	maximal EMG suppression prior to 80 ms post-stimulation
IPSP	inhibitory post-synaptic potential
mAsh	modified Ashworth score
MD	mean diffusivity
MEG	magnetoencephalography
MEP	motor evoked potential
$MEP_{1.2}$	the MEP at 1.2 x aMT
MEP_{max}	the largest MEP independent of testing conditions
MEP_{peak}	the largest MEP on the recruitment curve
M_{max}	maximal M-wave
MRC	Medical Research Council score
MRI	magnetic resonance imaging
MSO	maximum stimulator output
MVA	maximal voluntary activity
MVC	maximum voluntary contraction
NI	neurologically intact
Penn	Penn Spasm Frequency
PSF	peri-stimulus frequencygram
PSTH	post-stimulus time histogram
PVWMI	periventricular white matter injury
ROI	region of interest
Rx	medication
SD	standard deviation
SICI	short interval intracortical inhibition
TL	target leg
TMS	transcranial magnetic stimulation
Δ %BKD EMG	percent change relative to the mean background EMG
$\lambda_{//}$	parallel diffusivity
$\lambda_{\perp}$	perpendicular diffusivity

Chapter 1. Introduction

Foreword

Pathophysiological mechanisms underlying neuromotor impairments in spastic cerebral palsy (CP) span from cerebral cortex to muscle. This thesis will probe a few of these mechanisms, but first begin with a brief overview of the etiology and classification of CP followed by a description of motor function in spastic CP (Chapter 1). This will be followed by a detailed discussion of the current understanding of the pathophysiology underlying neuromotor impairments in spastic CP (Chapter 2) and our three studies that contribute to this knowledge base (Chapters 3, 4, 5). The experiments in this thesis probe mechanisms behind both reduced voluntary muscle activity and increased involuntary muscle activity in adults with spastic CP. As ankle plantarflexors exhibit many characteristics of spastic CP, namely weakness and involuntary muscle activity, and contribute to gait dysfunction, the three studies focus on the soleus.

The first study presented (Chapter 3) explores the recruitment of descending motor pathways activated by transcranial magnetic stimulation (TMS) and the voluntary modulation of TMS-evoked responses. Impairments are demonstrated in both. The anatomic integrity of descending motor pathways is also explored using diffusion tensor imaging (DTI). Again, impairments are noted in people with spastic CP. The subsequent two studies explore inhibitory mechanisms that may contribute to the excessive involuntary motor activity characteristic of spastic CP. Reduced inhibition of spinal motoneurons by sensory pathways (Chapter 4), as demonstrated by motor unit and surface electromyography recordings, was found to be associated with reduced motor function. In contrast, short-interval intracortical inhibition, known as SICI (Chapter 5), was found to be similar in adults with spastic CP and their neurologically-intact (NI) peers. Finally the implications of these findings for the general population with spastic CP, limitations in their interpretation and potential future investigations will be discussed (Chapter 6).

Cerebral Palsy

CP "describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour, by epilepsy, and by secondary musculoskeletal problems" (Rosenbaum et al., 2007). The underlying etiology, timing of injury, manifestations of the motor disorder and associated conditions can vary greatly. A recent meta-analysis calculated the prevalence of CP as 2.9 per 1000 children (Oskoui et al., 2013), making CP the most common cause of motor disability in childhood (Sewell et al., 2014). More than 85% of people with CP survive into adulthood (Blair et al., 2001). However, life expectancy for an individual is related to the degree of impairments. A 30 year-old with CP who can walk independently has a normal or near-normal life-expectancy whereas a 30 year-old with CP who cannot lift his/her head and requires tube feeding is only expected to live 14 more years (Strauss et al., 2008). The high rate of at least mild disability within this lifelong disorder contributes to a lifetime cost over $1 million per person (Centers for Disease Control and Prevention [CDC], 2004; Kruse et al., 2009).

Etiology of CP as Revealed by Brain Imaging

CP does not correspond to a single disease, but rather a *group* of disorders (see above for definition). Though there are many etiologies that can cause CP, the specific etiology for an individual is frequently unknown (Nelson, 2003). As reviewed by Krageloh-Mann and Horber (2007), brain imaging, especially magnetic resonance imaging (MRI), reveals an underlying brain injury in most cases and can provide insight into the cause of CP. However, 13-15% of people with cerebral palsy have no abnormalities detected by a standard clinical MRI (Reid et al., 2014b).

Most abnormal MRIs demonstrate a brain malformation, injury to the white matter adjacent to the ventricles, or grey matter lesions. Brain malformations occur early in fetal life during the first or second trimester. At this stage, as the brain undergoes cell proliferation, migration and organization of neuronal precursors, even a single disruptive event can lead to diffuse abnormalities in brain structure (Marret et al., 2013).

Periventricular white matter injury (PVWMI) is frequently the result of ischemia or inflammation leading to the loss of premyelinating oligodendrocytes (Khwaja and Volpe, 2008). In addition to the development of cerebral white matter pathways early in the third trimester, the vascular supply to the periventricular regions is immature, producing a region of selective vulnerability in the white matter adjacent to the ventricles (Khwaja and Volpe, 2008). Given these factors, it is unsurprising that PVWMI is the most common MRI finding in children with CP born preterm (<37 weeks) (Reid et al., 2014b). However, PVWMI can also be seen in 12-32% of children with CP born at term presumably due to in-utero injury (Krageloh-Mann and Horber, 2007; Reid et al., 2014b).

Later in the third trimester and following birth, superficial cortical and deep grey matter structures are particularly susceptible to injury including stroke or acute hypoxia-ischemia (Kirton and Deveber, 2013; Krageloh-Mann and Horber, 2007; Reid et al., 2014b). Some population studies of brain imaging patterns differentiate focal vascular insult (commonly a stroke in the middle cerebral artery distribution) from other causes of grey matter injury such as hypoxic-ischemic encephalopathy or severe hyperbilirubinemia (Hoon, 2005; Reid et al., 2014b).

Classification of Cerebral Palsy

As etiology is often elusive, CP is frequently classified based on the clinical presentation. The current recommendation is a descriptive classification that includes the dominant type of motor disorder, regions of the body that are primarily affected (topography), and functional motor abilities (Rosenbaum et al., 2007).

Classification by Dominant Motor Disorder

While weakness is found in most people with CP (Mockford and Caulton, 2010), features that distinguish motor impairments are used to describe the type of dominant motor disorder: spastic, dyskinetic or ataxic (Rosenbaum et al., 2007). An individual may have more than one of these motor disorders. When this occurs, people are classified based on their dominant motor impairment. Spasticity is by far the most common, occurring in almost 90% of people with CP (Reid et al., 2011). Its clinical characteristics are discussed in the "Motor Function in Spastic CP" section below. People with spastic CP may have any of the imaging findings described above, though PVWMI is the most common lesion pattern (Reid et al., 2014b). Dyskinesia and ataxia each account for the dominant motor impairment in roughly 5% of people with CP (Reid

et al., 2011). Dyskinetic CP, which includes the subcategories of dystonia and choreoathetosis, is most frequently associated with deep gray matter injury in the basal ganglia or thalamus (Marret et al., 2013). In dyskinetic CP, fluctuating levels of involuntary movement and muscle tone produce either stiff (dystonic) or writhing and jerky (choreoathetoid) movements (Sewell et al., 2014). Ataxic CP, characterized by abnormal coordination of force and rhythm, is most frequently associated with either normal imaging or a cerebral malformation (Reid et al., 2014b).

Classification by Topography

The current recommendation is to describe the regions of the body that are impaired: whether impairments can be found unilaterally or bilaterally, which limbs are involved, and if there is any asymmetry (left vs right or upper vs lower extremity) (Rosenbaum et al., 2007). Like the type of motor impairment, similar topographic patterns often result from similar brain lesions. For instance, people with comparable involvement of all four limbs more frequently have lesions in grey matter structures than those with purely lower extremity impairments (Reid et al., 2014b). People with unilateral, upper extremity-dominated impairments have frequently experienced a stroke (Kirton and deVeber, 2006). However, these generalizations are far from absolute and it is important to note that people with unilateral impairments frequently have bilateral lesions in the brain (Tsao et al., 2014).

Classification by Functional Motor Abilities

The Gross Motor Functional Classification System (GMFCS) uses five levels (I-V) to categorize the severity of movement impairment, focusing on mobility and posture in an age-specific manner (Palisano et al., 1997; Palisano et al., 2008). GMFCS-I corresponds to someone who moves about the home and school similarly to their peers with adaptations only needed for more challenging tasks such as sports. This is the most common level of function with 44% of people with CP at GMFCS-I, whereas the proportion of people functioning at each of the other levels (GMFCS-II to GMFCS-IV) is roughly evenly distributed at 10-18% each (Shevell et al., 2009).

Independent mobility is also characteristic of GMFCS-II, but adaptations may be needed for common settings. For example, people at GMFCS-II may require railings to climb stairs beyond their preschool years. Unilateral motor impairments or lower extremity-dominated bilateral impairments are most common for GMFCS-I & II (Shevell et al., 2009). The highest

rates of normal imaging (17%) are found at the higher-functioning levels (GMFCS-I & II) and most people with CP secondary to a stroke function at GMFCS-I or II. However, the most common finding of people at higher-functioning level is PVWMI (22-50%) depending on the registry (Reid et al., 2014b; Towsley et al., 2011).

People at GMFCS-III require assistive devices for mobility in most settings. While the development of walking may be delayed, they are eventually capable of walking with a cane, crutches or a walker in most indoor environments. In contrast, wheeled mobility is the primary mode of transportation for people functioning at GMFCS-IV, and assistance may be necessary for truncal support even from a young age. People with CP who are unable to mobilize independently without extensive adaptations to a power wheelchair are classified at GMFCS-V. People at GMFCS-IV & V frequently have all four limbs involved (Shevell et al., 2009) and an underlying grey matter injury or brain malformation, though no abnormality is still found on MRI in 7-8% (Reid et al., 2014b). Similar scales have been created to classify fine motor control of the upper extremities (Manual Ability Classification System) (Eliasson et al., 2006) and communication (Communication Function Classification System) (Hidecker et al., 2011).

Accompanying disorders

While all people with CP have a disorder of movement and posture, the brain lesion causing CP also causes other disorders for many people. In addition to skeletal motor impairments, motor function of the bladder and gastrointestinal tract can also be impaired (Sewell et al., 2014). Other common accompanying disorders (and their prevalence in CP when known) that also likely result from the underlying brain injury include: cognitive impairment (49%), seizures (25%), functional blindness (11%) and severe hearing impairment or deafness (4%) (Novak et al., 2012). Reduced sensory perception (Aisen et al., 2011), and higher rates of hypogonadism and osteoporosis (Trinh et al., 2016), have also been found in people with CP though their prevalence has not been established.

Additional complications arise secondary to the primary neuromotor impairments. Abnormal postures and activity patterns throughout development result in alterations in bone structure and deformities such as scoliosis and hip subluxation (Chan and Miller, 2014). In addition to contributing to motor dysfunction these musculoskeletal disorders can contribute to pain. In fact, the majority (75%) of people with CP experience pain (Novak et al., 2012).

Difficulties with swallowing and control of oral secretions can contribute to poor nutrition and increased rates of chest infections and dental problems (Dougherty, 2009; Sewell et al., 2014). Motor, sensory, and cognitive impairments can all contribute to difficulties with expressive and receptive language (Novak et al., 2012). Similarly, while the cause is multifaceted, sleep disorders are >4x more common in children with CP than in neurologically intact children (Newman et al., 2006). Presence of these associated and secondary disorders frequently varies with the type of underlying motor impairment, and functional level according to the GMFCS (Novak et al., 2012; Sewell et al., 2014).

Motor Function in Spastic CP

Involuntary Muscle Activation and Weakness

Regulation of muscle activation including muscle selection, timing and degree of activation are abnormal in spastic CP (Berger et al., 1982; Crenna, 1998; Damiano et al., 2000; Fowler et al., 2009). Impaired selective motor control, i.e. the inability to isolate the activation of muscles in a selected pattern, contributes to difficulties with gait and other voluntary movements such as reaching and grasping in spastic CP (Cahill-Rowley and Rose, 2014; Ostensjo et al., 2004; Sanger et al., 2006; Sukal-Moulton et al., 2014). Deficits in sensorimotor integration, likely contribute to inappropriate regulation of muscle activation (Gordon and Duff, 1999). Involuntary co-contraction of antagonist muscles is a common finding and can contribute to difficulty in moving a joint voluntarily or passively (Elder et al., 2003; Milner-Brown and Penn, 1979; O'Sullivan et al., 1998; Stackhouse et al., 2005). However, problems with muscle selection extend beyond a single joint. Muscles crossing multiple joints are frequently activated inappropriately (Cahill-Rowley and Rose, 2014; Sukal-Moulton et al., 2014; Tedroff et al., 2006). Even mirror movements of a contralateral limb, which are common in young children, are stronger and persist to adulthood in some cases of unilateral and bilateral spastic CP (Koerte et al., 2011; Kuhtz-Buschbeck et al., 2000; Norton et al., 2008). When the appropriate muscle is voluntarily activated, increased time is needed to voluntarily activate or relax the muscle and the magnitude of activation is controlled less consistently (Downing et al., 2009).

In addition to the increased activation of antagonist muscles mentioned above, there is reduced activation of spinal motoneurons (Hussain et al., 2014; Reid et al., 2014a; Rose and

McGill, 2005). However weakness, particularly of distal muscles, is a multifaceted impairment (Ross and Engsberg, 2002; Wiley and Damiano, 1998). In addition to reduced voluntary activation of muscle, there are also differences in the muscle available to be activated. Muscles in people with spastic CP are smaller as measured by muscle length, thickness, cross-sectional area and volume (Barber et al., 2011; Barrett and Lichtwark, 2010; Herskind et al., 2015; Hussain et al., 2014). Not only are the muscles smaller, but they are generally comprised of less contractile muscle cells and more fat and extracellular matrix (Barrett and Lichtwark, 2010; Noble et al., 2014; Smith et al., 2012). Lastly, skeletal deformities contribute to weakness by changing the location or angle of tendon insertions relative to a joint's axis of rotation, a phenomenon known as lever-arm dysfunction (Theologis, 2013)

Evolution of Motor Function and Gait

The motor dysfunction produced by difficulties with involuntary muscle activation and weakness evolves with growth and development (Palisano et al., 2008). Babies with spastic CP typically display delays in gross motor development, persistence of primitive reflexes such as the asymmetric tonic neck reflex, and abnormalities in muscle tone such as increased resistance to passive movement (Hirtz, 2007). Absent or abnormal spontaneous movements may be noticed in the first weeks after birth (Campbell and Hedeker, 2001; Prechtl et al., 1997). As babies grow into childhood, asymmetrical movements and/or developing a strong hand preference prior to 12 months are common symptoms of unilateral CP (Wu et al., 2006) and crawling entirely with the arms while dragging the legs is common in children with leg-dominant bilateral CP (Yokochi et al., 1990). When walking does develop, toe-walking is common though not pathognomonic (Rose et al., 1999). Eventually 56% of people with CP walk independently and 16% of people with CP walk with assistive devices. (Novak et al., 2012).

Many attempts have been made to classify distinctive gait patterns in people with spastic CP based on joint movements or muscle activation patterns during the gait cycle (Dobson et al., 2007). Often people with unilateral spastic CP walk with excessive plantarflexion in the stance phase, where as in people with bilateral spastic CP, both thighs adduct (to the extreme of crossing or scissoring) (Wren et al., 2005). Weakness particularly, but not exclusively, in plantarflexion (Eek et al., 2011), increased passive muscle stiffness (Willerslev-Olsen et al.,

2014), limitations in joint range of motion, and skeletal deformities (Theologis, 2013) have all been shown to contribute to gait dysfunction in spastic CP.

Relevance

I hope this brief introduction conveys that people with spastic CP are an important group to study and provides you with the background relevant to studies in this thesis. In summary, CP is a common neurologic disorder characterized by lifelong motor dysfunction. It can result from multiple types of brain injuries and it can mildly affect a single limb or disturb motor function significantly throughout the entire body. Almost all people with CP have spasticity as their dominant motor impairment. The problems with involuntary muscle activation and weakness impact gait and other motor tasks. In short, it is worthwhile to review the mechanisms known to contribute to neuromotor impairments in spastic CP in the next chapter and to probe additional mechanisms in the body of this thesis.

References

Aisen ML, Kerkovich D, Mast J, Mulroy S, Wren TA, Kay RM, Rethlefsen SA. Cerebral palsy: Clinical care and neurological rehabilitation. Lancet neurology 2011;10:844-852.

Barber L, Hastings-Ison T, Baker R, Barrett R, Lichtwark G. Medial gastrocnemius muscle volume and fascicle length in children aged 2 to 5 years with cerebral palsy. Dev Med Child Neurol 2011;53:543-548.

Barrett RS, Lichtwark GA. Gross muscle morphology and structure in spastic cerebral palsy: A systematic review. Dev Med Child Neurol 2010;52:794-804.

Berger W, Quintern J, Dietz V. Pathophysiology of gait in children with cerebral palsy. Electroencephalogr Clin Neurophysiol 1982;53:538-548.

Blair E, Watson L, Badawi N, Stanley FJ. Life expectancy among people with cerebral palsy in western australia. Dev Med Child Neurol 2001;43:508-515.

Cahill-Rowley K, Rose J. Etiology of impaired selective motor control: Emerging evidence and its implications for research and treatment in cerebral palsy. Dev Med Child Neurol 2014;56:522-528.

Campbell SK, Hedeker D. Validity of the test of infant motor performance for discriminating among infants with varying risk for poor motor outcome. J Pediatr 2001;139:546-551.

Centers for Disease Control and Prevention [CDC]. Economic costs associated with mental retardation, cerebral palsy, hearing loss, and vision impairment--united states, 2003. MMWR Morb Mortal Wkly Rep 2004;53:57-59.

Chan G, Miller F. Assessment and treatment of children with cerebral palsy. Orthop Clin North Am 2014;45:313-325.

Crenna P. Spasticity and 'spastic' gait in children with cerebral palsy. Neurosci Biobehav Rev 1998;22:571-578.

Damiano DL, Martellotta TL, Sullivan DJ, Granata KP, Abel MF. Muscle force production and functional performance in spastic cerebral palsy: Relationship of cocontraction. Arch Phys Med Rehabil 2000;81:895-900.

Dobson F, Morris ME, Baker R, Graham HK. Gait classification in children with cerebral palsy: A systematic review. Gait Posture 2007;25:140-152.

Dougherty NJ. A review of cerebral palsy for the oral health professional. Dent Clin North Am 2009;53:329-338.

Downing AL, Ganley KJ, Fay DR, Abbas JJ. Temporal characteristics of lower extremity moment generation in children with cerebral palsy. Muscle Nerve 2009;39:800-809.

Eek MN, Tranberg R, Beckung E. Muscle strength and kinetic gait pattern in children with bilateral spastic CP. Gait Posture 2011;33:333-337.

Elder GC, Kirk J, Stewart G, Cook K, Weir D, Marshall A, Leahey L. Contributing factors to muscle weakness in children with cerebral palsy. Dev Med Child Neurol 2003;45:542-550.

Eliasson AC, Krumlinde-Sundholm L, Rosblad B, Beckung E, Arner M, Ohrvall AM, Rosenbaum P. The manual ability classification system (macs) for children with cerebral palsy: Scale development and evidence of validity and reliability. Dev Med Child Neurol 2006;48:549-554.

Fowler EG, Staudt LA, Greenberg MB, Oppenheim WL. Selective control assessment of the lower extremity (scale): Development, validation, and interrater reliability of a clinical tool for patients with cerebral palsy. Dev Med Child Neurol 2009;51:607-614.

Gordon AM, Duff SV. Relation between clinical measures and fine manipulative control in children with hemiplegic cerebral palsy. Dev Med Child Neurol 1999;41:586-591.

Herskind A, Ritterband-Rosenbaum A, Willerslev-Olsen M, Lorentzen J, Hanson L, Lichtwark G, Nielsen JB. Muscle growth is reduced in 15-month-old children with cerebral palsy. Dev Med Child Neurol 2015.

Hidecker MJ, Paneth N, Rosenbaum PL, Kent RD, Lillie J, Eulenberg JB, Chester K, Jr., Johnson B, Michalsen L, Evatt M, Taylor K. Developing and validating the communication function classification system for individuals with cerebral palsy. Dev Med Child Neurol 2011;53:704-710.

Hirtz D. Classification of cerebral palsy: Collaborative study perspective. Dev Med Child Neurol 2007;49:23-23.

Hoon AH, Jr. Neuroimaging in cerebral palsy: Patterns of brain dysgenesis and injury. J Child Neurol 2005;20:936-939.

Hussain AW, Onambele GL, Williams AG, Morse CI. Muscle size, activation and coactivation in adults with cerebral palsy. Muscle Nerve 2014;49:76-83.

Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. Arch Dis Child Fetal Neonatal Ed 2008;93:F153-161.

Kirton A, deVeber G. Cerebral palsy secondary to perinatal ischemic stroke. Clin Perinatol 2006;33:367-386.

Kirton A, Deveber G. Life after perinatal stroke. Stroke 2013;44:3265-3271.

Koerte I, Pelavin P, Kirmess B, Fuchs T, Berweck S, Laubender RP, Borggraefe I, Schroeder S, Danek A, Rummeny C, Reiser M, Kubicki M, Shenton ME, Ertl-Wagner B, Heinen F. Anisotropy of transcallosal motor fibres indicates functional impairment in children with periventricular leukomalacia. Dev Med Child Neurol 2011;53:179-186.

Krageloh-Mann I, Horber V. The role of magnetic resonance imaging in elucidating the pathogenesis of cerebral palsy: A systematic review. Dev Med Child Neurol 2007;49:144-151.

Kruse M, Michelsen SI, Flachs EM, Bronnum-Hansen H, Madsen M, Uldall P. Lifetime costs of cerebral palsy. Dev Med Child Neurol 2009;51:622-628.

Kuhtz-Buschbeck JP, Sundholm LK, Eliasson AC, Forssberg H. Quantitative assessment of mirror movements in children and adolescents with hemiplegic cerebral palsy. Dev Med Child Neurol 2000;42:728-736.

Marret S, Vanhulle C, Laquerriere A. Pathophysiology of cerebral palsy. Handb Clin Neurol 2013;111:169-176.

Milner-Brown HS, Penn RD. Pathophysiological mechanisms in cerebral palsy. J Neurol Neurosurg Psychiatry 1979;42:606-618.

Mockford M, Caulton JM. The pathophysiological basis of weakness in children with cerebral palsy. Pediatr Phys Ther 2010;22:222-233.

Nelson KB. Can we prevent cerebral palsy? N Engl J Med 2003;349:1765-1769.

Newman CJ, O'Regan M, Hensey O. Sleep disorders in children with cerebral palsy. Dev Med Child Neurol 2006;48:564-568.

Noble JJ, Charles-Edwards GD, Keevil SF, Lewis AP, Gough M, Shortland AP. Intramuscular fat in ambulant young adults with bilateral spastic cerebral palsy. BMC musculoskeletal disorders 2014;15:236.

Norton JA, Thompson AK, Chan KM, Wilman A, Stein RB. Persistent mirror movements for over sixty years: The underlying mechanisms in a cerebral palsy patient. Clin Neurophysiol 2008;119:80-87.

Novak I, Hines M, Goldsmith S, Barclay R. Clinical prognostic messages from a systematic review on cerebral palsy. Pediatrics 2012;130:e1285-1312.

O'Sullivan MC, Miller S, Ramesh V, Conway E, Gilfillan K, McDonough S, Eyre JA. Abnormal development of biceps brachii phasic stretch reflex and persistence of short latency heteronymous reflexes from biceps to triceps brachii in spastic cerebral palsy. Brain 1998;121 (Pt 12):2381-2395.

Oskoui M, Coutinho F, Dykeman J, Jette N, Pringsheim T. An update on the prevalence of cerebral palsy: A systematic review and meta-analysis. Dev Med Child Neurol 2013;55:509-519.

Ostensjo S, Carlberg EB, Vollestad NK. Motor impairments in young children with cerebral palsy: Relationship to gross motor function and everyday activities. Dev Med Child Neurol 2004;46:580-589.

Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Dev Med Child Neurol 1997;39:214-223.

Palisano RJ, Rosenbaum P, Bartlett D, Livingston MH. Content validity of the expanded and revised gross motor function classification system. Dev Med Child Neurol 2008;50:744-750.

Prechtl HF, Einspieler C, Cioni G, Bos AF, Ferrari F, Sontheimer D. An early marker for neurological deficits after perinatal brain lesions. Lancet 1997;349:1361-1363.

Reid SL, Pitcher CA, Williams SA, Licari MK, Valentine JP, Shipman PJ, Elliott CM. Does muscle size matter? The relationship between muscle size and strength in children with cerebral palsy. Disabil Rehabil 2014a:1-6.

Reid SM, Carlin JB, Reddihough DS. Distribution of motor types in cerebral palsy: How do registry data compare? Dev Med Child Neurol 2011;53:233-238.

Reid SM, Dagia CD, Ditchfield MR, Carlin JB, Reddihough DS. Population-based studies of brain imaging patterns in cerebral palsy. Dev Med Child Neurol 2014b;56:222-232.

Rose J, Martin JG, Torburn L, Rinsky LA, Gamble JG. Electromyographic differentiation of diplegic cerebral palsy from idiopathic toe walking: Involuntary coactivation of the quadriceps and gastrocnemius. J Pediatr Orthop 1999;19:677-682.

Rose J, McGill KC. Neuromuscular activation and motor-unit firing characteristics in cerebral palsy. Dev Med Child Neurol 2005;47:329-336.

Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M. A report: The definition and classification of cerebral palsy - april 2006. Dev Med Child Neurol 2007;49:8-14.

Ross SA, Engsberg JR. Relation between spasticity and strength in individuals with spastic diplegic cerebral palsy. Dev Med Child Neurol 2002;44:148-157.

Sanger TD, Chen D, Delgado MR, Gaebler-Spira D, Hallett M, Mink JW. Definition and classification of negative motor signs in childhood. Pediatrics 2006;118:2159-2167.

Sewell MD, Eastwood DM, Wimalasundera N. Managing common symptoms of cerebral palsy in children. BMJ 2014;349:g5474.

Shevell MI, Dagenais L, Hall N. The relationship of cerebral palsy subtype and functional motor impairment: A population-based study. Dev Med Child Neurol 2009;51:872-877.

Smith LR, Chambers HG, Subramaniam S, Lieber RL. Transcriptional abnormalities of hamstring muscle contractures in children with cerebral palsy. PLoS One 2012;7:e40686.

Stackhouse SK, Binder-Macleod SA, Lee SC. Voluntary muscle activation, contractile properties, and fatigability in children with and without cerebral palsy. Muscle Nerve 2005;31:594-601.

Strauss D, Brooks J, Rosenbloom L, Shavelle R. Life expectancy in cerebral palsy: An update. Dev Med Child Neurol 2008;50:487-493.

Sukal-Moulton T, Krosschell KJ, Gaebler-Spira DJ, Dewald JP. Motor impairments related to brain injury timing in early hemiparesis. Part ii: Abnormal upper extremity joint torque synergies. Neurorehabil Neural Repair 2014;28:24-35.

Tedroff K, Knutson LM, Soderberg GL. Synergistic muscle activation during maximum voluntary contractions in children with and without spastic cerebral palsy. Dev Med Child Neurol 2006;48:789-796.

Theologis T. Lever arm dysfunction in cerebral palsy gait. J Child Orthop 2013;7:379-382.

Towsley K, Shevell MI, Dagenais L. Population-based study of neuroimaging findings in children with cerebral palsy. Eur J Paediatr Neurol 2011;15:29-35.

Trinh A, Wong P, Fahey MC, Brown J, Churchyard A, Strauss BJ, Ebeling PR, Fuller PJ, Milat F. Musculoskeletal and endocrine health in adults with cerebral palsy: New opportunities for intervention. J Clin Endocrinol Metab 2016;101:1190-1197.

Tsao H, Pannek K, Fiori S, Boyd RN, Rose S. Reduced integrity of sensorimotor projections traversing the posterior limb of the internal capsule in children with congenital hemiparesis. Res Dev Disabil 2014;35:250-260.

Wiley ME, Damiano DL. Lower-extremity strength profiles in spastic cerebral palsy. Dev Med Child Neurol 1998;40:100-107.

Willerslev-Olsen M, Andersen JB, Sinkjaer T, Nielsen JB. Sensory feedback to ankle plantar flexors is not exaggerated during gait in spastic hemiplegic children with cerebral palsy. J Neurophysiol 2014;111:746-754.

Wren TA, Rethlefsen S, Kay RM. Prevalence of specific gait abnormalities in children with cerebral palsy: Influence of cerebral palsy subtype, age, and previous surgery. J Pediatr Orthop 2005;25:79-83.

Wu YW, Lindan CE, Henning LH, Yoshida CK, Fullerton HJ, Ferriero DM, Barkovich AJ, Croen LA. Neuroimaging abnormalities in infants with congenital hemiparesis. Pediatr Neurol 2006;35:191-196.

Yokochi K, Hosoe A, Shimabukuro S, Kodama K. Gross motor patterns in children with cerebral palsy and spastic diplegia. Pediatr Neurol 1990;6:245-250.

Chapter 2. Pathophysiology of Neuromotor Impairments in Spastic Cerebral Palsy

Introduction

As discussed in Chapter 1, spastic cerebral palsy (CP) results from an injury to the developing brain causing disorders of movement and posture. In children and adults with spastic CP, changes relevant to motor function are found throughout the nervous system including within the brain's cerebral cortex and subcortical structures, in ascending and descending pathways and within the spinal cord. While motor impairments are also influenced by changes within the musculoskeletal system, these have been well reviewed previously (Chan and Miller, 2014; Lieber and Smith, 2014).

This chapter focuses on neural mechanisms contributing to motor dysfunction in spastic CP, particularly with difficulties in regulating muscle activation. This manifests as weakness (Mockford and Caulton, 2010) and involuntary muscle activity, particularly following voluntary activity (Downing et al., 2009), in response to passive stretch (Poon and Hui-Chan, 2009), and within antagonistic muscles (Milner-Brown and Penn, 1979; Stackhouse et al., 2005). The characteristics of each pathological finding, its causal factors (where possible), and the implications for motor function are discussed. These neuromotor impairments can result from the original brain injury or from secondary changes throughout the nervous system.

Cortical Changes

The typical human motor cortex has a consistent *contralateral* somatotopic organization. Motor representation was originally demonstrated with invasive electrical stimulation but can now be studied non-invasively using transcranial magnetic stimulation (TMS) or functional magnetic resonance imaging (fMRI). When applied over the motor cortex, TMS results in a response recorded in a muscle represented by the cortical area that was stimulated (Di Lazzaro and Ziemann, 2013; Mills, 1991). The response, known as the motor evoked potential (MEP), is recorded using surface electromyography (EMG). fMRI identifies regions within the brain that are active during a task by recording changes in blood oxygenation (Ogawa et al., 1990).

Representation of the lower extremities is medial, along the cortex adjacent to the interhemispheric fissure, and the upper extremities are represented farther from the midline toward the Sylvian fissure (Penfield and Boldrey, 1937). While projections from the motor cortex are bilateral at birth, most ipsilateral pathways are withdrawn during development, leaving only 8-15% of axons within the corticospinal tract (CST) projecting to ipsilateral muscles (Eyre, 2007). The ability of TMS to activate bilateral monosynaptic responses is usually lost, potentially as a result of activity-dependent pruning of the ipsilateral pathway to distal muscles (Eyre et al., 2007; Martin et al., 2011). The maturation of pathway lateralization (contralateral vs ipsilateral) occurs before the development of somatotopic maps (Martin et al., 2011), and maturation of interneuronal circuits within the motor cortex continues into adolescence (Walther et al., 2009a). As discussed below, lesions to the developing brain causing CP can result in changes in lateralization of the cortical motor representation (i.e. somatotopic organization within a cortex) and the excitability of the motor cortex.

Persistence of Ipsilateral Motor Representations

Representation within the brain of motor activity in ipsilateral muscles has been demonstrated frequently in unilateral and bilateral CP using both TMS and fMRI (Carr, 1996; Carr et al., 1993; Eyre et al., 2007; Eyre et al., 2001; Farmer et al., 1991; Kesar et al., 2012; Maegaki et al., 1999; Nezu et al., 1999; Staudt et al., 2002; Vandermeeren et al., 2009; Vandermeeren et al., 2003b). The lower extremities, which are frequently more impaired than the upper extremities in bilateral CP, are more likely to have an ipsilateral cortical representation (Maegaki et al., 1999). Note, ipsilateral and contralateral motor representation is not mutually exclusive; ipsilateral representations can exist along with contralateral maps (i.e. motor representation is bilateral) or in isolation (i.e. motor representation is purely ipsilateral) (Carr et al., 1993; Maegaki et al., 1999).

While MEPs activated by TMS targeting the ipsilateral cortex can be elicited in neurologically intact (NI) adults, they are felt to have minimal functional significance and are unlikely to be conveyed by monosynaptic corticospinal projections (Eyre et al., 2001; Ziemann et al., 1999). When ipsilateral MEPs occur in NI adults, they often require strong voluntary activation of the ipsilateral muscle, higher intensities of stimulation and occur at longer latencies than contralateral MEPs (Ziemann et al., 1999). These properties suggest they involve pathways

other than the CST's fast-conducting monosynaptic pathway, potentially the cortico-reticulospinal tract (Fisher et al., 2012; Ziemann et al., 1999). Similarly, following adult stroke, ipsilateral MEPs in the affected limb occur at longer latencies suggestive of oligosynaptic projections (Eyre et al., 2001; Netz et al., 1997; Turton et al., 1996). In contrast, in people with CP at least some of the pathways originating in the ipsilateral motor representations are fast-conducting, monosynaptic pathways. Their ipsilateral MEPs occur at rest, with similar or lower stimulation thresholds, and at similar or slightly shorter latency than contralateral responses from the same cortex (Eyre et al., 2001; Kesar et al., 2012; Maegaki et al., 1999; Staudt et al., 2002; Staudt et al., 2004). These fast-conducting ipsilateral pathways may represent persistence of the neonatal ipsilateral pathway through competitive, activity-dependent plasticity (Eyre et al., 2007; Friel and Martin, 2007; Martin et al., 2011). Whether persistence of the ipsilateral representation and the pathway projecting from it is a punitive compensatory response or a form of adaptive motor recovery in more severe injury has been debated (Stoeckel and Binkofski, 2010). It occurs more frequently when there is greater damage to key areas such as the contralateral corticospinal pathways (Lotze et al., 2009; Staudt et al., 2002; van der Aa et al., 2013). However this does not definitively demonstrate if the ipsilateral pathway persists because the contralateral pathway is too badly damaged or if activity in the ipsilateral pathway beyond the neonatal period contributes to further degeneration of the contralateral pathway.

Regardless of the etiology, the presence of an ipsilateral motor representation (Holmstrom et al., 2010; Nezu et al., 1999) and the absence of a contralateral motor representation, are both associated with greater impairments in the hands (Carr et al., 1993; Eyre et al., 2007; Kesar et al., 2012; Staudt et al., 2002). Higher scores in the Gross Motor Function Measure, which reflect better function, largely in the lower extremities and trunk muscles, occur in people with a contralateral representation (Kesar et al., 2012). In fact, in the upper extremities it appears that as the motor representation shifts from purely contralateral to bilateral to purely ipsilateral, there is a corresponding decrease in motor function as observed in multiple functional outcome measures (Holmstrom et al., 2010; Mackey et al., 2014; van der Aa et al., 2013). Mirror movements represent another functional implication of the lateralization of motor representation. While ipsilateral representations do occur in people without mirror movements, people displaying mirror movements consistently have a purely ipsilateral representation (Carr, 1996; Carr et al., 1993; Eyre et al., 2001; Nezu et al., 1999; Staudt et al., 2004).

An interventional study provides a final line of evidence in support of the concept that the side of motor representation is functionally relevant. In participants with unilateral CP evaluating the neurophysiological response to constraint-induced movement therapy, neuroplastic adaptations differed based on the side of their motor representation. Improvements in movement quality and increased contralateral sensory activation as recorded with magnetoencephalography (MEG) were comparable in both groups, independent of the lateralization of the motor representation. However, the group with *contralateral* short-latency MEPs demonstrated increases in movement speed and in motor cortex excitability (as documented by TMS and fMRI) whereas the group with *ipsilateral* short-latency MEPs demonstrated decreases in movement speed and motor cortex excitability (Juenger et al., 2013; Kuhnke et al., 2008).

Shifting of the Motor Maps within the Contralateral Cortex

Within the cortex contralateral to an affected limb, the somatotopic organization is altered in spastic CP, at least in some individuals. Specifically, there can be a lateral shift away from the midline in the optimal site for TMS evoked responses in the tibialis anterior muscle in the leg (Kesar et al., 2012; Maegaki et al., 1999). In the upper extremities, which are often less impaired in bilateral CP but more impaired in unilateral CP, findings differ. One study of 17 participants with spastic CP failed to demonstrate a shift of the motor maps to the upper extremities (Maegaki et al., 1999). However, only one participant had unilateral motor impairments and no comment was made about the presence of any upper extremity motor dysfunction in the participants. In contrast, a study in which over half of the participants had unilateral motor impairments and 10/14 participants had impaired upper extremity motor function, the motor maps projecting to an upper extremity muscle were farther from the midline compared to historical normative data (Kesar et al., 2012). When lower extremity maps could be elicited (n=4), these laterally shifted maps were abnormally close to the upper extremity maps (Kesar et al., 2012). Finally, in addition to lateral shifts in motor maps found with TMS, small fMRI studies demonstrate increased activity in the more anterior premotor and/or supplementary motor cortices (Lee et al., 2013; Norton et al., 2008b). These findings all demonstrate that motor maps in people with spastic CP are frequently present in areas where they are not found in NI populations.

The impact of changes in motor maps on motor function are not well known. One study found a strong association (spearman's r=-0.841) between impairments in hand function (Melbourne Assessment) and the lateral displacement of hand motor maps in participants with bilateral CP (Kesar et al., 2012). It is possible that a shift results in greater overlap of the motor maps representing distinct muscle groups and contributes to involuntary co-activation of neighbouring muscle groups. Evidence from people with focal hand dystonia, a disorder characterized by involuntary muscle co-activation restricting the independent control of fingers during a skilled task, supports that theory. Specifically, there was a decreased distance between the somatosensory representation of fingers as recorded by MEG (Elbert et al., 1998).

Changes in Cortical Circuitry

In addition to changes in the location of the motor maps within the cortex, changes in the excitability of neurons projecting to the spinal cord or cortical-brainstem pathways and their interneuronal inputs may also exist in people with spastic CP. As reviewed by Hallett (2007), the lowest stimulation intensity required to produce an MEP is known as the motor threshold. At this threshold intensity, a single-pulse of TMS depolarizes the axons of interneurons with excitatory synapses on cortical projection neurons (when oriented to produce a posterior to anterior current with the brain as is commonly done). In spastic CP, motor thresholds are increased (Berweck et al., 2008; Eyre et al., 2007; Heinen et al., 1999; Koerte et al., 2011; Vry et al., 2008). The excitatory interneurons that synapse on projection neurons may be damaged, aligned in an orientation less conducive to activation by TMS, or their synaptic efficacy may be decreased. However, MEPs are not recorded from the interneurons or even the projection neurons, they are recorded at the muscle. Thus, altered function in other components of the pathway from cortex to muscle, such as damage to the descending motor tracts, may also contribute to these findings. In fact higher thresholds are also seen for muscles distal to incomplete spinal cord injuries (Davey et al., 1998). These possibilities will be discussed further in Chapters 3 & 5.

Interestingly, inhibitory circuitry within the motor cortex may also be less excitable in spastic CP. The transition of gamma-aminobutyric acid (GABA) from an excitatory to an inhibitory neurotransmitter shortly after birth is dependent on a co-transporter (KCC2) that has reduced expression in a rat model of CP leading to reduced cortical inhibition (Jantzie et al., 2015). In humans, the function of inhibitory circuits can be explored non-invasively using TMS.

Cortical inhibitory circuits contribute to a period of reduced muscle activation known as a silent period following an MEP in active muscles. However, interpretation of changes in the silent period is limited by the fact that spinal circuits also contribute (Chen et al., 1999). An alternative is to use a paired-pulse stimulation protocol known as short-interval intracortical inhibition (SICI) that specifically evaluates the impact of cortical inhibitory interneurons on MEPs (Hallett, 2007). These techniques have been used to compare inhibitory circuitry between CP and NI populations and have found reduced contralateral silent period durations in children with bilateral CP (Vry et al., 2008) and reduced SICI in children with purely unilateral CP (Berweck et al., 2008). People with spastic CP also have shorter ipsilateral silent periods (Heinen et al., 1999; Koerte et al., 2011). These are recorded as the suppression of ongoing muscle activity in muscles ipsilateral to the TMS and are attributed to transcallosal inhibition (Chen et al., 2003).

Alterations in the excitability of cortical circuits are functionally relevant in people with unilateral CP. (Studies in people with bilateral CP are lacking.) The absence of contralateral silent periods and less transcallosal inhibition are both associated with measures of upper extremity dysfunction (Mackey et al., 2014). This is similar to the finding in adults post-stroke, where higher motor function is associated with more normal activation of SICI (Edwards et al., 2013; Honaga et al., 2013). Lastly, coincident improvements in motor function and cortical excitability suggest a mechanism of motor recovery. Specifically, motor thresholds were reduced following constraint-induced movement therapy in children with unilateral CP (Walther et al., 2009b).

Changes to White Matter Pathways

Descending motor pathways projecting from the motor cortex directly to the spinal motoneurons aid in the control of voluntary movements including walking in humans (Matsuyama et al., 2004; Nielsen, 2003). These pathways can be direct, via the CST, or indirect through pathways such as those that synapse in the brainstem. Motor function is also impacted by sensory and transcallosal pathways. Traditionally, the integrity of brain pathways was assessed by evaluating their size. Newer non-invasive techniques, particularly the MRI techniques based on diffusion tensor imaging (DTI) as reviewed by Johansen-Berg and Rushworth (2009), enable the evaluation of anatomic integrity (i.e. microstructure) and can be based on markers of a tract's direction in addition to location. DTI can be processed for a two-

dimensional region-of-interest, or using tractography, it can be processed as a pathway such as the tract projecting from the motor cortex. For a region or tract, the DTI parameter of fractional anisotropy (FA) describes the degree to which diffusion occurs in random directions (FA = 0) or is constrained to a single direction (FA = 1). The amount of diffusion can be presented as a mean in all directions, mean diffusivity (MD), or parsed into its parallel and perpendicular components, parallel and perpendicular diffusivity respectively. Using these techniques, differences have been noted within the corticospinal pathway and other pathways in people with CP compared to NI peers. Tractography extending into the corticobulbar tracts is still largely experimental (Yang et al., 2011; Yeo et al., 2012), but the role of these corticobulbar and the bulbospinal pathways can be explored with neurophysiological techniques (Baker, 2011).

Damage to Corticospinal Pathways

As recently reviewed by Scheck et al. (2012), the preponderance of evidence suggests that the integrity of the corticospinal pathways is impaired in people with spastic CP. While the findings are not universal across all measures of diffusion and all studies, findings from DTI studies of reduced FA, increased MD and decreased tract volume are common. Results for MD are particularly variable. However, MD represents an average diffusion across all directions independent of the tract direction. Diffusion parallel to the tract may be normal or even reduced whereas studies that evaluate perpendicular diffusivity demonstrate increases suggestive of tract damage (Glenn et al., 2003; Glenn et al., 2007; Holmstrom et al., 2011). Additional evidence of damage in corticospinal pathways comes from the use of a recently developed technique, probabilistic tractography, which facilitates tracking a specific pathway through regions of crossing fibres including within the cortex (Johansen-Berg and Rushworth, 2009). Using this technique, two studies of corticospinal pathways in children with unilateral CP revealed impairments in diffusion parameters including FA and MD (Lennartsson et al., 2015; Tsao et al., 2014). While these studies frequently attribute their findings to the CST specifically, within the brain the corticobulbar pathways follow a similar course and thus may also be represented by these findings (Wakana et al., 2004).

When combined with reductions in FA, increased perpendicular diffusivity is suggestive of reduced myelination of the corticospinal pathways or axonal loss within them (Concha et al., 2006; Song et al., 2002). The damage to premyelinating oligodendrocytes that is typical of

periventricular white matter injury (Khwaja and Volpe, 2008) could reduce myelination and cause this pattern of diffusion abnormalities. Interestingly, a rabbit model of CP, specifically prenatal ischemia resulting in hypertonia, has demonstrated this same pattern of decreased FA and increased perpendicular diffusivity; however, this occurred prior to the onset of myelination (Drobyshevsky et al., 2007). This may be explained by recognizing that differences in other structural components such as neurofilaments, which support an axon's structure and shape, and the axon packing density can also impact diffusion (Beaulieu, 2002). In other words, abnormalities seen in DTI parameters are consistent with damage caused by the original brain injury and/or Wallerian degeneration beyond the original area of brain injury.

While the precise pathophysiology underlying damage to the corticospinal pathways is unclear, it is clear that integrity of these tracts is relevant to motor function. Many studies have evaluated the asymmetry of structures in unilateral CP and their relationship to motor function. Asymmetry in volume of the medulla oblongata and cerebral peduncle is associated with impairment scores for the upper and lower extremities (Duque et al., 2003; Staudt et al., 2000) and asymmetry of FA in the posterior limb of the internal capsule is associated with impaired hand function (Mackey et al., 2014). Also in unilateral CP, FA of the corticospinal pathway contralateral to the impaired limb is associated with hand function (Holmstrom et al., 2011; Mackey et al., 2014; Tsao et al., 2014). In bilateral CP, FA within both corticospinal pathways is associated with motor function as assessed by the Gross Motor Function Classification System (GMFCS), a five level scale that assesses the sitting and walking abilities of people with CP (Lee et al., 2011; Trivedi et al., 2010). In addition, increases in FA or reduced asymmetry of FA have been used to document spontaneous recovery in the impaired hemisphere of babies with unilateral CP (Baek et al., 2013) and treatment-induced improvements in children with unilateral (Kim et al., 2015; Kwon et al., 2014) or bilateral CP (Chaturvedi et al., 2013; Trivedi et al., 2008).

Damage to other Cortical Pathways

As reviewed by Scheck et al. (2012), impairments in DTI measures have also been found in ascending sensory tracts, in transcallosal pathways (commissural tracts) and in association tracts such as the superior longitudinal fasciculus. However, these are less consistent than the impairments measured in the corticospinal pathways. Nevertheless, the bulk of the evidence

suggests diffusion tractography of sensory pathways frequently demonstrates reductions in FA in people with spastic CP (Tsao et al., 2015; Tsao et al., 2014; Yoshida et al., 2011; Yoshida et al., 2010). In support of changes within somatosensory pathways, MEG responses are delayed and smaller in children with spastic CP compared to NI peers (Guo et al., 2012; Pihko et al., 2014) and electroencephalography patterns suggest deficits in somatosensory processing (Kurz et al., 2015).

DTI findings within the corpus callosum as a whole are conflicting (Scheck et al., 2012). However, a study that specifically parsed out the transcallosal motor tracts did show a profound reduction in FA and increase in mean diffusivity (Koerte et al., 2011). This is supported by a review of MRI's in a very large cohort of children with CP (n=272) that found thinning of the corpus callosum in 74% of their population. Further, the reductions in transcallosal inhibition (Heinen et al., 1999; Koerte et al., 2011) discussed above, may in fact be mediated by damage to the transcallosal pathway rather than reduced the activity in the cortical components of the circuit mediating transcallosal inhibition (Chen et al., 2003).

Changes in DTI measures may result directly from the primary lesion or through altered developmental processes. Reduced thalamic volumes in CP suggest the primary lesion contributes to somatosensory pathway damage (Scheck et al., 2014). On the other hand, reduced FA in transcallosal motor fibers in conjunction with reduced transcallosal inhibition (Koerte et al., 2011), a circuit that develops throughout childhood (Heinen et al., 1999), suggests an abnormal developmental process has occurred.

Associations with motor function have also been noted from these non-corticospinal pathways. In ascending sensory pathways, FA is associated with hand function (Tsao et al., 2014) and GMFCS (Trivedi et al., 2010; Yoshida et al., 2010). In the corpus callosum, FA in the posterior body is associated with GMFCS (Lee et al., 2011). Further, in children with bilateral spastic CP (but not in children with unilateral CP), volume of motor and sensory sub-sections of the corpus callosum was associated with lower extremity spasticity and gait abnormalities (Meyns et al., 2016).

Changes within Brainstem Pathways

The potential role of corticobulbospinal pathways in impaired selective motor control has long been theorized (Cahill-Rowley and Rose, 2014; Lawrence and Kuypers, 1965). Following CST lesions in monkeys, brainstem pathways contribute to functional motor recovery. However, subsequent lesions to brainstem pathways produced sustained deficits in motor control (Lawrence and Kuypers, 1965). Recently direct evidence has begun to emerge. The cortex does have direct corticobulbar projections (Fisher et al., 2012). Further, stimulation of the reticulospinal pathway within the brainstem results in bilateral muscle activity (Baker, 2011) that is increased following a CST lesion (Zaaimi et al., 2012). DTI evidence suggests the rubrospinal tract that is present in babies and almost absent in NI adults (Hicks and Onodera, 2012), also contributes to motor recovery. Compared with NI peers, FA in the rubrospinal tract's red nucleus was lower and correlated with upper-extremity function in adults post stroke (Ruber et al., 2012).

The evidence for increased dependence on oligo- or poly-synaptic brainstem pathways in spastic CP is indirect and largely based on demonstrating less involvement of monosynaptic pathways. As discussed above, ipsilateral MEPs in NI adults and adults post-stroke are likely produced by oligo- or polysynaptic projections suggesting involvement of cortico-reticulospinal pathways (though propriospinal pathways may also contribute) (Turton et al., 1996; Ziemann et al., 1999). In spastic CP while many ipsilateral MEPs occur at short-latencies, others have demonstrated MEPs at longer latencies similar to those seen in NI populations and in adults post-stroke (Carr et al., 1993; Cincotta et al., 2000; Maegaki et al., 1999; Vandermeeren et al., 2003a). Additional evidence for the role of polysynaptic pathways in spastic CP comes from a study that evaluated pairs of surface EMG signals from the same muscle in the time and frequency-domain (Petersen et al., 2013). Frequency bands normally attributed to common drive from cortical sources (without polysynaptic processing) had reduced coherence. In addition, smaller cumulant density peaks of longer duration suggest there are more variable delays in the descending drive, consistent with a polysynaptic pathway.

Changes within Spinal Circuits

While the lesion in CP is in the brain, secondary changes occur in the functionality of spinal circuits. These circuits are explored using peripheral nerve stimulation evoking a direct

motor response (M-wave), a monosynaptic reflex in motoneurons in response to Ia afferent stimulation (H-reflex), and/or changes in ongoing voluntary activity and resultant torque. Evidence from research in cats has clearly demonstrated that intact signalling in descending pathways is necessary for the appropriate distribution of spinal interneurons and thus, the development of spinal circuits (Chakrabarty et al., 2009). Changes within spinal circuits in people with spastic CP may result from abnormal interneuron development as in the cats. However, spasticity also occurs following central nervous system damage in adults. In these cases, where the normal developmental processes would have occurred prior to injury, chronic changes in descending pathways lead to neuroplastic changes in the mature spinal cord. Thirdly, changes in the function of spinal circuits may result from reduced tonic signaling from descending pathways rather than changes within the spinal circuits themselves. Changes within spinal circuits are discussed relative to their end effect: decreased inhibition or increased excitation.

Decreased Inhibitory Circuitry

Decreased inhibition of motoneurons could contribute to the excessive involuntary muscle activity characteristic of spasticity. Previous activation of motoneurons through voluntary contractions or repeated activation of H-reflexes results in long-lasting (>10 seconds) post-activation depression, also known as homosynaptic depression (Hultborn et al., 1996). It appears to be universally impaired in all central nervous system lesions causing spasticity including CP (Achache et al., 2010; Aymard et al., 2000; Lamy et al., 2009; Pierrot-Deseilligny and Burke, 2012). Presynaptic inhibition of a synapse, such as the Ia-afferent to motoneuron synapse, is also reduced in adults with spastic CP (Achache et al., 2010). Interestingly, reduced presynaptic inhibition occurs following spinal cord injury, but is not consistently observed in other populations of people with spasticity following cerebral damage (Aymard et al., 2000; Faist et al., 1994; Lamy et al., 2009).

Changes in reciprocal inhibition, which involves inhibition of an antagonist muscle by activation of the agonist through disynaptic short-latency and polysynaptic long-latency mechanisms (Crone, 1993), are also inconsistent. Within groups of people with CP at rest both mechanisms appear to be intact (Achache et al., 2010; Leonard et al., 2006), however this is not the case during voluntary muscle activation. During activity, measures of reciprocal inhibition

were either increased in the tibialis anterior (Berbrayer and Ashby, 1990; Brouwer and Smits, 1996) or decreased in the soleus (Leonard et al., 2006). Reduced disynaptic reciprocal inhibition in spastic plantarflexors has also been demonstrated at rest in people with a spinal cord injury or stroke (Crone et al., 2003) and in people with multiple sclerosis (Crone et al., 1994). Lastly EMG recordings of polysynaptic cutaneomuscular reflexes comparing children and adolescents with spastic CP to NI peers suggest spinally-mediated inhibition occurs less frequently in the legs (Gibbs et al., 1999) and has a reduced magnitude in the hands (Evans et al., 1991). Interestingly, a complete absence of inhibition was noted when evaluating motor unit recordings of cutaneomuscular reflexes in adults with spasms secondary to spinal cord injury (Norton et al., 2008a). This will be discussed further in Chapter 4.

Loss of inhibition is in fact associated with measures of spasticity, though not universally. Impairments in post-activation depression are associated with measures of spasticity. Specifically it's associated with both the degree of resistance to passive stretch, as measured by the Ashworth scale, and the duration of clonus in adults with spastic CP (Achache et al., 2010). The normal modulation of post-activation depression during voluntary motor tasks was also lost (Leonard and Moritani, 1992). However, post-activation depression is not associated with motor function as assessed by the GMFCS, nor are there any associations with spasticity or voluntary movement found with presynaptic inhibition (Achache et al., 2010). These findings may not be definitive. Associations may be missed due to difficulties in identifying and clinically assessing the relevant aspect of spasticity such as increased resistance to passive stretch, or clonus. Interestingly H-reflex modulation during gait, a normal phenomenon attributed to presynaptic inhibition and/or reciprocal inhibition (Yang et al., 1991), is generally reduced in children with spastic CP but improved by gait training (Hodapp et al., 2009).

Increased Excitatory Circuitry

There are two known spinal circuits that have increased excitation in people with spastic CP. Peroneal nerve stimulation produces an early and a late facilitation of the quadriceps H-reflex through the activation of propriospinal-like interneurons by group I and group II afferents (Marque et al., 1996). While the earlier facilitation activated by group I afferents is similar in adults with spastic CP compared to NI peers, the later facilitation activated by group II afferents is enhanced in spastic CP (Achache et al., 2010). Interestingly both phases are enhanced post-

stroke (Marque et al., 2001). Similarly, enhancement of the early excitatory component of cutaneomuscular reflexes that is spinally-mediated can occur post-stroke (Nadler et al., 2004; Rowlandson and Stephens, 1985) and chronically post-incomplete spinal cord injury (Jones and Yang, 1994), but findings particularly in CP are inconsistent. Early excitatory responses recorded in the hand of children with CP were reduced, normal or enhanced (Evans et al., 1991). In the lower extremity, early excitatory responses were recorded more frequently in children and teens with spastic CP than in their NI peers (Gibbs et al., 1999). The implications of increased excitation are unclear. While theoretically it can contribute to movement impairments through excessive involuntary activity, no associations have been found with measures of spasticity or motor function (Achache et al., 2010; Evans et al., 1991; Gibbs et al., 1999).

Decreased Activation of Motor Units

As discussed in Chapter 1, indirect evidence suggests that decreased activation of spinal motoneurons contributes to weakness in spastic CP. The relationship between strength and muscle volume is weaker in people with spastic CP than in NI peers suggesting the muscle tissue in CP is either less efficient at producing force or is incompletely activated (Reid et al., 2014). When attempting a maximal voluntary activation, people with spastic CP activate a smaller proportion of the muscle as estimated by a ratio of the voluntary activation (measured by EMG) to maximal muscle response evoked by nerve stimulation (M_{max}) (Rose and McGill, 2005). Also at maximal effort, electrical stimulation of the peripheral nerve is able to evoke more additional torque in people with spastic CP (Hussain et al., 2014). These lines of evidence suggest that at maximal effort, a greater proportion of motor units are not activated compared to NI populations. To compare the performance of submaximal contractions, the ratio of EMG/ M_{max} can be used to approximate neuronal drive to the muscle. When neuronal drive to the muscles is matched in CP and NI groups, the degree of motor unit recruitment and the firing rates are similar. In other words, the relationship between recruitment and firing rate modulation is intact. However, at matched levels of activation relative to each individual's maximal effort (e.g. 20% of maximal EMG), motor unit recruitment and firing rates are lower, suggesting decreased descending and/or peripheral activation of motoneurons also exists during submaximal contractions (Rose and McGill, 2005).

Unfortunately none of these studies assessed motor function directly. However, other studies have looked for associations between weakness and motor dysfunction using maximal torque as an indicator of strength. Difficulty with gross motor function, as measured by the Gross Motor Function Measure, are indeed associated with weakness. Similarly, there are associations between weakness and reductions in gait speed, and measures of joint angles and forces during gait (Eek et al., 2011; Ross and Engsberg, 2007). From these finding it seems plausible to suggest that decreased activation of motor units contributes to impaired motor function in people with spastic CP.

Summary

Alterations in the physiology of CP are noted throughout the neuromotor system. These changes can occur in areas beyond the regions impacted by the brain injury causing spastic CP. In general there is damage to the contralateral corticospinal projections to motoneurons. In some people with spastic CP, functional contralateral motor pathways are absent. When present, their maps are frequently altered, they require higher threshold of stimulation for activation and have reduced structural integrity with the end result being less voluntary activation of spinal motoneurons and their innervated muscles.

Perhaps as a compensatory mechanism, there appears to be an increased dependence on alternative motor systems such as ipsilateral projections, brainstem pathways and a shift toward more excitable spinal circuitry. Many of these changes are associated with motor dysfunction in people with spastic CP. Investigations of how these individual changes are integrated together to produce the complete picture of weakness and involuntary muscle activity are ongoing. This thesis contributes three studies to this growing pool of knowledge.

Understanding the role of each of these pathophysiological mechanisms may help us design new treatments and optimize the treatment program for an individual by providing clinicians more mechanistic information than can be determined by clinical assessments alone. Understanding where and how the neuromotor system is different in people with spastic CP provides targets for evaluating responses to interventions both in animal models and in people. Physiologic changes may be recordable prior to functional gains and help predict if further

benefit may be achieved by a lengthy therapy program. Already, treatment studies are starting to monitor physiological responses and have been shown to differentiate mechanisms of recovery.

References

Achache V, Roche N, Lamy JC, Boakye M, Lackmy A, Gastal A, Quentin V, Katz R. Transmission within several spinal pathways in adults with cerebral palsy. Brain 2010;133:1470-1483.

Aymard C, Katz R, Lafitte C, Lo E, Penicaud A, Pradat-Diehl P, Raoul S. Presynaptic inhibition and homosynaptic depression: A comparison between lower and upper limbs in normal human subjects and patients with hemiplegia. Brain 2000;123 (Pt 8):1688-1702.

Baek SO, Jang SH, Lee E, Kim S, Hah JO, Park YH, Lee JM, Son SM. Cst recovery in pediatric hemiplegic patients: Diffusion tensor tractography study. Neurosci Lett 2013;557 Pt B:79-83.

Baker SN. The primate reticulospinal tract, hand function and functional recovery. J Physiol 2011;589:5603-5612.

Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 2002;15:435-455.

Berbrayer D, Ashby P. Reciprocal inhibition in cerebral palsy. Neurology 1990;40:653-656.

Berweck S, Walther M, Brodbeck V, Wagner N, Koerte I, Henschel V, Juenger H, Staudt M, Mall V. Abnormal motor cortex excitability in congenital stroke. Pediatr Res 2008;63:84-88.

Brouwer B, Smits E. Corticospinal input onto motor neurons projecting to ankle muscles in individuals with cerebral palsy. Dev Med Child Neurol 1996;38:787-796.

Cahill-Rowley K, Rose J. Etiology of impaired selective motor control: Emerging evidence and its implications for research and treatment in cerebral palsy. Dev Med Child Neurol 2014;56:522-528.

Carr LJ. Development and reorganization of descending motor pathways in children with hemiplegic cerebral palsy. Acta Paediatr Suppl 1996;416:53-57.

Carr LJ, Harrison LM, Evans AL, Stephens JA. Patterns of central motor reorganization in hemiplegic cerebral palsy. Brain 1993;116 (Pt 5):1223-1247.

Chakrabarty S, Shulman B, Martin JH. Activity-dependent codevelopment of the corticospinal system and target interneurons in the cervical spinal cord. J Neurosci 2009;29:8816-8827.

Chan G, Miller F. Assessment and treatment of children with cerebral palsy. Orthop Clin North Am 2014;45:313-325.

Chaturvedi SK, Rai Y, Chourasia A, Goel P, Paliwal VK, Garg RK, Rathore RK, Pandey CM, Gupta RK. Comparative assessment of therapeutic response to physiotherapy with or without

botulinum toxin injection using diffusion tensor tractography and clinical scores in term diplegic cerebral palsy children. Brain Dev 2013;35:647-653.

Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Exp Brain Res 1999;128:539-542.

Chen R, Yung D, Li JY. Organization of ipsilateral excitatory and inhibitory pathways in the human motor cortex. J Neurophysiol 2003;89:1256-1264.

Cincotta M, Borgheresi A, Liotta P, Montigiani A, Marin E, Zaccara G, Ziemann U. Reorganization of the motor cortex in a patient with congenital hemiparesis and mirror movements. Neurology 2000;55:129-131.

Concha L, Gross DW, Wheatley BM, Beaulieu C. Diffusion tensor imaging of time-dependent axonal and myelin degradation after corpus callosotomy in epilepsy patients. Neuroimage 2006;32:1090-1099.

Crone C. Reciprocal inhibition in man. Dan Med Bull 1993;40:571-581.

Crone C, Johnsen LL, Biering-Sorensen F, Nielsen JB. Appearance of reciprocal facilitation of ankle extensors from ankle flexors in patients with stroke or spinal cord injury. Brain 2003;126:495-507.

Crone C, Nielsen J, Petersen N, Ballegaard M, Hultborn H. Disynaptic reciprocal inhibition of ankle extensors in spastic patients. Brain 1994;117 (Pt 5):1161-1168.

Davey NJ, Smith HC, Wells E, Maskill DW, Savic G, Ellaway PH, Frankel HL. Responses of thenar muscles to transcranial magnetic stimulation of the motor cortex in patients with incomplete spinal cord injury. J Neurol Neurosurg Psychiatry 1998;65:80-87.

Di Lazzaro V, Ziemann U. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. Front Neural Circuits 2013;7:18.

Downing AL, Ganley KJ, Fay DR, Abbas JJ. Temporal characteristics of lower extremity moment generation in children with cerebral palsy. Muscle Nerve 2009;39:800-809.

Drobyshevsky A, Derrick M, Wyrwicz AM, Ji X, Englof I, Ullman LM, Zelaya ME, Northington FJ, Tan S. White matter injury correlates with hypertonia in an animal model of cerebral palsy. J Cereb Blood Flow Metab 2007;27:270-281.

Duque J, Thonnard JL, Vandermeeren Y, Sebire G, Cosnard G, Olivier E. Correlation between impaired dexterity and corticospinal tract dysgenesis in congenital hemiplegia. Brain 2003;126:732-747.

Edwards JD, Meehan SK, Linsdell MA, Borich MR, Anbarani K, Jones PW, Ferris J, Boyd LA. Changes in thresholds for intracortical excitability in chronic stroke: More than just altered intracortical inhibition. Restorative neurology and neuroscience 2013;31:693-705.

Eek MN, Tranberg R, Beckung E. Muscle strength and kinetic gait pattern in children with bilateral spastic CP. Gait Posture 2011;33:333-337.

Elbert T, Candia V, Altenmuller E, Rau H, Sterr A, Rockstroh B, Pantev C, Taub E. Alteration of digital representations in somatosensory cortex in focal hand dystonia. Neuroreport 1998;9:3571-3575.

Evans AL, Harrison LM, Stephens JA. Cutaneomuscular reflexes recorded from the first dorsal interosseous muscle of children with cerebral palsy. Dev Med Child Neurol 1991;33:541-551.

Eyre JA. Corticospinal tract development and its plasticity after perinatal injury. Neurosci Biobehav Rev 2007;31:1136-1149.

Eyre JA, Smith M, Dabydeen L, Clowry GJ, Petacchi E, Battini R, Guzzetta A, Cioni G. Is hemiplegic cerebral palsy equivalent to amblyopia of the corticospinal system? Ann Neurol 2007;62:493-503.

Eyre JA, Taylor JP, Villagra F, Smith M, Miller S. Evidence of activity-dependent withdrawal of corticospinal projections during human development. Neurology 2001;57:1543-1554.

Faist M, Mazevet D, Dietz V, Pierrot-Deseilligny E. A quantitative assessment of presynaptic inhibition of ia afferents in spastics. Differences in hemiplegics and paraplegics. Brain : a journal of neurology 1994;117 (Pt 6):1449-1455.

Farmer SF, Harrison LM, Ingram DA, Stephens JA. Plasticity of central motor pathways in children with hemiplegic cerebral palsy. Neurology 1991;41:1505-1510.

Fisher KM, Zaaimi B, Baker SN. Reticular formation responses to magnetic brain stimulation of primary motor cortex. J Physiol 2012;590:4045-4060.

Friel KM, Martin JH. Bilateral activity-dependent interactions in the developing corticospinal system. J Neurosci 2007;27:11083-11090.

Gibbs J, Harrison LM, Stephens JA, Evans AL. Cutaneomuscular reflex responses recorded from the lower limb in children and adolescents with cerebral palsy. Dev Med Child Neurol 1999;41:456-464.

Glenn OA, Henry RG, Berman JI, Chang PC, Miller SP, Vigneron DB, Barkovich AJ. DTI-based three-dimensional tractography detects differences in the pyramidal tracts of infants and children with congenital hemiparesis. J Magn Reson Imaging 2003;18:641-648.

Glenn OA, Ludeman NA, Berman JI, Wu YW, Lu Y, Bartha AI, Vigneron DB, Chung SW, Ferriero DM, Barkovich AJ, Henry RG. Diffusion tensor MR imaging tractography of the pyramidal tracts correlates with clinical motor function in children with congenital hemiparesis. AJNR Am J Neuroradiol 2007;28:1796-1802.

Guo X, Xiang J, Mun-Bryce S, Bryce M, Huang S, Huo X, Wang Y, Rose D, Degrauw T, Gartner K, Song T, Schmit J, Vargus-Adams J. Aberrant high-gamma oscillations in the somatosensory cortex of children with cerebral palsy: A meg study. Brain Dev 2012;34:576-583.

Hallett M. Transcranial magnetic stimulation: A primer. Neuron 2007;55:187-199.

Heinen F, Kirschner J, Fietzek U, Glocker FX, Mall V, Korinthenberg R. Absence of transcallosal inhibition in adolescents with diplegic cerebral palsy. Muscle Nerve 1999;22:255-257.

Hicks TP, Onodera S. The mammalian red nucleus and its role in motor systems, including the emergence of bipedalism and language. Prog Neurobiol 2012;96:165-175.

Hodapp M, Vry J, Mall V, Faist M. Changes in soleus h-reflex modulation after treadmill training in children with cerebral palsy. Brain 2009;132:37-44.

Holmstrom L, Lennartsson F, Eliasson AC, Flodmark O, Clark C, Tedroff K, Forssberg H, Vollmer B. Diffusion MRI in corticofugal fibers correlates with hand function in unilateral cerebral palsy. Neurology 2011;77:775-783.

Holmstrom L, Vollmer B, Tedroff K, Islam M, Persson JK, Kits A, Forssberg H, Eliasson AC. Hand function in relation to brain lesions and corticomotor-projection pattern in children with unilateral cerebral palsy. Dev Med Child Neurol 2010;52:145-152.

Honaga K, Fujiwara T, Tsuji T, Hase K, Ushiba J, Liu M. State of intracortical inhibitory interneuron activity in patients with chronic stroke. Clin Neurophysiol 2013;124:364-370.

Hultborn H, Illert M, Nielsen J, Paul A, Ballegaard M, Wiese H. On the mechanism of the post-activation depression of the h-reflex in human subjects. Exp Brain Res 1996;108:450-462.

Hussain AW, Onambele GL, Williams AG, Morse CI. Muscle size, activation and coactivation in adults with cerebral palsy. Muscle Nerve 2014;49:76-83.

Jantzie LL, Corbett CJ, Firl DJ, Robinson S. Postnatal erythropoietin mitigates impaired cerebral cortical development following subplate loss from prenatal hypoxia-ischemia. Cereb Cortex 2015;25:2683-2695.

Johansen-Berg H, Rushworth MF. Using diffusion imaging to study human connectional anatomy. Annu Rev Neurosci 2009;32:75-94.

Jones CA, Yang JF. Reflex behavior during walking in incomplete spinal-cord-injured subjects. Exp Neurol 1994;128:239-248.

Juenger H, Kuhnke N, Braun C, Ummenhofer F, Wilke M, Walther M, Koerte I, Delvendahl I, Jung NH, Berweck S, Staudt M, Mall V. Two types of exercise-induced neuroplasticity in congenital hemiparesis: A transcranial magnetic stimulation, functional MRI, and magnetoencephalography study. Dev Med Child Neurol 2013;55:941-951.

Kesar TM, Sawaki L, Burdette JH, Cabrera MN, Kolaski K, Smith BP, O'Shea TM, Koman LA, Wittenberg GF. Motor cortical functional geometry in cerebral palsy and its relationship to disability. Clin Neurophysiol 2012;123:1383-1390.

Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. Arch Dis Child Fetal Neonatal Ed 2008;93:F153-161.

Kim JH, Kwon YM, Son SM. Motor function outcomes of pediatric patients with hemiplegic cerebral palsy after rehabilitation treatment: A diffusion tensor imaging study. Neural Regen Res 2015;10:624-630.

Koerte I, Pelavin P, Kirmess B, Fuchs T, Berweck S, Laubender RP, Borggraefe I, Schroeder S, Danek A, Rummeny C, Reiser M, Kubicki M, Shenton ME, Ertl-Wagner B, Heinen F. Anisotropy of transcallosal motor fibres indicates functional impairment in children with periventricular leukomalacia. Dev Med Child Neurol 2011;53:179-186.

Kuhnke N, Juenger H, Walther M, Berweck S, Mall V, Staudt M. Do patients with congenital hemiparesis and ipsilateral corticospinal projections respond differently to constraint-induced movement therapy? Dev Med Child Neurol 2008;50:898-903.

Kurz MJ, Becker KM, Heinrichs-Graham E, Wilson TW. Children with cerebral palsy have uncharacteristic somatosensory cortical oscillations after stimulation of the hand mechanoreceptors. Neuroscience 2015;305:67-75.

Kwon JY, Chang WH, Chang HJ, Yi SH, Kim MY, Kim EH, Kim YH. Changes in diffusion tensor tractographic findings associated with constraint-induced movement therapy in young children with cerebral palsy. Clin Neurophysiol 2014;125:2397-2403.

Lamy JC, Wargon I, Mazevet D, Ghanim Z, Pradat-Diehl P, Katz R. Impaired efficacy of spinal presynaptic mechanisms in spastic stroke patients. Brain 2009;132:734-748.

Lawrence DG, Kuypers HG. Pyramidal and non-pyramidal pathways in monkeys: Anatomical and functional correlation. Science 1965;148:973-975.

Lee JD, Park HJ, Park ES, Oh MK, Park B, Rha DW, Cho SR, Kim EY, Park JY, Kim CH, Kim DG, Park CI. Motor pathway injury in patients with periventricular leucomalacia and spastic diplegia. Brain 2011;134:1199-1210.

Lee JJ, Lee DR, Shin YK, Lee NG, Han BS, You SJ. Comparative neuroimaging in children with cerebral palsy using fMRI and a novel eeg-based brain mapping during a motor task--a preliminary investigation. NeuroRehabilitation 2013;32:279-285.

Lennartsson F, Holmstrom L, Eliasson AC, Flodmark O, Forssberg H, Tournier JD, Vollmer B. Advanced fiber tracking in early acquired brain injury causing cerebral palsy. AJNR Am J Neuroradiol 2015;36:181-187.

Leonard CT, Moritani T. H-reflex testing to determine the neural basis of movement disorders of neurologically impaired individuals. Electromyogr Clin Neurophysiol 1992;32:341-349.

Leonard CT, Sandholdt DY, McMillan JA, Queen S. Short- and long-latency contributions to reciprocal inhibition during various levels of muscle contraction of individuals with cerebral palsy. J Child Neurol 2006;21:240-246.

Lieber RL, Smith LR. Skeletal muscle changes due to cerebral palsy. In: Shepherd RB, editor. Cerebral palsy in infancy: Targeted activity to optimize early growth and development. Edinburgh; New York: Churchill Livingstone/Elsevier; 2014. p. 135-155.

Lotze M, Sauseng P, Staudt M. Functional relevance of ipsilateral motor activation in congenital hemiparesis as tested by fMRI-navigated TMS. Exp Neurol 2009;217:440-443.

Mackey A, Stinear C, Stott S, Byblow WD. Upper limb function and cortical organization in youth with unilateral cerebral palsy. Front Neurol 2014;5:117.

Maegaki Y, Maeoka Y, Ishii S, Eda I, Ohtagaki A, Kitahara T, Suzuki N, Yoshino K, Ieshima A, Koeda T, Takeshita K. Central motor reorganization in cerebral palsy patients with bilateral cerebral lesions. Pediatr Res 1999;45:559-567.

Marque P, Pierrot-Deseilligny E, Simonetta-Moreau M. Evidence for excitation of the human lower limb motoneurones by group ii muscle afferents. Exp Brain Res 1996;109:357-360.

Marque P, Simonetta-Moreau M, Maupas E, Roques CF. Facilitation of transmission in heteronymous group ii pathways in spastic hemiplegic patients. J Neurol Neurosurg Psychiatry 2001;70:36-42.

Martin JH, Chakrabarty S, Friel KM. Harnessing activity-dependent plasticity to repair the damaged corticospinal tract in an animal model of cerebral palsy. Dev Med Child Neurol 2011;53 Suppl 4:9-13.

Matsuyama K, Mori F, Nakajima K, Drew T, Aoki M, Mori S. Locomotor role of the corticoreticular-reticulospinal-spinal interneuronal system. Prog Brain Res 2004;143:239-249.

Meyns P, Van Gestel L, Leunissen I, De Cock P, Sunaert S, Feys H, Duysens J, Desloovere K, Ortibus E. Macrostructural and microstructural brain lesions relate to gait pathology in children with cerebral palsy. Neurorehabil Neural Repair 2016.

Mills KR. Magnetic brain stimulation: A tool to explore the action of the motor cortex on single human spinal motoneurones. Trends Neurosci 1991;14:401-405.

Milner-Brown HS, Penn RD. Pathophysiological mechanisms in cerebral palsy. J Neurol Neurosurg Psychiatry 1979;42:606-618.

Mockford M, Caulton JM. The pathophysiological basis of weakness in children with cerebral palsy. Pediatr Phys Ther 2010;22:222-233.

Nadler MA, Harrison LM, Stephens JA. Cutaneomuscular reflexes following stroke: A 2-year longitudinal study. J Neurol Sci 2004;217:195-203.

Netz J, Lammers T, Homberg V. Reorganization of motor output in the non-affected hemisphere after stroke. Brain 1997;120 (Pt 9):1579-1586.

Nezu A, Kimura S, Takeshita S, Tanaka M. Functional recovery in hemiplegic cerebral palsy: Ipsilateral electromyographic responses to focal transcranial magnetic stimulation. Brain Dev 1999;21:162-165.

Nielsen JB. How we walk: Central control of muscle activity during human walking. Neuroscientist 2003;9:195-204.

Norton JA, Bennett DJ, Knash ME, Murray KC, Gorassini MA. Changes in sensory-evoked synaptic activation of motoneurons after spinal cord injury in man. Brain 2008a;131:1478-1491.

Norton JA, Thompson AK, Chan KM, Wilman A, Stein RB. Persistent mirror movements for over sixty years: The underlying mechanisms in a cerebral palsy patient. Clin Neurophysiol 2008b;119:80-87.

Ogawa S, Lee TM, Kay AR, Tank DW. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci U S A 1990;87:9868-9872.

Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 1937;60:389-443.

Petersen TH, Farmer SF, Kliim-Due M, Nielsen JB. Failure of normal development of central drive to ankle dorsiflexors relates to gait deficits in children with cerebral palsy. J Neurophysiol 2013;109:625-639.

Pierrot-Deseilligny E, Burke DJ. Contribution of spinal pathways to the pathophysiology of movement disorders. The circuitry of the human spinal cord: Neuroplasticity and corticospinal mechanisms. Cambridge: Cambridge University Press; 2012. p. 565-579.

Pihko E, Nevalainen P, Vaalto S, Laaksonen K, Maenpaa H, Valanne L, Lauronen L. Reactivity of sensorimotor oscillations is altered in children with hemiplegic cerebral palsy: A magnetoencephalographic study. Hum Brain Mapp 2014;35:4105-4117.

Poon DM, Hui-Chan CW. Hyperactive stretch reflexes, co-contraction, and muscle weakness in children with cerebral palsy. Dev Med Child Neurol 2009;51:128-135.

Reid SL, Pitcher CA, Williams SA, Licari MK, Valentine JP, Shipman PJ, Elliott CM. Does muscle size matter? The relationship between muscle size and strength in children with cerebral palsy. Disabil Rehabil 2014:1-6.

Rose J, McGill KC. Neuromuscular activation and motor-unit firing characteristics in cerebral palsy. Dev Med Child Neurol 2005;47:329-336.

Ross SA, Engsberg JR. Relationships between spasticity, strength, gait, and the gmfm-66 in persons with spastic diplegia cerebral palsy. Arch Phys Med Rehabil 2007;88:1114-1120.

Rowlandson PH, Stephens JA. Cutaneous reflex responses recorded in children with various neurological disorders. Dev Med Child Neurol 1985;27:434-447.

Ruber T, Schlaug G, Lindenberg R. Compensatory role of the cortico-rubro-spinal tract in motor recovery after stroke. Neurology 2012;79:515-522.

Scheck SM, Boyd RN, Rose SE. New insights into the pathology of white matter tracts in cerebral palsy from diffusion magnetic resonance imaging: A systematic review. Dev Med Child Neurol 2012;54:684-696.

Scheck SM, Pannek K, Fiori S, Boyd RN, Rose SE. Quantitative comparison of cortical and deep grey matter in pathological subtypes of unilateral cerebral palsy. Dev Med Child Neurol 2014;56:968-975.

Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 2002;17:1429-1436.

Stackhouse SK, Binder-Macleod SA, Lee SC. Voluntary muscle activation, contractile properties, and fatigability in children with and without cerebral palsy. Muscle Nerve 2005;31:594-601.

Staudt M, Grodd W, Gerloff C, Erb M, Stitz J, Krageloh-Mann I. Two types of ipsilateral reorganization in congenital hemiparesis: A TMS and fMRI study. Brain 2002;125:2222-2237.

Staudt M, Krageloh-Mann I, Holthausen H, Gerloff C, Grodd W. Searching for motor functions in dysgenic cortex: A clinical transcranial magnetic stimulation and functional magnetic resonance imaging study. J Neurosurg 2004;101:69-77.

Staudt M, Niemann G, Grodd W, Krageloh-Mann I. The pyramidal tract in congenital hemiparesis: Relationship between morphology and function in periventricular lesions. Neuropediatrics 2000;31:257-264.

Stoeckel MC, Binkofski F. The role of ipsilateral primary motor cortex in movement control and recovery from brain damage. Exp Neurol 2010;221:13-17.

Trivedi R, Agarwal S, Shah V, Goyel P, Paliwal VK, Rathore RK, Gupta RK. Correlation of quantitative sensorimotor tractography with clinical grade of cerebral palsy. Neuroradiology 2010;52:759-765.

Trivedi R, Gupta RK, Shah V, Tripathi M, Rathore RK, Kumar M, Pandey CM, Narayana PA. Treatment-induced plasticity in cerebral palsy: A diffusion tensor imaging study. Pediatr Neurol 2008;39:341-349.

Tsao H, Pannek K, Boyd RN, Rose SE. Changes in the integrity of thalamocortical connections are associated with sensorimotor deficits in children with congenital hemiplegia. Brain Struct Funct 2015;220:307-318.

Tsao H, Pannek K, Fiori S, Boyd RN, Rose S. Reduced integrity of sensorimotor projections traversing the posterior limb of the internal capsule in children with congenital hemiparesis. Res Dev Disabil 2014;35:250-260.

Turton A, Wroe S, Trepte N, Fraser C, Lemon RN. Contralateral and ipsilateral EMG responses to transcranial magnetic stimulation during recovery of arm and hand function after stroke. Electroencephalogr Clin Neurophysiol 1996;101:316-328.

van der Aa NE, Verhage CH, Groenendaal F, Vermeulen RJ, de Bode S, van Nieuwenhuizen O, de Vries LS. Neonatal neuroimaging predicts recruitment of contralesional corticospinal tracts following perinatal brain injury. Dev Med Child Neurol 2013;55:707-712.

Vandermeeren Y, Bastings E, Fadiga L, Olivier E. Long-latency motor evoked potentials in congenital hemiplegia. Clin Neurophysiol 2003a;114:1808-1818.

Vandermeeren Y, Davare M, Duque J, Olivier E. Reorganization of cortical hand representation in congenital hemiplegia. Eur J Neurosci 2009;29:845-854.

Vandermeeren Y, Sebire G, Grandin CB, Thonnard JL, Schlogel X, De Volder AG. Functional reorganization of brain in children affected with congenital hemiplegia: fMRI study. Neuroimage 2003b;20:289-301.

Vry J, Linder-Lucht M, Berweck S, Bonati U, Hodapp M, Uhl M, Faist M, Mall V. Altered cortical inhibitory function in children with spastic diplegia: A TMS study. Exp Brain Res 2008;186:611-618.

Wakana S, Jiang H, Nagae-Poetscher LM, van Zijl PC, Mori S. Fiber tract-based atlas of human white matter anatomy. Radiology 2004;230:77-87.

Walther M, Berweck S, Schessl J, Linder-Lucht M, Fietzek UM, Glocker FX, Heinen F, Mall V. Maturation of inhibitory and excitatory motor cortex pathways in children. Brain Dev 2009a;31:562-567.

Walther M, Juenger H, Kuhnke N, Wilke M, Brodbeck V, Berweck S, Staudt M, Mall V. Motor cortex plasticity in ischemic perinatal stroke: A transcranial magnetic stimulation and functional MRI study. Pediatr Neurol 2009b;41:171-178.

Yang HS, Kwon HG, Hong JH, Hong CP, Jang SH. The rubrospinal tract in the human brain: Diffusion tensor imaging study. Neurosci Lett 2011;504:45-48.

Yang JF, Fung J, Edamura M, Blunt R, Stein RB, Barbeau H. H-reflex modulation during walking in spastic paretic subjects. Can J Neurol Sci 1991;18:443-452.

Yeo SS, Chang MC, Kwon YH, Jung YJ, Jang SH. Corticoreticular pathway in the human brain: Diffusion tensor tractography study. Neurosci Lett 2012;508:9-12.

Yoshida S, Hayakawa K, Oishi K, Mori S, Kanda T, Yamori Y, Yoshida N, Hirota H, Iwami M, Okano S, Matsushita H. Athetotic and spastic cerebral palsy: Anatomic characterization based on diffusion-tensor imaging. Radiology 2011;260:511-520.

Yoshida S, Hayakawa K, Yamamoto A, Okano S, Kanda T, Yamori Y, Yoshida N, Hirota H. Quantitative diffusion tensor tractography of the motor and sensory tract in children with cerebral palsy. Dev Med Child Neurol 2010;52:935-940.

Zaaimi B, Edgley SA, Soteropoulos DS, Baker SN. Changes in descending motor pathway connectivity after corticospinal tract lesion in macaque monkey. Brain 2012;135:2277-2289.

Ziemann U, Ishii K, Borgheresi A, Yaseen Z, Battaglia F, Hallett M, Cincotta M, Wassermann EM. Dissociation of the pathways mediating ipsilateral and contralateral motor-evoked potentials in human hand and arm muscles. J Physiol 1999;518 (Pt 3):895-906.

Chapter 3. Full Activation Profiles and Integrity of Corticospinal Pathways in Adults with Bilateral Spastic Cerebral Palsy

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Introduction

In cerebral palsy (CP), damage to motor areas of the brain occurs before or shortly after birth during a critical period of neuronal development (Rosenbaum et al., 2007). Such early damage results in the life-long impairment of voluntary movements, including weakness and gait dysfunction (Dobson et al., 2007; Mockford and Caulton, 2010). It has long been postulated that descending pathways from the brain that project to spinal motoneurons may contribute to motor impairments in cerebral palsy (Milner-Brown and Penn, 1979). However, the evidence of this contribution is limited.

Activation of corticospinal pathways from transcranial magnetic stimulation (TMS), which include the corticospinal and corticobulbospinal tracts (Fisher et al., 2012), has only been explored to a limited extent previously in people with spastic CP. Studies have focused on outcomes that can be determined at relatively low intensities of stimulation. They have shown that, compared to neurologically intact controls, contralateral motor evoked potentials (MEPs) in participants with CP are evoked from more lateral locations over the motor cortex (Kesar et al., 2012; Maegaki et al., 1999) and require higher stimulation intensities to activate (Berweck et al., 2008; Eyre et al., 2007; Koerte et al., 2011; Vry et al., 2008). There is also an increased prevalence of ipsilateral MEPs in people with spastic CP (Carr et al., 1993; Kesar et al., 2012; Maegaki et al., 1999). While smaller MEPs might be expected as a result of injury to the corticospinal pathways, findings in the single study evaluating MEP amplitudes in children and adolescents with bilateral spastic CP performing maximal contractions failed to detect a significant difference compared to NI controls (Vry et al., 2008). Most of the other previous studies of responses to TMS in spastic CP were performed with the participants at rest rather than during voluntary muscle activity and thus cannot comment on the function of the corticospinal pathways during motor function. In addition, anatomical abnormalities of the corticospinal pathways have been demonstrated, though mainly in children. Studies using diffusion tensor imaging (DTI) consistently suggest a loss of white matter integrity in corticospinal pathways as reviewed by Scheck et al. (2012).

Here we examined the activation and anatomic integrity of the corticospinal pathway using TMS and diffusion tractography. We studied adults with bilateral spastic CP, who

represent ~60% of people with CP (Koman et al., 2004; Shevell et al., 2009). We recorded TMS responses in the soleus muscle because plantarflexor muscles in CP exhibit greater weakness and spasticity contributing to gait impairments than the more commonly studied dorsiflexors (Berger et al., 1982; Eek et al., 2011). TMS responses to a large range of stimulation intensities evoked during voluntary muscle activity were compared to neurologically intact (NI) participants of similar age and sex distribution.

While increases in both cortical and spinal excitability occur with increasing voluntary muscle activity in neurologically intact populations (Di Lazzaro et al., 1998; Maertens de Noordhout et al., 1992; Morita et al., 2000; Ugawa et al., 1995), the effect of increased voluntary activity has not been explored in spastic CP. Unlike previous studies we also examined activation of corticospinal pathways throughout a full range of voluntary muscle activity (resting to maximal activity). Because our participants with CP had a spectrum of motor weakness, we were able to examine if maximal voluntary muscle activity was associated with the TMS measures. In a subgroup of these participants, we used diffusion tractography to determine if the anatomical integrity of the corticospinal pathway projecting to all muscles was also reduced in adults with CP compared to age and sex-matched NI participants.

Materials & Methods

Participants

This study was approved by the Health Research Ethics Board at the University of Alberta, in accordance with the Declaration of Helsinki. All participants provided written informed consent. Seventeen adults with spastic CP (10 female, 7 male) with a median (range) age of 32 (19-57) (Table 3-1) and 15 neurologically intact (NI) controls (9 female, 6 male) with a median age of 28 (19-59) participated in this study. No participants had absolute contraindications to TMS (Rossi et al., 2009) or botulinum toxin injections in the lower leg within the past two years. Because MEP responses are stable in the age ranges tested here (Pitcher et al., 2002), strict age-matching was not performed for the TMS components of this study; however, the mean and standard deviation of ages were very similar between the CP (33.5±11.1) and NI (32.0±11.4) groups.

Motor function of the participants in the CP group was evaluated by a physician (author EGC, see Table 3-1). The Gross Motor Function Classification System (GMFCS) (Palisano et al., 2008) was used to classify motor abilities in sitting and walking and the need for assistive devices. Plantarflexor strength was assessed with the Medical Research Council score (MRC) (Hislop et al., 2014). The presence of spasticity was confirmed by the modified Ashworth scale (Bohannon and Smith, 1987) and the Penn Spasm Frequency scale (Penn et al., 1989).

General TMS Experimental Set-Up

Each participant took part in three TMS protocols during a single experimental session as detailed below: 1) location of the soleus MEP hotspot, 2) stimulus-recruitment curve and 3) modulation of MEPs during voluntary soleus activity. In the CP group, the leg that interfered more with function in terms of weakness and spasticity (referred to as the target leg: TL) was tested. The right leg was tested in the NI group except in three participants with previous injuries to the right ankle. Throughout the experiment, participants were seated comfortably with their knee at 80-90° of flexion and their ankle stabilized at 90°. Surface electromyography (EMG) was recorded from the soleus muscle in the target leg using pairs of conductive adhesive hydrogel electrodes (3.81 x 2.24 cm, Covidien Ltd., Dublin, Ireland). EMG signals were amplified (1k gain), filtered (10-1000 Hz) (AMT-8, Bortec Biomedical Ltd., Calgary, AB), digitized and sampled at 5 kHz using Axon hardware (Digidata 1440A) and software (Axoscope 10.3, Molecular Devices LLC, Sunnyvale, CA). Visual feedback of soleus activity levels was provided on an oscilloscope. Analysis of EMG data was performed offline using Matlab (R2011b, The Mathworks Inc., Natick, MA), and statistical analysis was performed in SPSS (version 21, IBM, Armonk, NY). Because most data were not normally distributed, as determined from the Shapiro-Wilk test, groups were described by their median and range values and compared using non-parametric statistics as described for each protocol below. The alpha level for significance was set at 0.05.

The maximal voluntary activity (MVA) of the soleus muscle was determined for each participant at the start of the experiment. The maximum activity of rectified EMG, averaged over 1s, was calculated for each trial. At least 60 s of rest was provided between each trial. Typically 3 to 4 trials were needed so that the two highest trials were within 10% of each other. The

ID	Sex	Age	TL	Medication	MRI	GMFCS	MRC	mAsh	Penn	MVA
CP-1	F	21	R		PVWMI +	I	5	0	1	44
CP-2*	M	32	R	baclofen citalopram	PVWMI ++/CM	I	4	3	3	26
CP-3	M	26	L		PVWMI ++	I	5	1+	1	85
CP-4	F	32	R	venlafaxine cyclobenzaprine	---	II	5	1+	2	22
CP-5	F	35	L		PVWMI ++	IV	1	2	1	37
CP-6*	M	49	L	citalopram tolterodine	PVWMI ++	III	3	3	2	7
CP-7	F	28	L		Normal	II	5	1	1	28
CP-8*	F	33	R		PVWMI ++/DGMI	III	4	3	2	11
CP-9	F	21	L		PVWMI ++	III	1	1	1	14
CP-10	M	28	L		PVWMI +/CM	II	2	0	1	39
CP-11	F	19	L	clonazepam sertraline	PVWMI ++/CM	IV	2	1	2	11
CP-12	M	24	R	clonazepam escitalopram quetiapine	PVWMI +	IV	2	1	1	19
CP-13	M	42	R		PVWMI +	III	2	1	2	31
CP-14	F	39	L		Normal	III	1	1+	1	81
CP-15*	F	52	R	amitriptyline citalopram phenytoin flunarizine	PVWMI +++/CVA	II	1	2	2	7
CP-16	F	57	R		PVWMI +	I	5	0	1	69
CP-17	M	32	R		PVWMI ++	II	4	1	1	9

Table 3-1. Demographics and Clinical Measures of Participants with CP. Columns represent the participant gender (Sex), age at time of TMS (Age), side of the target leg (TL), daily medication that may alter TMS results (Medication), MRI findings (MRI), Gross Motor Functional Classification System rating (GMFCS), target leg plantarflexors strength (MRC), modified Ashworth score of the plantarflexors of the target leg (mAsh), Penn Spasm Frequency (Penn), and maximal voluntary activation in the soleus (MVA) in μV. Participants with bolded IDs are age/sex-matched to the 11 NI participants for DTI analysis. * indicates participants without contralateral responses to TMS. MRI Abbreviations: PVWMI = periventricular white matter injury with a mild (+), moderate (++) or severe (+++) rating, CM = cerebral malformation, DGMI = deep gray matter injury, CVA = cerebral vascular accident. --- indicates not imaged with MRI due to a contraindication.

average of these two trials was defined as the MVA and was used to set the target background contraction levels. The electrical activity recorded during an MVA in some of the participants with CP (5-10 μV) was slightly above the electrical noise recorded at rest (1-2 μV). Thus, noise was subtracted from all measures of voluntary muscle activity (i.e., MVA and pre-stimulus background EMG) in each participant.

The maximal soleus M-wave (M_{max}) was measured at the end of the experiment to normalize MEP responses. To evoke M_{max}, monopolar tibial nerve stimulation (1-ms pulse width, DS7A constant-current stimulator NL703, Digitimer, Welwyn Garden City, UK) was applied with a cathode probe in the popliteal fossa over the tibial nerve and a large anode over the patellar tendon. M_{max} values were used to normalize MEP values in all but 3 participants (1PC, 2NI) due to contamination of the M-wave with the stimulus artifact.

TMS was delivered using a MagStim 200 stimulator or its BiStim module (The Magstim Company Ltd, Carmarthenshire, UK) and a custom batwing coil (P/N 15857: 90-mm wing diameter), designed to produce focal activation of the leg motor cortex and oriented to induce posterior-anterior current in the brain. For consistency, any measure of the absolute intensity of stimulation, i.e. the active motor threshold (aMT) was only compared in participants who were stimulated with the MagStim 200 stimulator (9 CP & 13 NI).

Part 1. Location of Soleus MEP Hotspot

Participants wore a cap containing a 1-cm grid aligned to their vertex. The hotspot was defined as the position resulting in the largest MEP (average of 4 responses) produced by submaximal stimulation at a background contraction of 20% MVA. TMS was applied at least 2 cm lateral to vertex to reduce current spread to the opposite cortex. If consistent responses could not be elicited from the cortex contralateral to the target leg, as occurred in 4 of the participants with CP, TMS was applied to the ipsilateral cortex to verify that responses could be elicited in that muscle. The Euclidian distance between the vertex and hotspot was compared between the CP and NI groups using the non-parametric Mann-Whitney U-test.

Part 2. Stimulus-Recruitment Curve

To measure the relationship between stimulation intensity and peak-to-peak MEP amplitude, the intensity of stimulation, expressed as a percentage of the maximum stimulator output (% MSO), was systematically varied. Participants performed submaximal (20% MVA) contractions throughout testing. The active motor threshold (aMT), defined as the lowest stimulation intensity that produced a distinguishable MEP in 3/5 individual responses, was first determined. Stimulation intensities above and below aMT were then applied in steps of 0.2 x aMT. Because MEP amplitudes could decrease at the highest TMS intensities after reaching a peak, finer adjustments in stimulation intensity (0.05 to 0.1 x aMT) were used to verify that we obtained the largest MEP amplitude possible. Five stimulations were applied at each TMS intensity, and the amplitude of each MEP was averaged across the 5 trials. The latency of the largest MEPs evoked at a single intensity was determined by visual inspection from both the overlaid unrectified MEPs and from an average of the rectified MEPs.

The stimulus-recruitment curve was described using three parameters: aMT (in units of % MSO), the amplitude of the MEP at the intensity of 1.2 x aMT ($MEP_{1.2}$) and the largest MEP amplitude on the recruitment curve (MEP_{peak}). In 2 participants with CP, where 1.2 x aMT exceeded 100% MSO (i.e., when aMT was greater than 84% MSO), the response to stimulation at the 100% MSO intensity was used for both $MEP_{1.2}$ & MEP_{peak}. The three parameters of the stimulus-recruitment curve (aMT, $MEP_{1.2}$ and MEP_{peak}) were compared between the CP and NI groups using the non-parametric Mann-Whitney U-test. Associations between the MEP parameters and MVA within the CP group were explored with Spearman rank correlation.

Part 3. Voluntary Modulation of MEPs

The relationship between MEP amplitude and voluntary activity (expressed as a % MVA) was measured using a TMS intensity of 1.2 x aMT (or 100% MSO in the 2 participants with aMT > 84% MSO). Stimulations were applied with the participant at contraction levels between rest and 100% MVA, with a total of 34 stimulations on average. The voluntary activity (noise-subtracted) was measured as the rectified soleus EMG averaged over the 100 ms prior to TMS. For each individual, MEPs were plotted in ascending order of their respective voluntary activity.

To enable comparisons of MEP facilitation voluntary activity between participants with CP and NI control participants, the largest MEP (MEP_{max}) that could be evoked in each participant was determined and the MEP amplitudes were expressed as a percentage of MEP_{max} similar to (Davey et al., 1999). MEP_{max} was the largest average of 5 MEPs that could be evoked in a participant. It was recorded during the stimulus-recruitment curve at 20% MVA and varying stimulation intensities (Part 2), during the voluntary modulation of MEPs by varying the level of voluntary activity at a stimulation intensity of 1.2 x aMT (Part 3), or during a separate trial where both a high stimulation intensity and levels of voluntary activation were used.

The relationship between MEP amplitude (% MEP_{max}) and voluntary activity (% MVA) was positive and linear in participants from both groups. Thus, the slope of the increase in MEP amplitude relative to % MVA was measured for each participant and compared between the two groups using a Mann-Whitney U-test.

Effect of Medications on TMS Responses

There is little evidence regarding the effect of chronic medication use on TMS measures. Six participants with CP were chronically taking medications (listed in Table 3-1) that, in a single dose, have been shown to, or theoretically may, alter the results of TMS (Ziemann, 2013). To ensure *chronic* use of these medications did not influence the TMS results, all statistical tests were repeated without these 6 participants. Because the statistical results were unchanged, data from these 6 participants were included in the CP group. This also ensured that the population studied remained representative of people with spastic CP, many of whom require neuromodulatory medications.

General MRI Setup

On a separate day from the TMS experiments all participants with CP, except one with a contraindication to MRI (16/17), were scanned using a 1.5T Siemens Sonata. Eleven of the 15 NI participants were scanned; three were not included in the MRI protocol, and one declined to be imaged. Sequences included T1-weighted, T2-weighted, fluid-attenuated inversion recovery (FLAIR), and diffusion tensor imaging (DTI) for a total scan time of ~20 minutes. DTI was acquired using a dual spin-echo, single shot echo-planar imaging sequence with: 40 axial-oblique

slices, 3-mm slice thickness with no inter-slice gap; TR = 6900 ms; TE = 100 ms; 30 non-collinear gradient directions with b=1000 s/mm^2, 1 b=0 s/mm^2, 2 averages, FOV 220x220 mm^2, matrix of 128 x 128 with 75% phase partial Fourier zero-filled to 256 x 256, acquisition time 8:11 minutes.

A senior radiology resident and a neuroradiologist (authors DTJ & DJE) reviewed the T1, T2 and FLAIR sequences in both groups. Anatomical abnormalities were classified into categories relevant in CP, including periventricular white matter injury (PVWMI), cerebral malformations (CM), deep gray matter injury (DGMI) and normal anatomical imaging (Towsley et al., 2011). The severity of any periventricular white matter injury was rated qualitatively as mild, moderate or severe based on cerebral white matter volume loss and T2 hyper-intensities by consensus between the two radiologists, who were blinded to the clinical scores.

Diffusion Tractography

DTI sequences were pre-processed, and motion distortion was corrected in ExploreDTI v4.8.3 (Leemans et al., 2009). Deterministic whole brain tractography was performed using a fractional anisotropy threshold of 0.2 and an angle threshold of 50º. The corticospinal pathway contralateral to the leg where the MEPs were recorded (i.e., target leg) was analyzed. A 'seed' region of interest (ROI) was placed around the posterior limb of the internal capsule and a 'target' ROI around the corticospinal pathway below the basis pontis (Rha et al., 2012). Due to technical factors, in CP-14 the contralateral, right corticospinal pathway could not be reliably followed to the basis pontis, requiring placement of the 'target' ROI at the level of the cerebral peduncle. 'Not' ROI(s) were used to exclude spurious fibres. Partial tract analysis was performed only on the tract segment between the 'seed' and 'target' ROIs only rather than following the tracts all the way up to the cortex. This was done to control for the high variability in tract length in the CP group, as reflected in the larger standard deviation of tract length in the CP group (±27%) compared to the NI group (±10%). Fractional anisotropy (FA), mean diffusivity (MD), parallel diffusivity ($\lambda_{//}$) and perpendicular diffusivity ($\lambda_{\perp}$) of the partial tracts were determined for each individual. Because DTI parameters are affected within the age range tested here (Lebel et al., 2012), between-group comparisons were performed using the 11 participants with CP (median age 31, range 21-56, see bolded IDs in Table 3-1) who were individually sex- and age-

matched to the 11 NI participants (median age 32, range 18-59) who had MRIs. Between-group comparisons were made using the Mann-Whitney U-test. Associations between the MEP and DTI measures were explored with Spearman rank correlation.

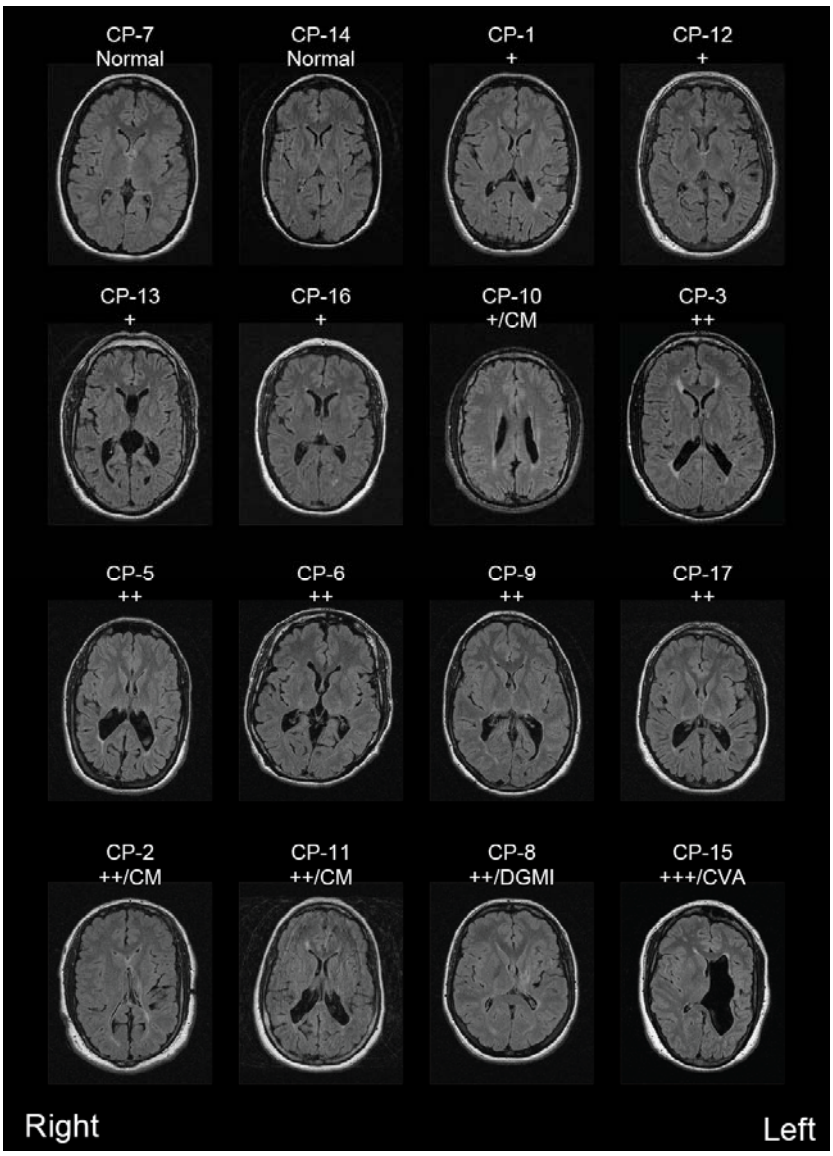

Figure 3-1. MRI of Participants with CP. Axial FLAIR slices illustrating the anatomical findings for each participant with CP imaged (16/17) in increasing order of severity. Two participants had normal imaging. All remaining participants had evidence of mild (+), moderate (++), or severe (+++) periventricular white matter injury (PVWMI) on both sides of the brain. Additional abnormalities were noted in 5 participants. Three participants had cerebral malformations (CM) in the form of polymicrogyria (CP-10 & CP-11) and partial corpus callosum agenesis (CP-2). CP-8 had deep gray matter injury (DGMI) particularly notable on the left. The image of CP-15 is consistent with a periventricular cerebrovascular accident (CVA). Figure published previously in (Condliffe et al., 2016).

Results

Participant Characteristics

The MRI findings in the participants with CP were fairly consistent whereby most (13/16) had evidence of mild (+) or moderate (++) PVWMI on both sides of the brain (Fig. 3-1). Two participants with CP (13%) had normal MRI scans, a proportion that is consistent with population studies of people with spastic CP (Reid et al., 2014). One participant (CP-15) had severe (+++) bilateral PVWMI along with evidence of a cerebrovascular accident (CVA). Three participants had additional findings suggestive of CM, such as polymicrogyria (CP-10 and CP-11) and partial corpus callosum agenesis (CP-2). One participant (CP-8) had evidence of DGMI.

Part 1. Location of Soleus MEP Hotspot

MEP responses in the soleus muscle were evoked from the contralateral cortex in all but 4 participants with CP (13/17) and in all NI participants (15/15). In general, soleus MEPs in the CP group were evoked from locations farther from the vertex over the motor cortex. The hotspots for the NI group were located 2 to 3 cm lateral from vertex in a fairly tight cluster (open circles, Fig. 3-2A). The median (range) distance of the hotspot from vertex, as marked by the length of the arrow for one participant in Fig. 3-2A, was 2.2 cm (2.0-3.2 cm) in the NI group (see individual values in Fig. 3-2B). The hotspot locations for the CP group were more dispersed and very lateral in some individuals (solid circles, Fig. 3-2A), with a median distance of the hotspot from vertex at 4.0 cm (2.8-9.1 cm), which was farther compared to NI controls ($p<0.001$, Fig. 3-2B).

Part 2. Stimulus-Recruitment Curve

MEPs evoked in the contralateral soleus muscle of participants with CP were generally smaller and more variable than the MEPs measured in NI controls. Sample MEPs evoked during submaximal contractions (20% MVA) and at increasing intensities of stimulation showed smaller MEPs (note the different vertical scales) with similar latencies for the two participants with CP versus the one participant in the NI group (Figure 3-3).

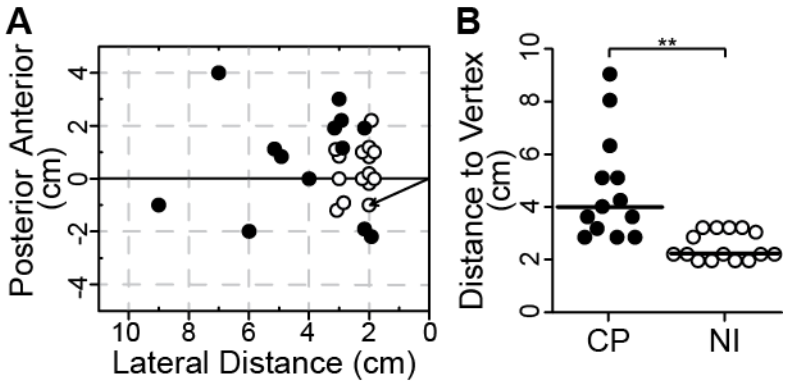

Figure 3-2. Location of Soleus MEP Hotspot. A) Cartesian coordinates of the soleus MEP hotspot for each CP (solid circles) and NI (open circles) participant relative to vertex (0, 0) where contralateral MEPs could be obtained. The Euclidian distance from vertex is marked by the arrow for one NI participant. **B)** Values of the Euclidian distance from vertex for each of the CP and NI participants shown in A. Horizontal lines represent the group medians. ** = p<0.001.

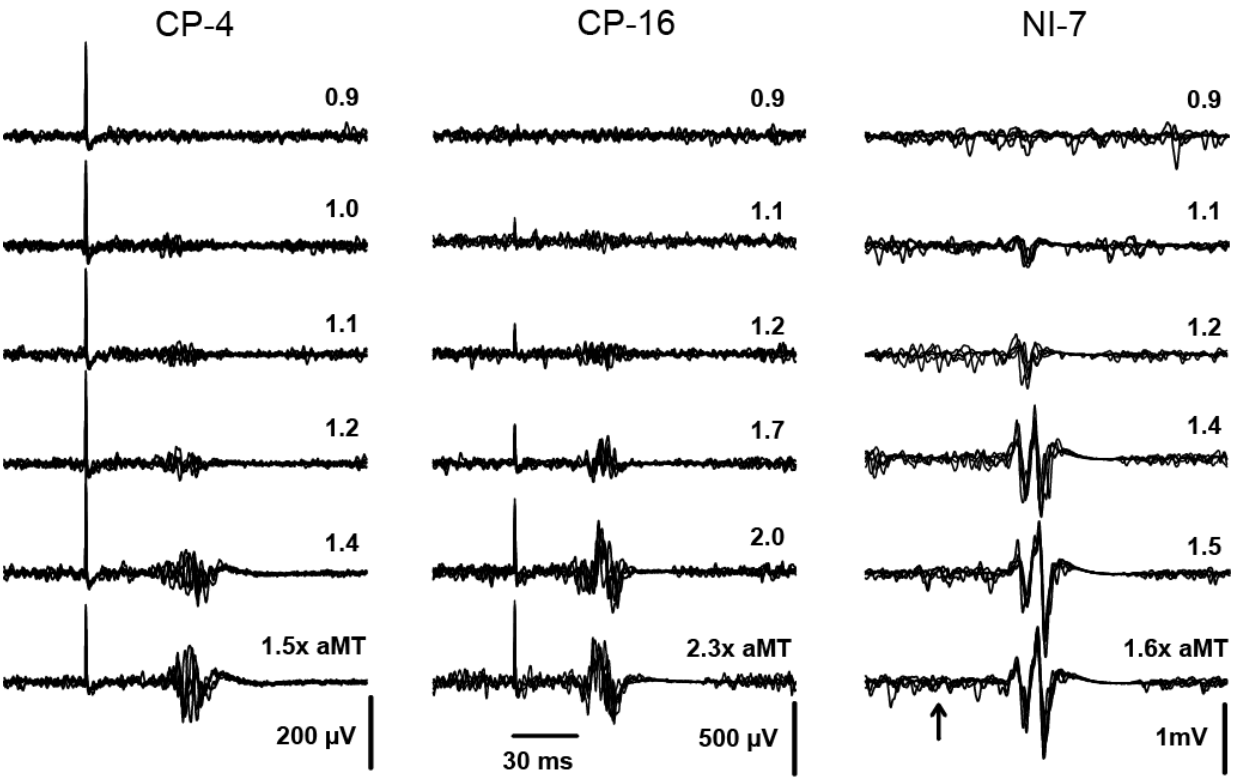

Figure 3-3. Example Soleus MEPs. Five superimposed traces of unrectified EMG showing MEP responses to incrementing intensities of TMS for two representative participants with CP and one NI participant. The intensity of stimulation shown above each trace is expressed as a multiple of active motor threshold (x aMT) obtained during a 20% MVA contraction. The time of stimulation is marked by the stimulus artifact in CP-4 & CP-16 and by the vertical arrow for NI-7. Note the different vertical scale bars.

The peak-to-peak MEP values of the three participants from Figure 3-3 are plotted against the corresponding intensity of stimulation in Figure 3-4A to construct stimulus-recruitment curves. The stimulus-recruitment curves measured in the CP group were of different amplitude and shape compared to the NI group. In all NI participants, MEP amplitudes increased for stimulation intensities above motor threshold (aMT) until reaching a peak (MEP_{peak}), beyond which the MEP amplitude either plateaued or decreased slightly, as shown for NI-7 in Figure 3-4A. In most individuals with CP, the MEP amplitude continued to increase up to maximum intensities of stimulation, as shown for CP-4 in Figure 3-4A. In the remaining two participants with CP with good motor function (Table 3-1), MEP amplitudes peaked prior to plateauing (CP-16, Fig. 3-4A) or decreased slightly (CP-3, not shown) as in NI controls (NI-7, Fig. 3-4A). While the stimulation intensity that produced MEP_{peak} in the CP group was frequently limited by the maximum stimulator output, the TMS intensity where MEP_{peak} was obtained appeared similar when expressed relative to aMT, with a median (range) of 1.5 x aMT (1.2-2.0 x aMT) in the CP group and 1.6 x aMT (1.4-2.1 x aMT, $p=0.56$) in the NI group. This suggests that the lack of plateau in the stimulus-recruitment curve in 11/13 participants with spastic CP was not purely due to a limitation in the maximal stimulation intensity, but due to the inability to reach higher stimulus intensities (relative to aMT) to reach the plateau in the group with spastic CP.

Overall, aMT was higher and MEP amplitudes were lower in the CP group compared to NI controls. Specifically, the median aMT was higher in the CP group at 65% MSO (50-87% MSO, Fig. 3-4B left graph) compared to the NI group at 52% MSO (30-70% MSO, $p=0.029$). MEP amplitudes when stimulating at 1.2 x aMT ($MEP_{1.2}$) were smaller in the CP group at 0.09 mV (0.0-0.32 mV, Fig. 3-4B middle graph) compared to the NI group at 0.54 mV (0.21-1.09 mV, $p<0.001$). The largest MEP amplitude from the stimulus recruitment curve (MEP_{peak}) was also smaller in the CP group at 0.14 mV (0.0-1.53 mV, Fig. 3-4B right graph) compared to the NI group at 1.32 mV (0.53-2.97 mV, $p<0.001$). Interestingly, the latencies of the soleus MEPs appeared quite similar between groups. Specifically, the median latency of the MEP_{peak} in the CP group was 30.6 ms (20.3-38.0 ms) whereas in the NI group it was 30.2 ms (26.7-37.5 ms, $p=0.85$).

In 4 participants with CP, it was not possible to evoke MEPs from any location over the contralateral motor cortex at the maximum stimulator output (MEPs = 0.0 mV, Fig. 3-4B), although responses from stimulation to the ipsilateral cortex were present (not shown). Note that all differences in MEP measures between the NI and CP groups remained when data from these

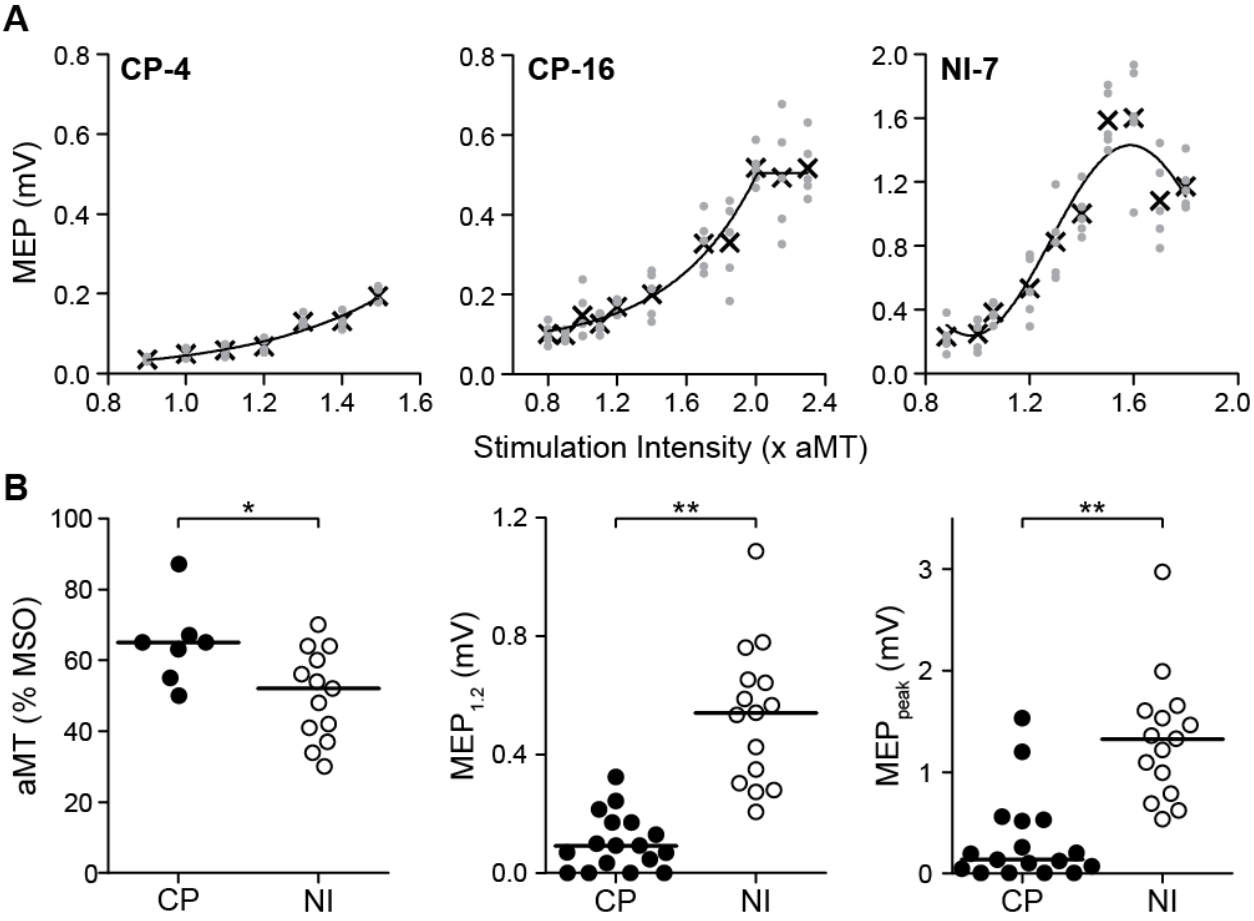

Figure 3-4. Stimulus-Recruitment Curves. A) Relationship between stimulation intensity (x aMT) and the peak-to-peak MEP amplitude for the same participants in Figure 3-3. Each gray dot represents a single MEP, X's indicate the mean MEP amplitude at each intensity and solid lines represent a best fit relationship. **B)** Left graph: comparison of active motor threshold (aMT) for participants tested with the MagStim 200 stimulator. Participants with CP without contralateral MEPs were not included. Middle and right graphs: comparison of $MEP_{1.2}$ and MEP_{peak} respectively. Solid circles represent the CP group and open circles represent the NI group. Horizontal lines represent the group medians. All responses for both groups were recorded at 20% MVA. * = $p<0.05$. ** = $p<0.001$.

4 participants (CP-2, CP-6, CP-8 and CP-15) were excluded. Interestingly, participants with no contralateral MEPs had evidence of comparatively more severe brain injury, with moderate to severe PVWMI and typically additional MRI findings such as cerebral malformations, deep grey matter injury and cerebrovascular accidents (Fig. 3-1). Some of these participants also had low MVA values (Table 3-1). As a whole, the TMS measures within the CP group were related to measures of voluntary muscle activity. For example, the amplitude of $MEP_{1.2}$ was positively associated with the amplitude of MVA ($r=0.86$, $p<0.001$). Likewise, MEP_{peak} was also associated with MVA ($r=0.71$, $p=0.001$).

Normalizing MEP by M_{max}

As expected, the CP group displayed motor weakness in the plantarflexors. This was reflected in the MRC grades and in the lower muscle activity levels where the median MVA for CP group was 26μV (7-85μV, see individual CP values in Table 3-1) compared to the NI group at 185μV (73-297μV, $p<0.001$). A peripheral contribution to this weakness was probable given the smaller responses evoked by direct stimulation of soleus motor axons (M_{max}) in the CP group at 5.1 mV (1.3-9.8 mV) compared to the NI group at 9.8 mV (4.5-12.2 mV, $p=0.002$). In contrast, the median latency of M_{max} appeared similar in the CP group at 5.33 ms (4.44-7.45 ms) compared to controls at 5.32 ms (4.98-7.13 ms, $p = 0.76$).

	CP	NI	p
$MEP_{1.2}$ / M_{max} (%)	1.4 (0 – 10.2)	5.2 (2.8 – 10.9)	<0.001
MEP_{peak} / M_{max} (%)	2.8 (0 – 18.7)	12.4 (8.6 – 32.9)	0.002
MEP_{max} / M_{max} (%)	5.7 (0 – 46.8)	19.9 (11.4 – 40.3)	0.002

Table 3-2. MEP Amplitudes Normalized to M_{max}. All differences between groups in MEP amplitudes persisted when the amplitude for each individual was normalized to the amplitude of their maximal M-wave.

Although M_{max} was smaller in the CP group, all differences in MEP measures between groups were maintained when expressing MEP amplitudes as a percentage of M_{max} (Table 3-2). In addition, even when accounting for peripheral contributions to weakness by normalizing to

M_{max}, there was a positive association between the amplitude of the MEPs and MVA ($MEP_{1.2}/M_{max}$ r=0.79, p<0.001 & MEP_{peak}/M_{max} r=0.76, p=0.001).

Part 3. Voluntary Modulation of MEPs

Increasing voluntary activity produced linearly increasing amplitudes of MEPs when a constant intensity of stimulation (1.2 xaMT) was applied in both CP and NI groups (Fig. 3-5A). It is worth noting here that levels of voluntary activity >100% MVA were obtained given that the voluntary activity measured 100 ms before the TMS pulse could be greater than the averaged 1000 ms of activity used to quantify the MVA at the beginning of the experiment. To compare the modulation of MEPs among individuals, MEPs were normalized to the largest MEP evoked from all trials (MEP_{max}). The slope of the relationship between MVA and modulation of the MEP amplitude was lower in the CP group at 0.58 (0.23-1.71, Fig. 3-5B) compared to the NI group at 0.72 (0.54-1.64, p=0.040). Similarly, MEP_{max}, the largest MEP produced by the participant throughout the testing session, was also lower in CP at 0.29 mV (0.0-1.83 mV) compared to NI controls at 1.83 mV (0.67-3.14 mV, p<0.001) (see also Table 3-2 for normalized values).

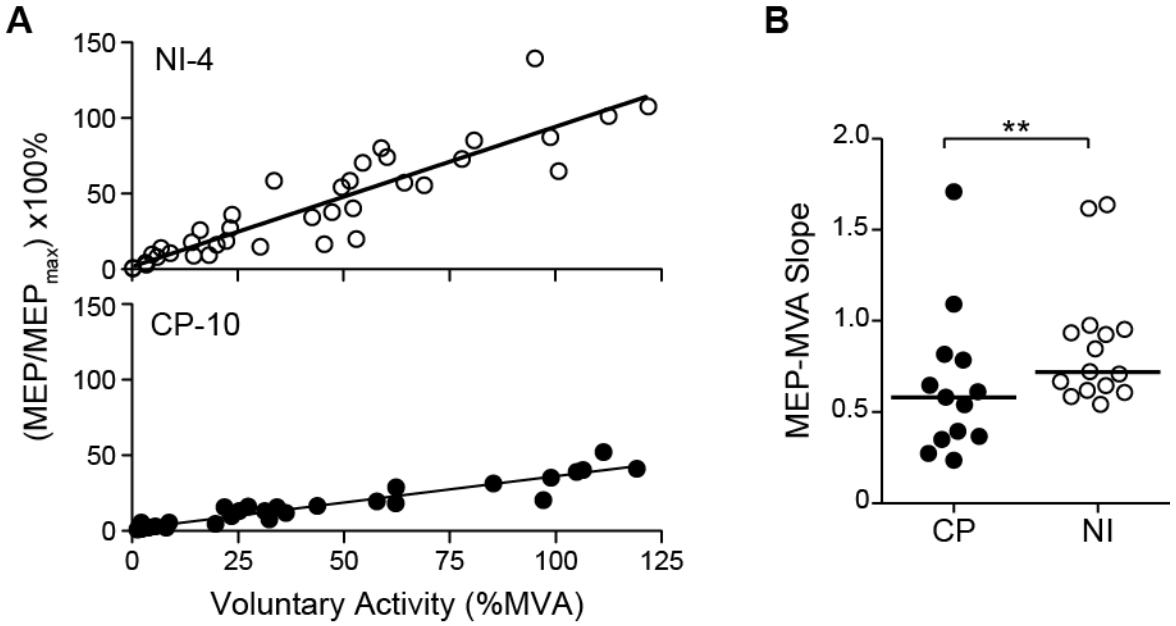

Figure 3-5. Voluntary Modulation of MEPs. A) The relationship between voluntary activity (% MVA) and MEP amplitude (expressed as a percentage of MEP_{max}) plotted for a representative NI (top) and CP (bottom) participant. TMS was applied at 1.2 x aMT. Recall voluntary activity over 100 ms prior to stimulation could exceed 100% MVA measured over 1 s.

B) Slope of the relationship between voluntary activity and MEP amplitude for each CP (solid circles) and NI (open circles) participant. Horizontal lines represent the group medians. ** = $p<0.001$.

Diffusion Tractography

Tractography successfully generated streamlines attributed to the corticospinal pathway that was contralateral to the target leg in all 11 age- and sex-matched pairs of CP and NI participants (examples in Figure 3-6A). Two of the four diffusion tensor parameters differed for the corticospinal pathways between the two groups (Fig. 3-6B). The tendency for water molecules to diffuse in a preferred direction, i.e., fractional anisotropy (FA), was lower in the CP group at 0.55 (0.42-0.61) compared to the NI group at 0.60 (0.53-0.66, p=0.023), and the perpendicular diffusivity ($\lambda_{\perp}$) was significantly higher in the CP group at 0.56 $x10^{-3}$ mm^2/s (0.47-0.80 $x10^{-3}$ mm^2/s) compared to the NI group at 0.49 $x10^{-3}$ mm^2/s (0.43-0.64 $x10^{-3}$ mm^2/s, p=0.028). In contrast, the average motion of water molecules, i.e., mean diffusivity (MD, p = 0.11), and its parallel component ($\lambda_{//}$, p = 0.34) were not significantly different. In the CP group only, both MEP_{peak} and MEP_{max} were correlated with fractional anisotropy (r's = 0.62 and 0.65, p's = 0.040 and 0.30, respectively).

Discussion

This is the first study in participants with spastic CP to examine a full activation profile of corticospinal pathways using a large range of TMS intensities and levels of voluntary muscle activity. Using adult participants made applying high intensities of TMS and requiring controlled contractions more feasible. MEP amplitudes recorded from the soleus muscle were consistently reduced at both low and high intensities of TMS compared to neurologically intact controls. Further, increasing the level of voluntary muscle activity provided less facilitation of MEP responses. These novel findings, suggest alterations to the corticospinal pathways may contribute to motor dysfunction in people with spastic CP.

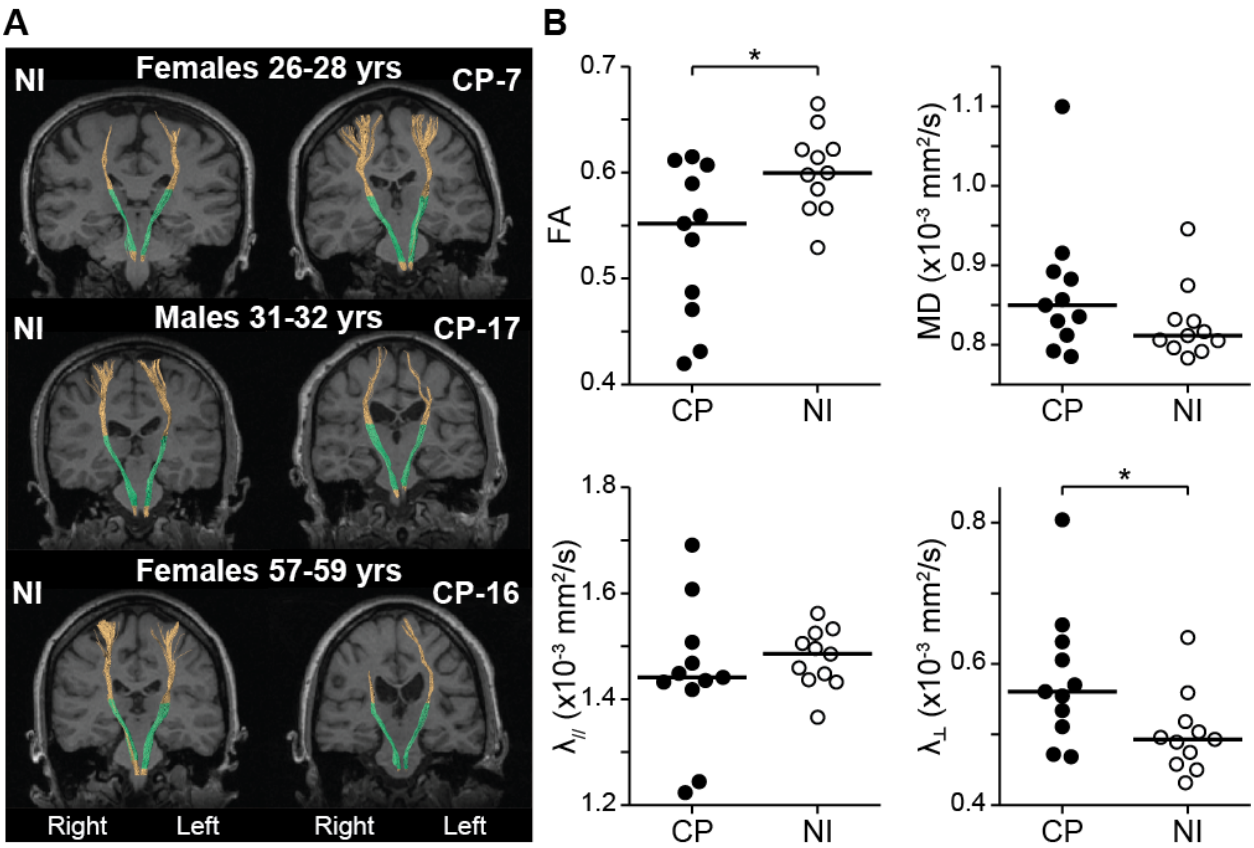

Figure 3-6. Tractography of Corticospinal Pathway. **A)** Tractography of the corticospinal pathway superimposed on a coronal T1-weighted image for three representative sex- and age-matched pairs of NI (left) and CP (right) participants. The sex and age-range is indicated above each pair. Full tracts are shown in orange and the analyzed partial tracts between the 'seed' and 'target' ROI are displayed in green. **B)** Fractional anisotropy (FA, top left), mean diffusivity (MD, top right), parallel diffusivity ($\lambda_{//}$, bottom left) and perpendicular diffusivity ($\lambda_{\perp}$, bottom right) values for each CP (solid circles) and NI (open circles) participant. Horizontal lines represent the group medians. * = $p<0.05$.

Impaired Activation of Soleus MEPs

Relationship to Previous Findings

Similar to the hotspot for the tibialis anterior in children and adolescents (Kesar et al., 2012; Maegaki et al., 1999), adults with CP had a soleus hotspot that was farther from vertex, potentially due to a relocation of the leg representation to more intact, lateral corticospinal

pathways. In addition to a relocation of the leg corticospinal pathways. In addition to a relocation of the leg motor area, there is an increase in the threshold to activate MEPs in both hand (Berweck et al., 2008; Eyre et al., 2007; Heinen et al., 1999; Koerte et al., 2011) and leg (Vry et al., 2008) muscles in children and adolescents with CP. As mentioned in the introduction, MEP amplitudes evoked at relatively low TMS intensities in children and adolescents with bilateral spastic CP were not significantly different compared to a neurologically intact control group; however, while there was high variability in both groups, the mean MEP amplitude in the CP group was about half of that in the control group (Vry et al., 2008). Responses were recorded while participants performed a maximal voluntary effort which may have contributed to the high variability and insignificant difference. In the present study, when evoking MEPs in the soleus muscle at 20% MVA, significant increases in the threshold and decreases in the amplitude of MEPs at both low and high TMS intensities were observed. Likewise, the largest MEPs evoked throughout the experiments (i.e., MEP_{max}), were six times smaller in CP compared to NI controls.

The onset latencies of the MEPs in the CP group were similar to NI controls. Given that the latency of M_{max} was also similar between the two groups, this suggests that the conduction velocity of the corticospinal pathways in this cohort of adults with bilateral CP was normal. This is consistent with findings in children and adolescents (Vry et al., 2008), but in contrast to a 2-4 ms delay in the central motor conduction time of contralateral MEPs in toddlers and infants (Eyre et al., 2007) with bilateral spastic CP. Thus, the conduction velocity of corticospinal pathways in bilateral CP, functioning at GMFCS I-IV, may normalize during development to reach intact values prior to adulthood.

Potential Mechanisms Contributing to Impaired Activation

The reduced amplitude of soleus MEPs in the present study could have resulted from damage to the axons of corticospinal or corticobulbospinal pathways, the latter including the corticoreticular tract that is also activated by TMS to the primary motor cortex (Fisher et al., 2012). Damage or alterations to excitatory interneurons in the gray matter of the motor cortex that synapse onto corticospinal or corticobulbar neurons could also contribute. Because TMS preferentially activates horizontally aligned axons that transynaptically activate corticospinal

projection neurons, a more vertical orientation of these axons due to diffuse brain injury or ventricle enlargement may have contributed to the reduced MEP responses observed in our CP group. Such alterations to both input and output neurons in the motor cortex, as indirectly assessed from these TMS measures, may contribute to the impaired muscle activation observed in people with spastic CP.

In addition to damage of corticospinal pathways, peripheral factors such as reduced muscle size and increased subcutaneous tissue could have contributed to the reduced MEP amplitudes in the CP group. Although M_{max} was lower in the CP group compared to NI controls, that the differences in MEP amplitudes between the two groups persisted when MEPs were normalized by M_{max}, suggests a central nervous system cause contributes to smaller MEPs. Therefore, the higher thresholds and reduced MEP amplitudes observed in the participants with CP in this study were likely mediated by the decreased activation of both low- and high-threshold corticospinal pathways by TMS.

Variability within the CP group and Associations of TMS Measures to MVA

As is typical of bilateral spastic CP (Shevell et al., 2009), there was some variability in the clinical presentation of the participants with CP. For example, there was variability in the use and type of medications taken, pattern of brain injury and residual muscle strength as reflected by the MRC grades, and maximal voluntary activity in the soleus. However, when separately removing participants taking neuromodulators that could affect neuronal excitability or the four participants with additional MRI findings, MEP measures were still lower in the CP group compared to NI controls. Thus, the inclusion of individuals with these potentially confounding factors did not drive the findings of reduced MEPs measured in this study. In contrast, the amount of residual muscle activity appeared to have an influence over MEP amplitudes in the CP group. For example, higher functioning individuals, as indicated by their GMFCS and MVA scores, had MEP amplitudes within the range of the NI controls. Moreover, as a group, measures of MEP amplitude were all associated with MVA, the latter a potential indicator of corticospinal pathway function.

Reduced Voluntary Modulation of Soleus MEPs

MEP amplitudes increased to a lesser degree with increasing voluntary muscle activity in the CP group, even after normalizing MEPs to each participant's maximal response (MEP_{max}). Facilitation of MEPs by voluntary contractions is mediated by both cortical and spinal mechanisms whereby cortical mechanisms appear to dominate at contractions of 50% MVA or more (Di Lazzaro et al., 2008; Ugawa et al., 1995). For instance, voluntary contractions increase the amplitude and number of I-waves recorded in descending volleys evoked by TMS (Di Lazzaro et al., 1998) to provide a larger activation of spinal motoneurons and, thus, larger MEPs. The degree to which voluntary muscle activity facilitates descending volleys evoked by TMS is reduced in the CP group. This is likely due to disturbances in the voluntary activation of cortical circuits and/or damage to the descending tracts themselves as described below. Given that spinal circuits are either more excitable or unchanged in people with spastic CP (Achache et al., 2010; Condliffe et al., 2016), the reduced voluntary facilitation of MEPs may be predominantly mediated by a reduced facilitation of descending volleys. Previous findings support this argument. At matched levels of MVA, the weaker *voluntary* drive in the CP group likely resulted in lower recruitment of soleus motoneurons compared to the NI controls (Rose and McGill, 2005). As a result, voluntary activity must be increased further before the proportion of the motoneuron pool available for activation by TMS reaches a comparable magnitude to that of the NI controls. The reduced efficacy of voluntary effort to facilitate the activation of the soleus motoneuron pool by corticospinal pathways may contribute to the inefficient voluntary activation of muscles in CP (Hussain et al., 2014; Stackhouse et al., 2005).

Anatomical Integrity of Corticospinal Pathways

This is one of the few studies in CP to examine the anatomical integrity of corticospinal pathways in participants over the age of 18 (Scheck et al., 2012), and it reveals that measures of DTI remain abnormal into adulthood. The pattern of reduced FA and increased $\lambda_{\perp}$ suggests reduced myelination of the corticospinal pathways or axonal loss within them (Concha et al., 2006; Song et al., 2002), the latter more probable given the similar MEP latencies between the CP and NI groups. In addition, alterations in other structural components, such as axonal diameter and packing density, could play a role (Beaulieu, 2002). Such anatomical abnormalities

in the corticospinal pathways may have mediated, in part, the reductions in soleus MEP responses in this study. This is supported by the finding that fractional anisotropy was associated with maximal MEP amplitudes.

Implications

We can conclude that adults with bilateral spastic CP demonstrate impaired activation and recruitment of low- and high-threshold corticospinal pathways to soleus motoneurons by TMS. These impairments may be indicative of reduced function in corticospinal pathways that mediate difficulties with voluntary movement including weakness, particularly as it contributes to reduced push-off in gait (Eek et al., 2011) and during graded contractions required for skilled walking. However it is important to note that there are likely differences in the activation of corticospinal pathways by TMS compared to the physiological activation of this pathway during functional movements. Future studies examining TMS responses during different phases of movement may shed light on how early brain injury affects the functional organization and recruitment of corticospinal pathways in adult CP.

References

Achache V, Roche N, Lamy JC, Boakye M, Lackmy A, Gastal A, Quentin V, Katz R. Transmission within several spinal pathways in adults with cerebral palsy. Brain 2010;133:1470-1483.

Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 2002;15:435-455.

Berger W, Quintern J, Dietz V. Pathophysiology of gait in children with cerebral palsy. Electroencephalogr Clin Neurophysiol 1982;53:538-548.

Berweck S, Walther M, Brodbeck V, Wagner N, Koerte I, Henschel V, Juenger H, Staudt M, Mall V. Abnormal motor cortex excitability in congenital stroke. Pediatr Res 2008;63:84-88.

Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther 1987;67:206-207.

Carr LJ, Harrison LM, Evans AL, Stephens JA. Patterns of central motor reorganization in hemiplegic cerebral palsy. Brain 1993;116 (Pt 5):1223-1247.

Concha L, Gross DW, Wheatley BM, Beaulieu C. Diffusion tensor imaging of time-dependent axonal and myelin degradation after corpus callosotomy in epilepsy patients. Neuroimage 2006;32:1090-1099.

Condliffe EG, Jeffery DT, Emery DJ, Gorassini MA. Spinal inhibition and motor function in adults with spastic cerebral palsy. J Physiol 2016.

Davey NJ, Smith HC, Savic G, Maskill DW, Ellaway PH, Frankel HL. Comparison of input-output patterns in the corticospinal system of normal subjects and incomplete spinal cord injured patients. Exp Brain Res 1999;127:382-390.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol 1998;508 (Pt 2):625-633.

Di Lazzaro V, Ziemann U, Lemon RN. State of the art: Physiology of transcranial motor cortex stimulation. Brain stimulation 2008;1:345-362.

Dobson F, Morris ME, Baker R, Graham HK. Gait classification in children with cerebral palsy: A systematic review. Gait Posture 2007;25:140-152.

Eek MN, Tranberg R, Beckung E. Muscle strength and kinetic gait pattern in children with bilateral spastic CP. Gait Posture 2011;33:333-337.

Eyre JA, Smith M, Dabydeen L, Clowry GJ, Petacchi E, Battini R, Guzzetta A, Cioni G. Is hemiplegic cerebral palsy equivalent to amblyopia of the corticospinal system? Ann Neurol 2007;62:493-503.

Fisher KM, Zaaimi B, Baker SN. Reticular formation responses to magnetic brain stimulation of primary motor cortex. J Physiol 2012;590:4045-4060.

Heinen F, Kirschner J, Fietzek U, Glocker FX, Mall V, Korinthenberg R. Absence of transcallosal inhibition in adolescents with diplegic cerebral palsy. Muscle Nerve 1999;22:255-257.

Hislop HJ, Avers D, Brown M, Daniels L. Daniels and worthingham's muscle testing : Techniques of manual examination and performance testing. 9th ed. St. Louis, Mo.: Elsevier/Saunders; 2014.

Hussain AW, Onambele GL, Williams AG, Morse CI. Muscle size, activation and coactivation in adults with cerebral palsy. Muscle Nerve 2014;49:76-83.

Kesar TM, Sawaki L, Burdette JH, Cabrera MN, Kolaski K, Smith BP, O'Shea TM, Koman LA, Wittenberg GF. Motor cortical functional geometry in cerebral palsy and its relationship to disability. Clin Neurophysiol 2012;123:1383-1390.

Koerte I, Pelavin P, Kirmess B, Fuchs T, Berweck S, Laubender RP, Borggraefe I, Schroeder S, Danek A, Rummeny C, Reiser M, Kubicki M, Shenton ME, Ertl-Wagner B, Heinen F. Anisotropy of transcallosal motor fibres indicates functional impairment in children with periventricular leukomalacia. Dev Med Child Neurol 2011;53:179-186.

Koman LA, Smith BP, Shilt JS. Cerebral palsy. Lancet 2004;363:1619-1631.

Lebel C, Gee M, Camicioli R, Wieler M, Martin W, Beaulieu C. Diffusion tensor imaging of white matter tract evolution over the lifespan. Neuroimage 2012;60:340-352.

Leemans A, Jeurissen G, Sijbers J, Jones DK. Exploredti: A graphical toobox for processing, analyzing, and visualizing diffusion MR data. 17th Annual Meeting of Intl Soc Mag Reson Med. Hawaii, USA2009. p. 3537.

Maegaki Y, Maeoka Y, Ishii S, Eda I, Ohtagaki A, Kitahara T, Suzuki N, Yoshino K, Ieshima A, Koeda T, Takeshita K. Central motor reorganization in cerebral palsy patients with bilateral cerebral lesions. Pediatr Res 1999;45:559-567.

Maertens de Noordhout A, Pepin JL, Gerard P, Delwaide PJ. Facilitation of responses to motor cortex stimulation: Effects of isometric voluntary contraction. Ann Neurol 1992;32:365-370.

Milner-Brown HS, Penn RD. Pathophysiological mechanisms in cerebral palsy. J Neurol Neurosurg Psychiatry 1979;42:606-618.

Mockford M, Caulton JM. The pathophysiological basis of weakness in children with cerebral palsy. Pediatr Phys Ther 2010;22:222-233.

Morita H, Olivier E, Baumgarten J, Petersen NT, Christensen LO, Nielsen JB. Differential changes in corticospinal and ia input to tibialis anterior and soleus motor neurones during voluntary contraction in man. Acta Physiol Scand 2000;170:65-76.

Palisano RJ, Rosenbaum P, Bartlett D, Livingston MH. Content validity of the expanded and revised gross motor function classification system. Dev Med Child Neurol 2008;50:744-750.

Penn RD, Savoy SM, Corcos D, Latash M, Gottlieb G, Parke B, Kroin JS. Intrathecal baclofen for severe spinal spasticity. N Engl J Med 1989;320:1517-1521.

Pitcher JB, Ogston KM, Miles TS. Age and sex differences in human motor cortex input-output characteristics. J Physiol 2002;546:605-613.

Reid SM, Dagia CD, Ditchfield MR, Carlin JB, Reddihough DS. Population-based studies of brain imaging patterns in cerebral palsy. Dev Med Child Neurol 2014;56:222-232.

Rha DW, Chang WH, Kim J, Sim EG, Park ES. Comparing quantitative tractography metrics of motor and sensory pathways in children with periventricular leukomalacia and different levels of gross motor function. Neuroradiology 2012;54:615-621.

Rose J, McGill KC. Neuromuscular activation and motor-unit firing characteristics in cerebral palsy. Dev Med Child Neurol 2005;47:329-336.

Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M. A report: The definition and classification of cerebral palsy - april 2006. Dev Med Child Neurol 2007;49:8-14.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008-2039.

Scheck SM, Boyd RN, Rose SE. New insights into the pathology of white matter tracts in cerebral palsy from diffusion magnetic resonance imaging: A systematic review. Dev Med Child Neurol 2012;54:684-696.

Shevell MI, Dagenais L, Hall N. The relationship of cerebral palsy subtype and functional motor impairment: A population-based study. Dev Med Child Neurol 2009;51:872-877.

Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 2002;17:1429-1436.

Stackhouse SK, Binder-Macleod SA, Lee SC. Voluntary muscle activation, contractile properties, and fatigability in children with and without cerebral palsy. Muscle Nerve 2005;31:594-601.

Towsley K, Shevell MI, Dagenais L. Population-based study of neuroimaging findings in children with cerebral palsy. Eur J Paediatr Neurol 2011;15:29-35.

Ugawa Y, Terao Y, Hanajima R, Sakai K, Kanazawa I. Facilitatory effect of tonic voluntary contraction on responses to motor cortex stimulation. Electroencephalogr Clin Neurophysiol 1995;97:451-454.

Vry J, Linder-Lucht M, Berweck S, Bonati U, Hodapp M, Uhl M, Faist M, Mall V. Altered cortical inhibitory function in children with spastic diplegia: A TMS study. Exp Brain Res 2008;186:611-618.

Ziemann U. Pharmaco-transcranial magnetic stimulation studies of motor excitability. Handb Clin Neurol 2013;116:387-397.

Chapter 4. Spinal Inhibition and Motor Function in Adults with Spastic Cerebral Palsy

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Key Points Summary

- Abnormal activation of motoneurons in the spinal cord by sensory pathways is thought to contribute to impaired movement control and spasticity in persons with cerebral palsy.
- Here we use single motor unit recordings to show how individual motoneurons in the spinal cord respond to sensory inputs in a group of participants with cerebral palsy having different degrees of motor dysfunction.
- In participants who had problems walking independently and required assistive devices such as wheelchairs, sensory pathways only excited motoneurons in the spinal cord.
- In contrast, in participants with cerebral palsy who walked independently for long distances, sensory inputs both inhibited and excited motoneurons in the spinal cord, similar to what we found in uninjured control participants.
- These findings demonstrate that in individuals with severe cerebral palsy, inhibitory control of motoneurons from sensory pathways is reduced and may contribute to motor dysfunction and spasticity.

Introduction

Spasticity in cerebral palsy (CP) contributes to deficits in the control of posture and movement and is the primary motor impairment for 90% of those affected with CP (Reid et al., 2011; Shevell et al., 2009). The clinical symptoms of spasticity in CP include hyperreflexia, clonus, spasms, co-contraction and improper timing of muscle activity (Downing et al., 2009; Milner-Brown and Penn, 1979; Poon and Hui-Chan, 2009). The mechanisms producing spasticity in CP are not completely understood. However, we know that damage to the brain and descending motor pathways during early development can result in enhanced activation of spinal motoneurons by peripheral reflex pathways (Evans et al., 1991; Gibbs et al., 1999). In a study of 28 participants with mainly bilateral spastic CP, indirect evidence from reflex recordings measured at rest suggest reduced presynaptic inhibition of Ia afferents and increased activation of excitatory propriospinal pathways but no evidence of reduced excitability of inhibitory

pathways to motoneurons (Achache et al., 2010). Here, we examine more directly with single motor unit recordings if there are systematic abnormalities in the activation of inhibitory post synaptic potentials (IPSPs) in motoneurons by sensory pathways in a similar population of participants with bilateral, spastic CP as examined in the Achache et al. 2010 study. It is important to understand if inhibition of motoneurons by sensory inputs is abnormal in CP to determine if this may be a contributing factor to the production of spasticity and abnormal motor function.

To estimate the profile of excitatory and inhibitory post-synaptic potentials (EPSP/IPSPs) evoked in motoneurons, we used the technique of peri-stimulus frequencygrams (PSFs) whereby the tonic discharge rate of single motor units is plotted time-locked to the occurrence of a sensory stimulation (Turker and Powers, 2005). Unlike surface EMG profiles, the firing rate profile of a motoneuron is a more accurate representation of its underlying post-synaptic potential. For example, during an EPSP the firing rate of a motoneuron increases above the mean tonic discharge rate and the overlay of multiple frequency profiles closely follows the trajectory of the underlying membrane potential, especially for profiles lasting longer than 50 ms (Norton et al., 2008; Turker and Powers, 1999). Likewise, during a very weak IPSP, the firing rate of a motoneuron drops below the tonic discharge rate in line with the reduced synaptic potential {Turker, 1999 #3067}. A strong IPSP on the other hand is marked by a pause in the tonic discharge of the motoneuron during the hyperpolarizing phase of the IPSP with a resumption of firing occurring at rates near or slightly below the tonic discharge rate during the repolarization phase of the IPSP (Norton et al., 2008; Turker and Powers, 1999). Thus, the PSF can provide an indication of the amplitude (i.e. profile) of the underlying post-synaptic potential. In contrast, surface EMG activity is dominated by the occurrence, and not frequency, of motor unit discharge so that its profile during a reflex response can give misleading information about the profile of the post-synaptic potential (Powers and Turker, 2010a). This is especially problematic during the later components of a post-synaptic potential given that a large transient input, such as an EPSP or IPSP, can synchronize the discharge of a tonically-firing motoneuron to produce false indications of repeated IPSPs and EPSPs in the surface EMG.

The use of PSFs have revealed that IPSP activation in motoneurons from stimulation of cutaneomuscular afferents is reduced after spinal cord injury, resulting in the activation of a pure, prolonged EPSP. This prolonged EPSP increases the probability of triggering self-sustained discharge of motoneurons during involuntary muscle spasms, a common feature of spasticity in spinal cord injury (Norton et al., 2008). In this study, we also examined post-synaptic potentials in motoneurons that were activated by cutaneomuscular afferents given that both excitatory and inhibitory interneurons are activated in this reflex pathway (Pinter et al., 1982). This allowed us to examine if there was a general loss of sensory-evoked IPSPs in spinal motoneurons to help explain why reflexes are heightened in CP where damage to descending motor pathways occurs during early development. Moreover, we could examine the activation of IPSPs during voluntary contractions given that impairments of inhibitory pathways, like reciprocal and recurrent inhibition, are only revealed in CP and stroke during voluntary contractions (Crone et al., 2003; Katz and Pierrot-Deseilligny, 1999) compared to when measured at rest (Achache et al., 2010; Leonard et al., 2006). We evaluated sensory activation of motoneurons supplying the soleus muscle which is particularly spastic in CP (Elder et al., 2003) and involved in gait impairment (Eek et al., 2011). Finally, given that the strength of inhibition evoked in motoneurons can be reduced by inactivity following spinal cord injury (Boulenguez et al., 2010; Murray et al., 2011) and restored by intensive exercise (Cote et al., 2014), we recruited a group of participants with CP with varying levels of motor dysfunction to examine if the strength of motoneuron inhibition was associated with clinical measures of gross motor and walking function.

Methods

Ethical Approval and Participants

This study was approved by the Health Research Ethics Board at the University of Alberta, in accordance with the Declaration of Helsinki. All participants provided informed consent in writing. Seventeen adults with bilateral spastic CP (10 female, 7 male) and with an average age of 32.8 ± 11.1 years (range 19-56) participated in this study. Fifteen participants who were neurologically intact (NI) and with a similar gender distribution (10 female, 5 male) and average age (30.4 ± 11.6, range 18-59) were also recruited. The presence and degree of brain

injury in the participants with CP was assessed with Magnetic Resonance Imaging (MRI, described below). Participants with CP having injections of botulinum toxin into the lower leg within 6 months prior to the experiment were excluded although none had such treatment for at least 2 years. Medications taken by the participants with CP which may affect neuronal excitability are listed in Table 4-1.

Motor Assessments

Motor function of the participants with CP was evaluated by a physician (author EGC) with the results presented in Table 4-1. The Gross Motor Function Classification System (GMFCS) (Palisano et al., 2008) was used to measure functional abilities in sitting and walking and the need for assistive devices. The Functional Mobility Scale (FMS) was used to categorize the type of mobility a participant uses on a scale of 1 to 6, with 1 = uses wheelchair to 6 = independent on all surfaces, at three distances of 5, 50 and 500 m (Graham et al., 2004). The combined FMS score for all three distances (FMS_{TOTAL}) is presented in Table 4-1 with a possible maximum score of 18. Although only FMS scores at the individual distances have been validated (Adair et al., 2012), here we summed all the FMS scores because it produced a broader distribution of scores to better compare the functional walking abilities in the participants with CP than any one of the individual distances. Plantarflexor and dorsiflexor strength at the ankle was assessed with the Medical Research Council score (MRC) (Hislop et al., 2014). The modified Ashworth scale (mAsh) (Bohannon and Smith, 1987) and the Penn Spasm Frequency scale (Penn) (Penn et al., 1989) were used to confirm the presence, and not extent, of spasticity given the limited range of these scores.

MRI scans

On a separate day from the reflex experiments, all participants with CP except CP-4 with a contraindication to MRI (16/17) and 11 of the NI participants were scanned using a 1.5T Siemens Sonata scanner. Sequences included T1-weighted, T2-weighted and fluid-attenuated inversion recovery (FLAIR). A senior radiology resident and a neuroradiologist (authors DTJ and DJE respectively) reviewed the T1, T2 and FLAIR sequences. Anatomical abnormalities were classified into categories relevant for our CP population, including periventricular white matter injury (PVWMI), cerebral malformations (CM), deep gray matter injury (DGMI) and

ID	Sex	Age	Rx	MRI	GMFCS	FMS_{TOTAL}	MRC		mAsh	Penn
							Plantar	Dorsi		
CP-1	F	19		PVWMI+	I	17	5	4	0	1
CP-2	M	29	baclofen citalopram	PVWMI++/CM	I	16	4	4	3	3
CP-3	M	25		PVWMI ++	I	18	5	5	1+	1
CP-4	F	31	venlafaxine	Contraindication	II	10	5	4	1+	2
CP-5	F	34		PVWMI ++	IV	3	1	2	2	1
CP-6	M	48	citalopram tolterodine	PVWMI ++	III	3	3	1	3	2
CP-7*	F	28		Normal	II	14	5	4	1	1
CP-8*	F	33		PVWMI ++/DGMI	III	6	4	4	3	2
CP-9	F	20		PVWMI ++	III	5	1	0	1	1
CP-10	M	26		PVWMI +/CM	II	7	2	1	0	1
CP-11*	F	19	sertraline clonazepam	PVWMI ++/CM	IV	3	2	1	1	2
CP-12	M	23		PVWMI +	IV	3	2	4	1	1
CP-13	M	42		PVWMI +	III	4	2	4	1	2
CP-14	F	38		Normal	III	9	1	2	1+	1
CP-15	F	51	amitriptyline citalopram phenytoin flunarizine	PVWMI +++/CVA	II	9	1	3	2	2
CP-16	F	56		PVWMI +	I	17	5	5	0	1
CP-17	M	30		PVWMI ++	II	14	4	1	1	1

Table 4-1 Characteristics of Participants with CP. Columns represent the participant demographics (Sex & Age), daily medications (Rx), the MRI findings, the Gross Motor Functional Classification System (GMFCS) rating, the total Functional Mobility Scale (FMS_{TOTAL}) score with a maximum score of 18, the strength (MRC) of the plantarflexors (Plantar) and dorsiflexors (Dorsi) with a maximum score of 5, the modified Ashworth score (mAsh) for the plantarflexors with a maximum score of 5 and the Penn Spasm Frequency scale (Penn) where 1= mild spasms induced by stimulation, 2 = infrequent full spasms occurring < 1/hr, 3 = spasms occurring > 1/hr). * indicates the three CP participants from whom motor unit data were not obtained. PVWMI = Periventricular White Matter Injury, CM = Cerebral Malformations, DGMI = Deep Gray Matter Injury, CVA = Cerebrovascular Accident.

normal brains (Towsley et al., 2011). The severity of any periventricular white matter injury was rated qualitatively as mild, moderate or severe based on cerebral white matter volume loss and T2 hyper-intensities by consensus between the two radiologists who were blinded to the clinical scores.

Cutaneomuscular Reflexes

In the participants with CP, the leg which interfered more with motor function was tested (n = 8 left, n = 9 right). The right leg was tested in the NI group except in 3 participants with previous injuries to the right ankle. Cutaneomuscular reflexes were elicited by a brief stimulus train (15 ms) delivered to the medial arch of the foot (5 pulses, 200 µs pulse width, 300 Hz with a 3-second interstimulus interval) using a DS7A constant-current stimulator (NL703, Digitimer, Welwyn Garden City, UK). The intensity of stimulation was set just below pain threshold to elicit a clear response in the surface EMG within the average of a few (<10) trials.

Motor Unit Recordings

Sixteen of the 17 participants with CP agreed to take part in this component of the experiment (CP-11 declined) and 13 NI controls were recruited to participate. Fine-wire electrodes made of stainless steel and with 50µm de-insulated ends (California Fine Wire, 304, H-ML) were used to record motor unit activity from the soleus muscle. A 21-gauge needle was used to guide the fine wires into the soleus muscle. Intronix hardware (Intronix Technology Corporation, Bolton, Canada) was used to amplify (5k gain) and filter (200 Hz-20 kHz) the fine wire EMG signal. A Power1401 A/D and Spike 2 software (version 6, Cambridge Electronic Design Ltd., Cambridge, UK) were used to digitize the fine-wire EMG recordings at a sampling rate of 25 kHz. Surface EMG from the soleus and lateral gastrocnemius was also recorded with Intronix hardware at 1k gain, 20 Hz - 2.5 kHz bandpass filter and at a sampling rate of 5 kHz. Participants were instructed to tonically plantarflex the ankle to produce steady firing of soleus motor units to a target of about 7 Hz. Auditory feedback of motor unit activity, as well as verbal cues, were provided to maintain sufficient and steady firing rates of the motor units. Most participants lay prone with their foot off the edge of a plinth pushing against a secured board. A few participants in each group were tested sitting or standing but this did not produce systematic differences in reflex responses. We aimed to deliver at least 300 stimuli to the medial arch of the foot (478 ± 190 stimuli was applied on average), typically in blocks of 50, and participants could rest at any time.

Surface EMG recordings

To quantify cutaneomuscular reflexes without the insertion of fine wires into the soleus muscle, which can produce added mechanical sensory activation during muscle contractions, all participants with CP (n = 17) returned for another experiment on a different day where only surface EMG was recorded along with 14 of the 15 NI controls. Surface EMG was recorded using pairs of conductive adhesive hydrogel electrodes (3.81 x 2.24 cm, Covidien Ltd., Dublin, Ireland) at 1 k gain and 10-1000 Hz bandpass filtering (AMT-8, Bortec Biomedical Ltd., Calgary, Canada), digitized and sampled at 5 kHz using Axon hardware (Digidata 1440A) and software (Axoscope 10.3, Molecular Devices LLC, Sunnyvale, USA). All participants were seated with their upper leg secured to a chair, the knee in 90° of flexion and the ankle secured in a custom binding at 0° of plantarflexion (±5°). With the knee positioned at 90° of flexion, all participants with CP were able to comfortably reach and maintain a consistent ankle position for the surface EMG recordings without significant dorsiflexion stretch to reduce the impact of contracture on the surface EMG recordings.

Cutaneomuscular reflexes were recorded while participants voluntarily activated their soleus to a target voluntary contraction of 20% maximum (MVC). Thirty-five cutaneomuscular reflexes at 20% MVC were collected and opportunities for rest were provided. A participant's MVC was measured at the start of the experiment. For MVC, the surface EMG was digitally band-pass filtered (10-500 Hz), rectified and then smoothed with a 1-s moving-window average applied every 1 ms. At least 60 s of rest was given between each MVC trial. The MVC was calculated from averaging the peak EMG, lasting at least 1 s, from two trials that were within 10% of each other.

Data Processing of Cutaneomuscular Reflexes (CMRs)

Peri-stimulus Frequencygrams (PSFs) and Post-Stimulus Time Histograms (PSTHs)

Motor units were fully identified off-line using Spike 2 wavemarks and manual discrimination. Single motor unit waveforms could be reliably identified in 14 of the 16 participants with CP tested and in all of the NI participants tested (n=13). PSFs and PSTHs were constructed using custom Matlab software (R2011b, The Mathworks Inc., Natick, USA). For the

PSFs, the reciprocal of the interval between spikes was calculated to give instantaneous firing rates which were then plotted at the end of each interspike interval and aligned to the onset of the stimulus train. Multiple stimulation trials were superimposed to generate the PSF. In 8 cases, the firing rate profiles of more than one motor unit were used to construct the PSF for a total of 35 motor units that were fully analyzed. Only trials with motor unit firing rates that were within 2 standard deviations of the mean pre-stimulus rate for at least 1 second before the stimulation were included. For visualization purposes, the mean firing rate of the PSF was calculated with a moving-window average over 10 ms of data and plotted every 1 ms. PSTHs were generated by counting the number of spikes occurring within a 10 ms bin and dividing by the number of stimulation trials to align count values between PSTHs having different trial numbers.

Surface EMG

Both excitatory and inhibitory components of the cutaneomuscular reflex were measured from the data collected on the second recording day. To mark the different components of the reflex, surface EMG from the Axoscope files were digitally bandpass filtered (10-500 Hz, 0 phase shift) prior to rectification using a custom Matlab program. The mean level of background EMG over the 100 ms prior to stimulation was calculated (dotted horizontal lines in Fig. 4-1). Only the first 25 trials having a background EMG that fluctuated <10% of MVC were averaged together. To compare cutaneomuscular reflexes between participants, the averaged EMG response was expressed as a percentage of the mean, pre-stimulus background EMG for each participant (i.e., as 100% of the background EMG). To more readily appreciate how EMG activity during the cutaneomuscular reflex fluctuated from the pre-stimulus background, the averaged EMG amplitude was then expressed as an absolute change from the mean background EMG (Fig. 4-1). For example, the mean background EMG was indicated as 0% change (Δ %BKD EMG) and any EMG activity above and below this were indicated by positive and negative values respectively.

In these experiments, the majority of cutaneomuscular reflexes started with an initial onset of EMG suppression that was termed I1 (Fig. 4-1A). The onset of I1 was determined visually as the time point when the EMG signal fell below 2 standard deviations of the mean, pre-stimulus EMG for 20 ms or more (I1 onset is marked by the first vertical line in Fig. 4-1A).

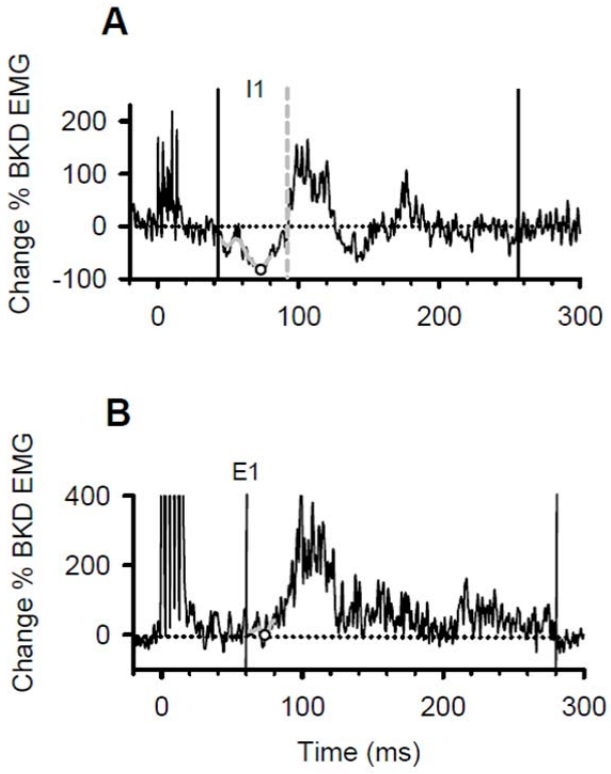

Figure 4-1 Components of the Cutaneomuscular Reflex. A) Example of cutaneomuscular reflex that started with an EMG suppression (I1). The onset of I1 is marked by the first black vertical line and its termination by the dashed vertical grey line as the EMG falls below and returns to the mean pre-stimulus EMG as marked by the dashed horizontal line. The pre-stimulus EMG is expressed as a 0% Change (Δ) BKD EMG value. The maximum suppression of EMG activity between reflex onset and before 80 ms ($I1_{max}$) is marked by the white circle taken from the moving-window average (solid grey line). The termination of the cutaneomuscular reflex is marked by the second black vertical line when the EMG response returns to the pre-stimulus baseline. **B)** Same as in A but for a cutaneomuscular reflex with no EMG suppression and only an excitatory response with the onset of the early component (E1) marked by the first black vertical line. Here I1max is marked by the lowest point on the moving-window average between the onset of the reflex and 80 ms. I1 = first inhibitory response; $I1_{max}$ = maximal amount of EMG suppression within I1; E1 = first excitatory response.

The termination of the reflex was determined when the EMG signal returned to baseline (at the gray dashed vertical line). The magnitude of EMG suppression ($I1_{ave}$) was measured as the average EMG during the duration of the I1 phase. To measure EMG suppression from mainly spinal mechanisms, the maximum amount of EMG suppression within I1 ($I1_{max}$) was measured

by applying a 10-ms moving-window average (grey line) that was plotted every 0.5 ms to filter out very fast transients and by measuring the minimum value from response onset to 80 ms post-stimulation (marked by circle in Fig. 4-1A). An 80 ms cut-off was used because components of the cutaneomuscular reflex 80 ms and earlier are likely spinal in origin and not activated by transcortical pathways (Nielsen et al., 1997). In some participants with CP, there was no clear I1 but rather a single excitatory response was evoked (Fig. 4-1B). Here, the onset of EMG facilitation above the pre-stimulus background was determined (E1: first solid vertical line) and the minimum value ($I1_{max}$) out to 80 ms was measured from the window average (marked by circle). The termination of the cutaneomuscular reflex was measured as the time point when the EMG returned to the pre-stimulus background (second solid vertical line, Fig. 4-1A and B).

Statistics:

Statistical analysis was performed in SPSS (version 21, IBM, Armonk, NY). Differences in motor unit firing rates and features of the cutaneomuscular reflex (e.g., $I1_{ave}$, $I1_{max}$, etc.) between the CP and NI groups was determined using a Mann-Whitney U-test because the data were not normally distributed as tested using the Shapiro-Wilk test. Median and range values are presented for this data. Mean and standard deviation (±SD) were used to describe normally distributed data. In the CP group, Spearman rank correlations were used to measure associations between motor assessments (GMFCS, FMS_{TOTAL}, MVC) and EMG-based physiological measures ($I1_{ave,}$ $I1_{max}$). The alpha level for significance was set at 0.05.

Results

Functional Motor Impairments

The cohort of participants with bilateral, spastic CP had a range of functional motor impairments (Table 4-1). General gross motor disabilities, as measured by the Gross Motor Function Classification System (GMFCS), ranged from visible impairments only when running (e.g., CP-3 functioning at a GMFCS level I) to the inability to walk, requiring a power wheelchair with trunk support (e.g., CP-12 functioning at a GMFCS level IV). In general, participants were somewhat evenly distributed between the four GMFCS categories with 4 in level I, 5 in levels II and III and 3 in level IV. In specific measures of walking function, 4 out of

17 participants with CP required a wheelchair to ambulate 5, 50 or 500 m, as indicated by a total Functional Mobility Scale (FMS_{TOTAL}) score of 3, whereas 4/17 could walk independently at all distances using no aids, resulting in a FMS_{TOTAL} score of ≥ 16. The remainder of participants with CP had a mixture of walking abilities in between.

Approximately half of the participants with CP (9/17) could, at a minimum, plantarflex their ankle against gravity throughout their available range of motion, as reflected in an MRC score of 3 or more. The remaining participants with CP (8/17) could only plantarflex their ankle through the full range of movement with gravity eliminated (MRC 2) or only produce a flicker or trace contraction (MRC 1). Plantarflexor MRC scores were often not equal to dorsiflexor scores (Table 4-1). Despite the range of motor disabilities, all participants with CP had spasticity as their primary motor impairment. In agreement with the diagnosis of spastic CP, most participants (14/17) had discernible muscle tone in response to passive ankle dorsiflexion (modified Ashworth Scores ≥1) and all reported the presence of involuntary muscle spasms as assessed by the Penn Spasm Frequency scale (Table 4-1).

Brain Imaging

Two of the 16 participants with CP who underwent MRI had scans comparable to the NI participants with no obvious signs of brain injury or structural malformations (CP-7, CP-14, Fig. 4-2). This is in agreement with previous reports demonstrating that ~13% of participants with CP have normal MRI scans (Reid et al., 2014; Towsley et al., 2011). The remainder had some evidence of periventricular white matter injury (PVWMI) on both sides of the brain as marked by hyperintensities (white areas) on the FLAIR sequences and loss of white matter. Most participants with CP (13/16) had mild (+) or moderate (++) periventricular white matter injury as determined qualitatively by two radiologists blinded to clinical presentation. Five participants with CP had additional findings including three with cerebral malformations (CM), such as polymicrogyria, and one with a deep gray matter injury (DGMI). Severe periventricular white matter injury (+++), particularly on the left but also present on the right, was observed in CP-15 along with evidence suggestive of periventricular ischemia.

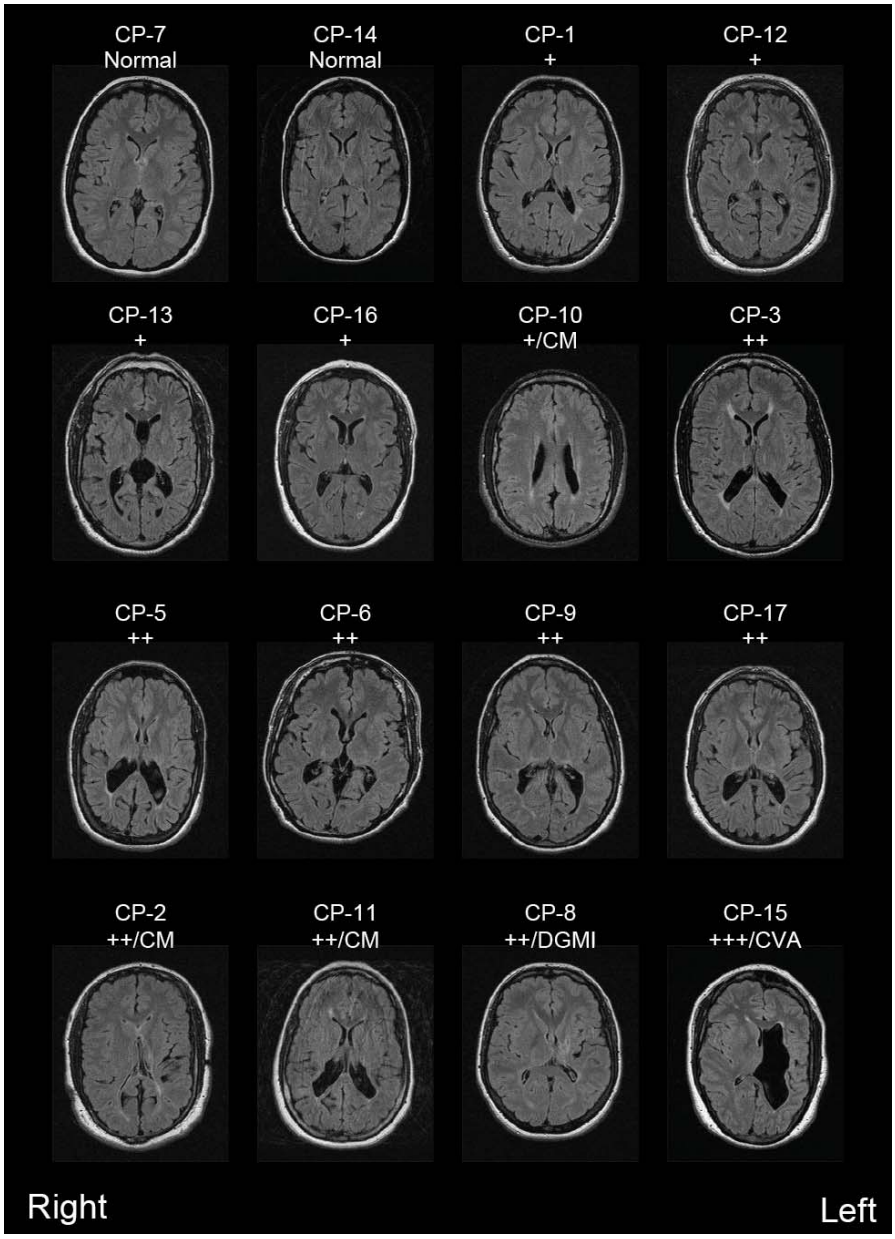

Figure 4-2 MRI Scans. Example axial FLAIR slices from each participant with CP imaged. Slices were selected at similar axial locations across participants except for those with cerebral malformations (CM) or deep gray matter injury (DGMI) where the slice was chosen to best display the abnormality. All participants with abnormal imaging had evidence of mild (+), moderate (++) or severe (+++) periventricular white matter injury (PVWMI) on both sides of the brain. Five participants had additional findings: CP-2 has partial agenesis of the posterior corpus callosum (a type of cerebral malformation) and evidence of a shunt tract; CP-10 & CP-11 have polymicrogyria (a type of cerebral malformation); CP-8 has injury to the deep gray matter on the left and CP-15 has evidence of a perinatal cerebral vascular accident (CVA).

Sensory Activation of Motoneurons: Peri-stimulus Frequencygrams (PSFs)

Similar to the clinical and MRI findings, there was a spectrum of PSF profiles in response to cutaneomuscular afferent stimulation in the CP group compared to the consistent responses produced in the NI group. Despite this, conditions for producing the PSF were well matched between the two groups. First, participants in both groups could maintain prolonged periods of steady motor unit discharge so that on average, 140±109 (SD) trials were used to construct PSF profiles in the CP group and 158±72 trials in the NI group (p=0.17). Second, the median pre-stimulus firing rate of the motor units in the NI group was 6.7 Hz (5.7 - 8.2 Hz) and appeared similar to the median rate maintained in the CP group at 6.8 Hz (4.9 - 10.0 Hz, p=0.94). Third, the median stimulation intensity applied to the medial arch of the foot to evoke the cutaneomuscular reflex appeared similar between the two groups [NI: 20.2 mA (15.0 - 30.0 mA) vs. CP: 22.0 mA (11.0 - 42.6 mA), p=0.57].

In all but one of the NI participants (12/13), there was a marked pause in motor unit firing near the onset of the cutaneomuscular reflex to reflect the activation of a strong IPSP. As shown in Figure 4-3A, in half of the NI participants, the pause in motor unit firing depicted in the PSF (bottom trace) and reflected in the corresponding suppression of EMG activity (top two traces), occurred *without* a prior excitatory (E1) response. This pause was also reflected in the reduced occurrence of motor unit discharge in the post-stimulus time histogram (PSTH: third trace, Fig. 4-3A). In the other half of NI participants, a brief increase in firing rate, occurrence of motor unit discharge and EMG activity (E1) preceded the pause (Fig. 4-3B). In both cases, the firing rates after the pause fell slightly below the mean pre-stimulus rate (marked by dotted horizontal line), indicating a resumption of firing on the repolarization phase of the IPSP. Following the pause or decrease in motor unit discharge, firing rates would often increase above the pre-stimulus rate to indicate a subsequent activation of an EPSP as readily seen in Figure 4-3B.

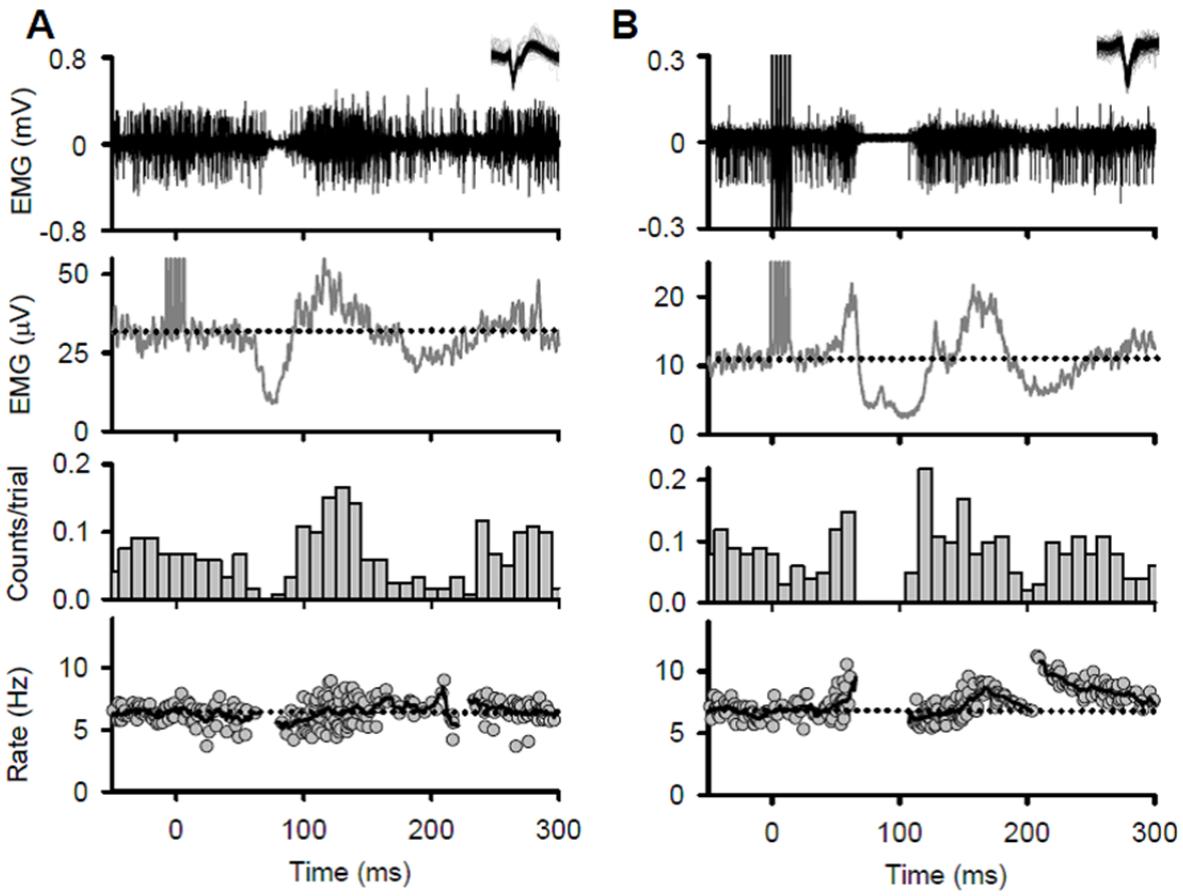

Figure 4-3 Representative PSFs in NI participants. Representative examples of the two main types of PSFs measured NI participants evoked from cutaneomuscular stimulation (at time = 0 ms). Within the PSF (bottom panel), each dot corresponds to the instantaneous firing rate of a motor unit and the thick black line is a moving-window average of those rates. Unrectified intramuscular EMG and an inset of the superimposed, isolated motor unit are displayed in the top panel (duration of motor unit waveforms 2-3 ms). **A)** An initial IPSP is indicated by a pause in firing ~60 ms post-stimulation in the PSF. A pause in firing was noted when there was a break in the PSF and low to no counts in the PSTH (third panel). Dashed horizontal lines indicate mean pre-stimulus EMG (second panel) and firing rates (bottom panel). **B)** PSF demonstrating an initial EPSP that preceded the IPSP with increased firing probability and rate near 50 ms post-stimulation. Subsequent IPSP is indicated by a pause in firing starting at 60 ms and a resumption of firing near 120 ms with firing rates slightly below baseline. PSF = peri-stimulus frequencygram; PSTH = peri-stimulus time histogram.

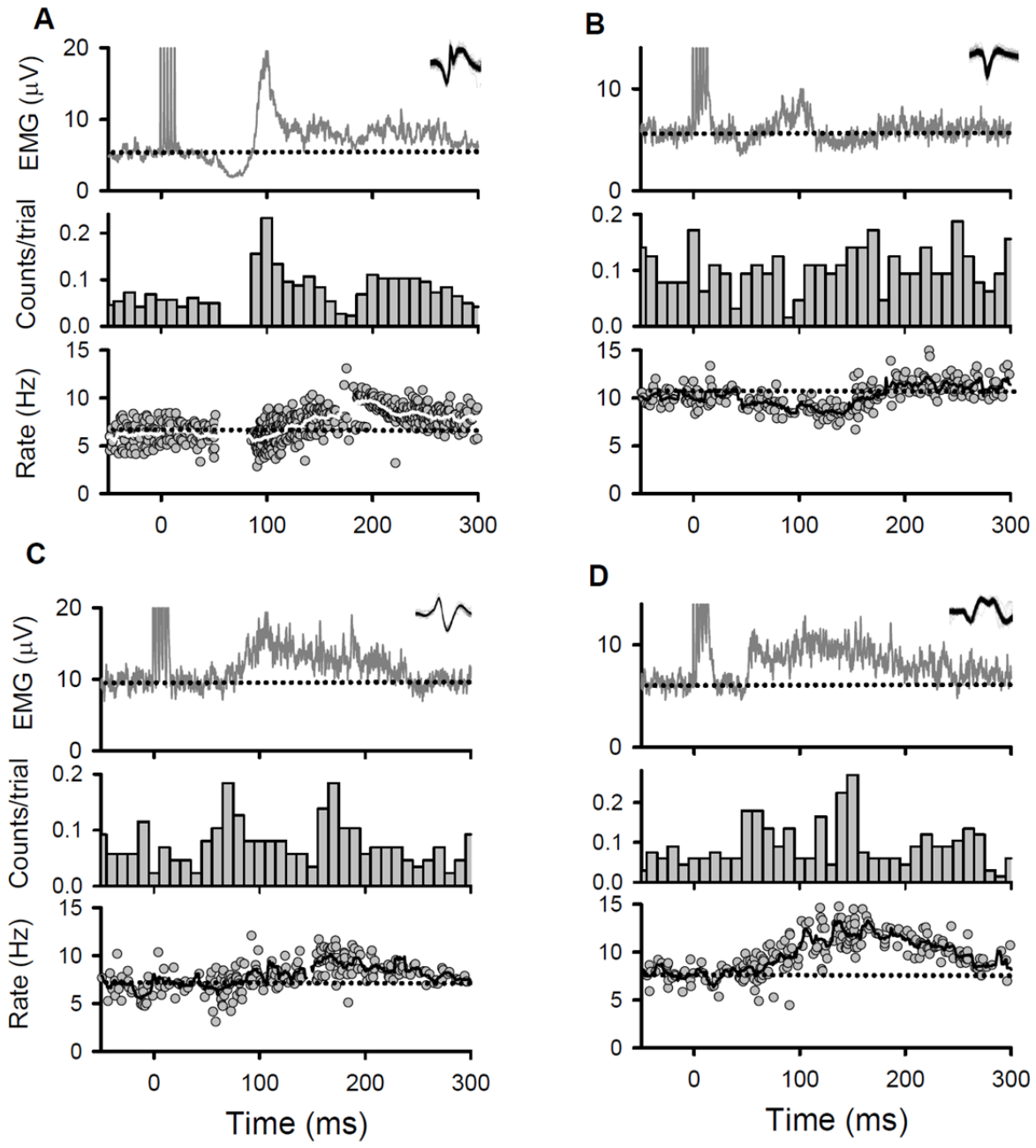

Figure 4-4 Representative PSFs in participants with CP. Same as in Figure 4-3 but for representative PSFs from four participants with CP. **A)** PSF indicating a strong initial IPSP with a pause in firing starting near 60 ms and followed by an EPSP (CP-2). Note the line in the PSF marking the moving window average is white for better visualization. **B)** PSF indicating a weak IPSP as marked by a decrease in the firing rate beginning near 45 ms post-stimulation (CP-17). **C** and **D)** Pure EPSP indicated by increases in the firing rate starting near 50 ms post-stimulation and continuing to 300 ms (CP-13, CP-6). PSF = peri-stimulus frequencygram.

In 8 of the 14 participants with CP, a pause (Fig. 4-4A) or transient decrease in motor unit firing rates below the pre-stimulus level (Fig. 4-4B) also occurred at the start of the cutaneomuscular reflex, signifying a strong and mild activation, respectively, of an IPSP. Similar to the NI control group, following the resumption of firing after the pause, motor unit firing rates would increase above the pre-stimulus level, as shown in Figure 4-4A, to indicate the presence of a subsequent EPSP. The participant in Figure 4-4A had good motor function with a GMFCS score of I and a FMS_{TOTAL} score of 16 and likewise, the participant in Figure 4-4B had a GMFCS score of II and FMS_{TOTAL} score of 14. Interestingly, in the remainder of the participants with CP (6/14), there were no consistent pauses or decreases in motor unit firing rates below the pre-stimulus level. Rather, the PSF consisted only of a gradual increase and then return of motor unit firing rates to the pre-stimulus level (Figs. 4-4C and D). Such a PSF profile is indicative of a single, long-duration EPSP and similar to the profiles previously recorded in participants with chronic spinal cord injury (Norton et al., 2008). The participants in Figures 4-4C and D had poor motor function, both having GMFCS scores of III and FMS_{TOTAL} scores of 4 and 3 respectively. In all cases for both CP and NI groups, the mean motor unit firing rates returned to the mean pre-stimulus rate by 600 ms.

In summary, in 8 of the 14 participants with CP there was evidence for the sensory-evoked activation of an IPSP followed by an EPSP in soleus motoneurons that was similar to NI controls. In the other 6 of participants with CP, only an EPSP was evident from the PSF profiles, similar to that shown previously in participants with chronic spinal cord injury.

Sensory Activation of Motoneurons: Surface EMG

Participants with CP that lacked evidence for the activation of an IPSP, namely a pause or decrease in motor unit firing rates below the pre-stimulus level, also had more severe deficits in voluntary muscle activation and walking function. Thus, to examine if the strength of IPSP activation was associated with motor dysfunction, we quantified the amount of early-onset EMG suppression (I1) within the cutaneomuscular reflex. Based on the PSF profiles we were confident that the magnitude of early-onset EMG suppression was likely related to the magnitude of IPSP activation, even for reflexes where EMG suppression occurred with a prior excitation (E1). To avoid extraneous sensory inputs that could affect the magnitude of EMG suppression,

cutaneomuscular reflexes were measured on a separate recording day without the insertion of fine wires into the muscle. Moreover, all participants were seated to better control positioning of the ankle and knee.

In all NI participants removing the sensory stimulation from the intramuscular wire abolished the occurrence of the early-onset excitation (E1) that could sometimes precede I1, as shown for three representative participants in Figure 4-5A. Thus, the amount of EMG suppression was likely produced by the activation of an IPSP and not influenced by motoneuron refractoriness or discharge synchronization as would occur when there is a prior motoneuron excitation (e.g., E1). To measure the amount of EMG suppression that was mediated at a *spinal* latency, the maximum amount of EMG suppression from response onset to 80 ms post-stimulation was determined ($I1_{max}$), as shown by the dots in Figure 4-5A (see *Surface EMG* in Methods for rationale). The presence of a marked EMG suppression was very consistent across all NI controls and the median $I1_{max}$ was 69.5% lower than the pre-stimulus background EMG (i.e., -69.5% Δ BKD EMG, see Table 4-2 for median and range values).

Reflex components	CP	NI	P-value
$I1_{max}$ (Δ % BKD EMG)	-36.1 (-66.7 – 68.0)	-69.5 (-87.7 – -30.3)	**<0.001**
$I1_{ave}$ (Δ % BKD EMG)	-26.4 (-46.3 – 0.0)	-48.6 (-63.6 – -21.7)	**0.001**
I1 onset (ms)	58.4 (40.7 – 60)	57.4 (43.3 – 70.9)	0.98
Cutaneomuscular reflex termination (ms)	286 (89 – 825)	273 (152 – 449)	0.30

Table 4-2 Characteristics of the Cutaneomuscular Reflex. Median (top row) and range values (bottom row) for various components of the cutaneomuscular reflex in both the CP and NI groups and the associated P-values from the Mann-Whitney U-test comparisons between the two groups. $I1_{max}$ = maximal amount of EMG suppression within the first inhibitory response. $I1_{ave}$ = average EMG during the first inhibitory response.

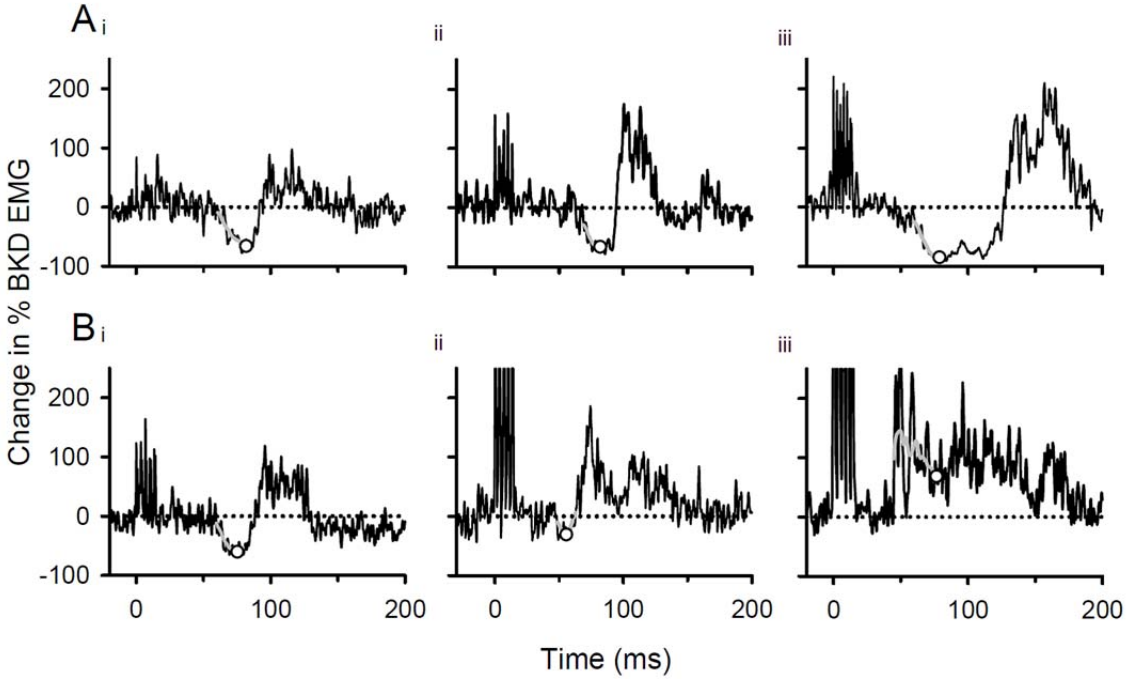

Figure 4-5 Cutaneomuscular reflexes: surface EMG. A) Black lines represent the averaged surface EMG response (25 trials) to cutaneomuscular stimulation at time = 0 ms for 3 representative NI participants (i to iii). The grey line represents the moving-window average from response onset to 80 ms post-stimulation with the lowest point on the moving-window average ($I1_{max}$) indicated by the white circle. EMG values are normalized to the mean pre-stimulation EMG (marked by the dotted horizontal line) and expressed as an absolute change (Δ) from that value (mean pre-stimulation EMG = 0% Δ). **B)** Same as in A but for 3 representative participants with CP showing progressively lower amounts of EMG suppression (i to ii) and EMG facilitation (iii). $I1_{max}$ = maximal amount of EMG suppression within the first inhibitory response.

In 10 of the 17 participants with CP, the cutaneomuscular reflex started with a sustained period of EMG suppression (I1) similar to that measured in NI participants. The amount of early-onset EMG suppression was strongest in the 4 participants with CP who could walk at least 500 m with no gait aids (e.g., Fig. 4-5Bi). In the other 6 participants with CP who had an initial I1 (6/10), the amount of EMG suppression was smaller, as shown for a representative example in Figure 4-5Bii. Very little to no sustained suppression of EMG activity was present in the remaining participants with CP (7/17). Instead, the cutaneomuscular reflex consisted solely of an

excitatory response (e.g., Fig. 4-5Biii) and this typically occurred in participants requiring wheeled mobility.

Overall, the maximum amount of EMG suppression ($I1_{max}$, see dots in Fig. 4-5) was less in the CP group at -36.1% compared to the NI control group at -69.5% ($p<0.001$). A positive $I1_{max}$ occurred when the EMG activity from response onset to 80 ms post-stimulation did not fall below the pre-stimulus background (as in Fig. 4-5Biii). Likewise, the average EMG over the entire period of EMG suppression ($I1_{ave}$) was also smaller in the CP group with a median $I1_{ave}$ of -26.4% compared to the NI control group at -48.6% ($p=0.001$, Table 4-2). Note, when there was no visible I1 in the participants with CP, an $I1_{ave}$ value of 0 was given. When present, the median onset of I1 was 58.4 ms in the CP group and appeared similar to the onset of I1 in the NI control group at 57.4 ms ($p = 0.56$, Table 4-2). Likewise, the total duration of the cutaneomuscular reflex appeared similar between the two groups with the reflex terminating at 279.2 ms post-stimulation in the CP group and at 285.7 ms in the NI group ($p = 0.78$, Table 4-2).

Associations of Spinal Inhibition to Motor Function in CP

To determine if our estimate of spinally-mediated IPSPs (i.e., $I1_{max}$) was related to the functional motor abilities of the participants with CP, both the Functional Mobility Score (FMS_{TOTAL}) and the Gross Motor Functional Classification System score (GMFCS) for each participant with CP was plotted against $I1_{max}$. There was a significant negative association between $I1_{max}$ and the FMS_{TOTAL} scores ($r = -0.82$, $p < 0.001$, Spearman Rank) indicating that as the walking ability of the participants with CP was more affected (low FMS scores), the less EMG suppression (more positive $I1_{max}$ values) and likely less IPSPs activated in the motoneurons (left graph, Fig. 4-6). Significant associations were also present for each individual FMS score at 5, 50 and 500 m (p all ≤ 0.001). In participants with CP who relied on wheeled mobility for even short distances (having $FMS_{TOTAL} = 3$), $I1_{max}$ was more positive than any value recorded in the NI group. These participants also had evidence of pure EPSPs in their PSF profiles. In the participants with CP who could walk for at least 500 m with no gait aides ($FMS_{TOTAL} \geq 16$), $I1_{max}$ values were all within the 25^{th} and 75^{th} percentile limits of the NI control group (i.e., within the grey bar of Fig. 4-6). These participants also had evidence of strong IPSP activation with marked pauses in motor unit firing in the PSF. There was also a positive

association between GMFCS and $I1_{max}$ (r=0.72, p < 0.001, Spearman Rank, right graph, Fig. 4-6), signifying that the greater impairment in functional sitting and walking abilities (as indicated by a higher GMFCS score), the lower the EMG suppression (more positive $I1_{max}$) from reduced IPSP activation. Associations remained significant when the 5 participants with CP who had additional MRI findings (e.g., cerebral malformations, deep grey matter injury and periventricular ischemia) were removed from the group (p all < 0.008). Similar to the FMS_{TOTAL} and GMFCS, there was a significant association between $I1_{max}$ and the Maximum Voluntary Contraction (MVC) that was generated during plantarflexion (not shown, r =-0.56, p=0.02). Significant associations were also produced when comparing these clinical measures with the average EMG measured across the entire EMG suppression period, i.e., $I1_{ave}$ (p all < 0.01).

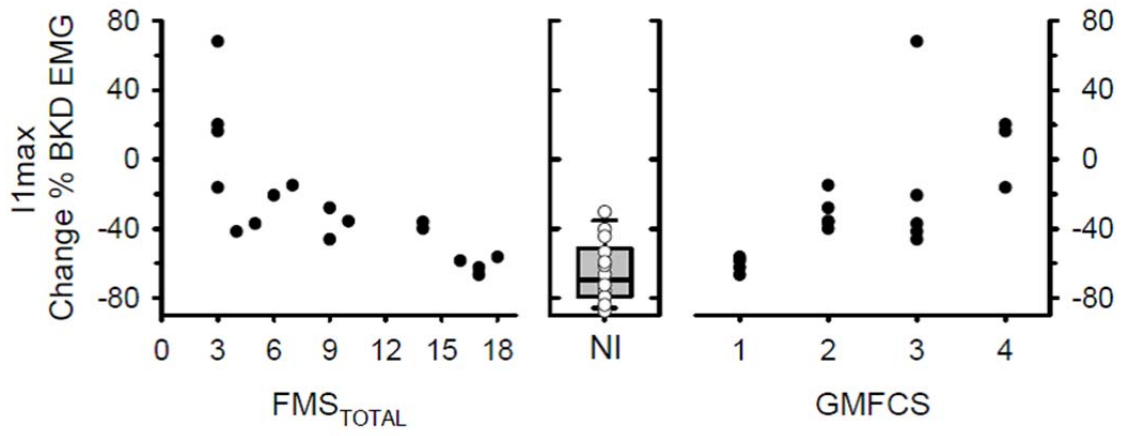

Figure 4-6 Spinal-Inhibition and Motor Function. The magnitude of $I1_{max}$ is plotted against FMS_{TOTAL} (left panel) and GMFCS (right panel) in the CP group (n = 17). The box plot in the middle panel illustrates the median (thick black line), 25th & 75th percentiles (box bounds), and 95th and 5th percentile (whiskers) of $I1_{max}$ in the NI group. The scatter of all data points for the NI group (n = 14) is also displayed over the box plot. FMS_{TOTAL} = total Functional Mobility Scale, GFMCS = Gross Motor Functional Classification System, $I1_{max}$ = maximal amount of EMG suppression within the first inhibitory response.

Discussion

General Summary

Similar to studies examining the activation of IPSPs in soleus motoneurons from tendon afferent stimulation (Binboga et al., 2011; Rogasch et al., 2012), there was consistent evidence for the activation of IPSPs in the PSF profiles of NI controls in response to cutaneomuscular afferent stimulation. In contrast, evidence for the activation of an IPSP was observed in only half of the participants with CP and in the other half, only a pure EPSP was indicated in the PSF profiles, consistent with findings from surface EMG recordings in children and adolescents with CP (Gibbs et al., 1999). As discussed below, the presence and magnitude of sensory-evoked IPSPs in the participants with CP was associated with their gross motor and walking function, suggesting that post-synaptic inhibition of motoneurons may be dependent upon motor activity.

PSFs and what they tell us about the profile of post-synaptic potentials

Although there are some limits with the PSF technique in providing an accurate measure of IPSP or EPSP amplitude (Powers and Turker, 2010b), data from intracellular experiments confirm that the *presence* of a long-lasting (> 50 ms) EPSP or IPSP can be accurately represented by the modulation in firing rate of a tonically discharging motor unit/motoneuron (Norton et al., 2008; Powers and Turker, 2010a; Turker and Powers, 2005). For example, during both the rising and falling phase of a single EPSP, the time it takes for a tonically discharging motoneuron to reach firing threshold is shortened, producing elevated firing rates above the pre-stimulus background on both the rising and falling phase of the EPSP (Norton et al., 2008; Turker and Powers, 1999). A pure, single EPSP evoked from cutaneomuscular stimulation was demonstrated in the PSF profiles for approximately half of the participants with CP (e.g., Figs. 4-4 C&D). Even though the baseline firing rates were low at ~7 Hz (Powers and Turker, 2010a), a clear EPSP-like profile emerged in the PSF. This may have been possible since at 7 Hz, the motor unit would fire every 140 ms so that two action potentials could occur over the course of a single 300 ms EPSP. Coupled with a large number of PSF trials (~150), a sufficient number of sampling points were likely obtained to measure the underlying membrane potential of the EPSP. Moreover, the PSF indicated a pure, single EPSP unlike the multiple peaks produced in the PSTH as a result of synchronization in motoneuron firing.

The profile of more complicated, multi-component post-synaptic potentials can also be reflected in the PSF profile. For example, when using current injection to mimic a strong IPSP followed by an EPSP, a PSF is produced whereby a sustained pause in motor unit firing occurs during the falling phase of the IPSP, especially when the rate of change in membrane potential is 100 mV/s or faster as this flattens and prolongs the upward trajectory of the afterhyperpolarization (AHP) (Norton et al., 2008; Turker and Powers, 2003; 2005). A cluster of resumed firing is then often produced near or slightly below the mean pre-stimulus rate as the rising phase of the IPSP accelerates the AHP potential to firing threshold (Norton et al., 2008). The firing rate then increases above the pre-stimulus level during both the rising and falling phase of the subsequent EPSP. A post-synaptic potential having an IPSP followed by an EPSP was reflected in the PSFs of both the NI control group (e.g., Fig. 4-3) and in some of the participants with CP (Fig. 4-4A).

In summary, there is robust intracellular data to support our claim for the presence or absence of an IPSP from the PSF profiles measured in the NI and CP groups in this study. However, it is more difficult to measure the *amplitude* of IPSPs from the PSF profile because the motor unit often stops firing with the rate going to 0 Hz. Instead, as discussed below, we quantified the amount of IPSP activation from the surface EMG to determine if the strength of motoneuron inhibition was related to motor function in the participants with CP.

Estimating early-onset IPSP amplitude from surface EMG

Because surface EMG activity is dominated by the occurrence, and not frequency, of motor unit discharge, its profile during a reflex response can give misleading information concerning the profile of the post-synaptic potential in the motoneuron (Powers and Turker, 2010a). This is especially problematic during the later components of a post-synaptic potential given that a large transient input, such as an EPSP or IPSP, can synchronize the discharge of a tonically-firing motoneuron to produce false indications of repeated IPSPs and EPSPs. However, this is not a problem for the first component of a post-synaptic potential whereby the surface EMG (and PSTH) reflect what is happening to the post-synaptic potential because it is not influence by a prior transient event. We propose that our estimation of IPSP amplitude from measuring the amplitude of the surface EMG during the first component of the cutaneomuscular

reflex ($I1_{max}$) is reasonable because a large IPSP will strongly suppress the occurrence of motor unit discharge (likely in multiple motoneurons), a small IPSP will reduce it to a lesser extent and an EPSP will increase the occurrence of motor unit discharge to modify, correspondingly, the surface EMG signal (Turker and Powers, 1999; 2003).

In the surface EMG profiles where we measured $I1_{max}$ (for both NI and CP participants), a prior excitatory response (E1) was not present when the first component of the cutaneomuscular reflex contained a sustained, marked suppression of EMG activity (e.g., Fig. 4-6). Thus, the EMG suppression in these cases was not affected by motoneuron refractoriness or firing synchronization from a prior excitatory response and likely resulted from the activation of an initial IPSP as reflected in the PSF profiles for these participants. In the remainder of trials, which occurred in the CP group only, a sustained increase in surface EMG activity was measured out to 80 ms post-stimulation (positive or near positive $I1_{max}$ values) and likely reflected the activation of an EPSP, as also indicated in the PSF profiles of these same participants. We took the measurement of $I1_{max}$ out to 80 ms to not only reflect changes in motoneuron activation from spinal mechanisms (Nielsen et al., 1997) but to also avoid effects from motoneuron synchronization which, at a mean discharge rate of 7 Hz (140 ms interspike interval) and with a reflex onset near 60 ms, would have occurred near 200 ms post-stimulation. Thus, based on the supporting PSF profiles, we are fairly confident that the magnitude of surface EMG suppression within the first 80 ms from stimulation onset is a reasonable estimate of the magnitude of IPSP (or EPSP) activation from within the entire soleus motoneuron pool.

IPSPs and Motor Function

The presence and magnitude of IPSP activation was strongest in participants with CP who had good motor function (e.g., daily walkers) and absent in those with poor motor function (e.g., wheelchair dependent). We cannot determine if decreases in IPSP activation contributed to the reduced motor function of the participants with CP or if motor activity itself is necessary for the development and maintenance of post-synaptic inhibition in motoneurons. Indeed, reduced inhibition at the spinal level during voluntary activity may represent a compensatory plastic response to decreased descending activation of spinal motoneurons. Work in rat models provide evidence for the importance of motor activity in maintaining the activation of IPSPs in

motoneurons. For example, following a complete spinal cord injury where motor activity is greatly reduced, insertion of potassium-chloride (KCC2) transporters into the motoneuron membrane is reduced (Boulenguez et al., 2010). This lowers the intracellular chloride concentration so that when glycine or GABA receptors are activated, chloride flows out of the motoneuron to reduce or abolish the activation of IPSPs. Increased activation of the spinal cord from passive cycling increases the insertion of KCC2 transporters into the motoneuron membrane and associated GABA-mediated inhibition (Cote et al., 2014). Likewise, in patients with incomplete spinal cord injury, the excitability of spinal inhibitory pathways can be facilitated by daily locomotor training (Knikou and Mummidisetty, 2014; Manella and Field-Fote, 2013; Zewdie et al., 2015). Thus, it would be interesting to determine if intensive motor training in the CP participants with poor motor function could increase the amount of post-synaptic inhibition of motoneurons and if this would decrease symptoms of spasticity in addition to improving motor control.

Regardless of the cause and effect, reduced activation of inhibitory interneurons and/or enhanced activation of excitatory interneurons within the cutaneomuscular reflex pathway may impair voluntary movements in persons with CP. During the generation of voluntary movements, descending and other peripheral afferent pathways may also utilize spinal interneurons that are contained within the cutaneomuscular reflex pathway studied here (Geertsen et al., 2011). For example, spinal interneurons that are activated by corticospinal pathways to the soleus muscle are implicated in setting the excitability levels of agonist/antagonist motoneuron pairs to aid in fast transitions of dorsi- and plantarflexion movements (Geertsen et al., 2010). An imbalance of excitation and inhibition in cutaneomuscular reflex pathways may directly impair the timing of agonist/antagonist muscle activation to explain, in part, why participants with CP have difficulty in making rapid, alternating movements (Milner-Brown and Penn, 1979). Likewise, reduced inhibition in cutaneous reflex pathways from the foot may also impede the descending and afferent control of both posture (Aniss et al., 1992) and walking (Bouyer and Rossignol, 2003) where cutaneous reflexes are thought to stabilize the ankle and mediate fine control of foot placement, respectively. Alternatively, the reduced inhibition in spinal pathways may be a

compensation for reduced descending activation of the spinal cord so that weakened inputs can more effectively activate spinal motoneurons.

Effects of Reduced IPSPs on Spasticity

A reduced level of IPSP activation and predominance of EPSP activation by sensory inputs may contribute to the activation of involuntary muscle spasms in people with spastic CP. Sustained involuntary activity during a muscle spasm is produced by the activation of persistent inward currents in the motoneuron that are triggered by a prolonged (>500 ms) EPSP evoked by cutaneous afferents in a rat model of spinal cord injury (Li et al., 2004). Similarly, stimulation of cutaneomuscular afferents in participants with incomplete spinal cord injury failed to evoke IPSPs (based on PSF profiles) but instead, produced a ~1000-ms EPSP which likely facilitated the triggering of persistent inward currents and involuntary muscle spasms (Norton et al., 2008). The reduced sensory-evoked IPSP and facilitated EPSP activation observed in the participants with CP could also facilitate the triggering of involuntary muscle spasms reported in this group. Although not as prolonged compared to spinal cord injury, the 300 to 600-ms *pure* EPSPs evoked from cutaneomuscular stimulation in some of the participants with CP, if temporally summed, may trigger the activation of persistent inward currents in motoneurons to produce self-sustained activity and prolonged, involuntary muscle spasms. Such pure EPSPs would more readily active persistent inward currents compared to a mixed IPSP-EPSP as measured in controls.

Two of the participants with CP were on anti-spastic medications that theoretically could have enhanced inhibition in the cutaneomuscular reflex (Table 4-1). Although intermittently taking clonazepam to enhance GABAa-receptor activity, participant CP-11 still had no evidence of IPSP activation. In contrast, CP-2 who was on a low dose of baclofen (10mg, 2x/day), a GABAb-receptor agonist to enhance pre-synaptic inhibition, had evidence of strong IPSP activation (-59%), similar to other participants with CP that had good motor function who were not on anti-spastics. Thus, it is difficult to determine with certainty if the strong IPSP activation in this participant was due to baclofen or preserved motor function.

Conclusion

We have found that in some adult participants with CP, post-synaptic inhibition of motoneurons is reduced or lost, most notably in those with more severe motor deficits. This is in contrast to previous studies showing that post-synaptic inhibitory pathways measured at rest, such as reciprocal inhibition, are normal in CP (Achache et al., 2010) and illustrates the importance of measuring the presence or strength of inhibition during voluntary contractions. Although spasticity in people with CP is multifactorial, ranging in causes from muscle and joint tissue changes to abnormal activation of brain and spinal cord pathways, data from this study suggest that in adults with CP, many years of reduced motor activity may affect how spinal motoneurons transduce sensory information which may contribute to motor dysfunction in this population.

References

Achache V, Roche N, Lamy JC, Boakye M, Lackmy A, Gastal A, Quentin V, Katz R. Transmission within several spinal pathways in adults with cerebral palsy. Brain 2010;133:1470-1483.

Adair B, Said CM, Rodda J, Morris ME. Psychometric properties of functional mobility tools in hereditary spastic paraplegia and other childhood neurological conditions. Dev Med Child Neurol 2012;54:596-605.

Aniss AM, Gandevia SC, Burke D. Reflex responses in active muscles elicited by stimulation of low-threshold afferents from the human foot. J Neurophysiol 1992;67:1375-1384.

Binboga E, Prasartwuth O, Pehlivan M, Turker KS. Responses of human soleus motor units to low-threshold stimulation of the tibial nerve. Experimental brain research 2011;213:73-86.

Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther 1987;67:206-207.

Boulenguez P, Liabeuf S, Bos R, Bras H, Jean-Xavier C, Brocard C, Stil A, Darbon P, Cattaert D, Delpire E, Marsala M, Vinay L. Down-regulation of the potassium-chloride cotransporter kcc2 contributes to spasticity after spinal cord injury. Nat Med 2010;16:302-307.

Bouyer LJ, Rossignol S. Contribution of cutaneous inputs from the hindpaw to the control of locomotion. I. Intact cats. J Neurophysiol 2003;90:3625-3639.

Cote MP, Gandhi S, Zambrotta M, Houle JD. Exercise modulates chloride homeostasis after spinal cord injury. J Neurosci 2014;34:8976-8987.

Crone C, Johnsen LL, Biering-Sorensen F, Nielsen JB. Appearance of reciprocal facilitation of ankle extensors from ankle flexors in patients with stroke or spinal cord injury. Brain 2003;126:495-507.

Downing AL, Ganley KJ, Fay DR, Abbas JJ. Temporal characteristics of lower extremity moment generation in children with cerebral palsy. Muscle Nerve 2009;39:800-809.

Eek MN, Tranberg R, Beckung E. Muscle strength and kinetic gait pattern in children with bilateral spastic CP. Gait Posture 2011;33:333-337.

Elder GC, Kirk J, Stewart G, Cook K, Weir D, Marshall A, Leahey L. Contributing factors to muscle weakness in children with cerebral palsy. Dev Med Child Neurol 2003;45:542-550.

Evans AL, Harrison LM, Stephens JA. Cutaneomuscular reflexes recorded from the first dorsal interosseous muscle of children with cerebral palsy. Dev Med Child Neurol 1991;33:541-551.

Geertsen SS, van de Ruit M, Grey MJ, Nielsen JB. Spinal inhibition of descending command to soleus motoneurons is removed prior to dorsiflexion. J Physiol 2011;589:5819-5831.

Geertsen SS, Zuur AT, Nielsen JB. Voluntary activation of ankle muscles is accompanied by subcortical facilitation of their antagonists. J Physiol 2010;588:2391-2402.

Gibbs J, Harrison LM, Stephens JA, Evans AL. Cutaneomuscular reflex responses recorded from the lower limb in children and adolescents with cerebral palsy. Dev Med Child Neurol 1999;41:456-464.

Graham HK, Harvey A, Rodda J, Nattrass GR, Pirpiris M. The functional mobility scale (FMS). J Pediatr Orthop 2004;24:514-520.

Grunt S, Fieggen AG, Vermeulen RJ, Becher JG, Langerak NG. Selection criteria for selective dorsal rhizotomy in children with spastic cerebral palsy: A systematic review of the literature. Dev Med Child Neurol 2014;56:302-312.

Hislop HJ, Avers D, Brown M, Daniels L. Daniels and worthingham's muscle testing : Techniques of manual examination and performance testing. 9th ed. St. Louis, Mo.: Elsevier/Saunders; 2014.

Katz R, Pierrot-Deseilligny E. Recurrent inhibition in humans. Prog Neurobiol 1999;57:325-355.

Knikou M, Mummidisetty CK. Locomotor training improves premotoneuronal control after chronic spinal cord injury. J Neurophysiol 2014;111:2264-2275.

Leonard CT, Sandholdt DY, McMillan JA, Queen S. Short- and long-latency contributions to reciprocal inhibition during various levels of muscle contraction of individuals with cerebral palsy. J Child Neurol 2006;21:240-246.

Li Y, Gorassini MA, Bennett DJ. Role of persistent sodium and calcium currents in motoneuron firing and spasticity in chronic spinal rats. J Neurophysiol 2004;91:767-783.

Manella KJ, Field-Fote EC. Modulatory effects of locomotor training on extensor spasticity in individuals with motor-incomplete spinal cord injury. Restorative neurology and neuroscience 2013;31:633-646.

Milner-Brown HS, Penn RD. Pathophysiological mechanisms in cerebral palsy. J Neurol Neurosurg Psychiatry 1979;42:606-618.

Murray KC, Stephens MJ, Rank M, D'Amico J, Gorassini MA, Bennett DJ. Polysynaptic excitatory postsynaptic potentials that trigger spasms after spinal cord injury in rats are inhibited by 5-ht1b and 5-ht1f receptors. J Neurophysiol 2011;106:925-943.

Nielsen J, Petersen N, Fedirchuk B. Evidence suggesting a transcortical pathway from cutaneous foot afferents to tibialis anterior motoneurones in man. J Physiol 1997;501 (Pt 2):473-484.

Norton JA, Bennett DJ, Knash ME, Murray KC, Gorassini MA. Changes in sensory-evoked synaptic activation of motoneurons after spinal cord injury in man. Brain 2008;131:1478-1491.

Palisano RJ, Rosenbaum P, Bartlett D, Livingston MH. Content validity of the expanded and revised gross motor function classification system. Dev Med Child Neurol 2008;50:744-750.

Penn RD, Savoy SM, Corcos D, Latash M, Gottlieb G, Parke B, Kroin JS. Intrathecal baclofen for severe spinal spasticity. N Engl J Med 1989;320:1517-1521.

Pinter MJ, Burke RE, O'Donovan MJ, Dum RP. Supraspinal facilitation of cutaneous polysynaptic epsps in cat medical gastrocnemius motoneurons. Exp Brain Res 1982;45:133-143.

Poon DM, Hui-Chan CW. Hyperactive stretch reflexes, co-contraction, and muscle weakness in children with cerebral palsy. Dev Med Child Neurol 2009;51:128-135.

Powers RK, Turker KS. Deciphering the contribution of intrinsic and synaptic currents to the effects of transient synaptic inputs on human motor unit discharge. Clin Neurophysiol 2010a;121:1643-1654.

Powers RK, Turker KS. Estimates of epsp amplitude based on changes in motoneuron discharge rate and probability. Experimental brain research 2010b;206:427-440.

Reid SM, Carlin JB, Reddihough DS. Distribution of motor types in cerebral palsy: How do registry data compare? Dev Med Child Neurol 2011;53:233-238.

Reid SM, Dagia CD, Ditchfield MR, Carlin JB, Reddihough DS. Population-based studies of brain imaging patterns in cerebral palsy. Dev Med Child Neurol 2014;56:222-232.

Rogasch NC, Burne JA, Turker KS. Comparison of the inhibitory response to tendon and cutaneous afferent stimulation in the human lower limb. J Neurophysiol 2012;107:564-572.

Shevell MI, Dagenais L, Hall N. The relationship of cerebral palsy subtype and functional motor impairment: A population-based study. Dev Med Child Neurol 2009;51:872-877.

Towsley K, Shevell MI, Dagenais L. Population-based study of neuroimaging findings in children with cerebral palsy. Eur J Paediatr Neurol 2011;15:29-35.

Turker KS, Powers RK. Effects of large excitatory and inhibitory inputs on motoneuron discharge rate and probability. J Neurophysiol 1999;82:829-840.

Turker KS, Powers RK. Estimation of postsynaptic potentials in rat hypoglossal motoneurones: Insights for human work. J Physiol 2003;551:419-431.

Turker KS, Powers RK. Black box revisited: A technique for estimating postsynaptic potentials in neurons. Trends Neurosci 2005;28:379-386.

Zewdie ET, Roy FD, Yang JF, Gorassini MA. Facilitation of descending excitatory and spinal inhibitory networks from training of endurance and precision walking in participants with incomplete spinal cord injury. Prog Brain Res 2015;218:127-155.

Authors' Translational Perspective

Abnormal activation of motoneurons in the spinal cord by sensory pathways is thought to contribute to impaired motor control and spasticity in persons with cerebral palsy (CP). We examined how spinal motoneurons respond to sensory inputs from the foot in a group of participants with CP who had different degrees of motor dysfunction. In the strongest participants who walked regularly, there was no evidence of abnormal sensory activation of their motoneurons. This might have resulted from less injury to descending systems that regulate spinal inhibition or because these participants generated sufficient motor activity to develop and maintain motoneuron inhibition. The latter possibility introduces the idea that intensive motor training may improve the control over motoneuron inhibition and spinal cord excitability as a means to reduce and treat spasticity in CP. Moreover, the assessment of sensory-evoked motoneuron inhibition may also be used as a screening tool. For example, selective dorsal rhizotomies are used to reduce aberrant sensory activation of the spinal cord to reduce spasticity in CP. The ability to walk is a commonly utilized criterion for such surgeries (Grunt et al., 2014). However, we demonstrate that individuals who walk regularly have normal sensory activation of their motoneurons. Thus, selecting only individuals who exhibit abnormal sensory-evoked activation of motoneurons may further optimize the selection criterion for dorsal rhizotomies. In summary, our findings shed light on spinal mechanisms involved in the production of spasticity and suggest a potential screening and assessment tool for interventions aimed at reducing spasticity in people with CP.

Chapter 5. Short-Interval Intracortical Inhibition in Adults with Bilateral Spastic Cerebral Palsy

Elizabeth G. Condliffe & Monica A. Gorassini

Introduction

Cerebral palsy (CP) arises from a non-progressive insult to the fetal or infant brain (Rosenbaum et al., 2007) and results in a group of disorders that include deficits in movement and posture. Spastic CP, characterized by intermittent or sustained involuntary muscle activity affects roughly 90% of people with CP (Pandyan et al., 2005; Reid et al., 2011; Shevell et al., 2009). A possible mechanism producing the excessive involuntary muscle activity in people with spastic CP is a reduction in the excitability of inhibitory networks in both the spinal cord (Achache et al., 2010; Condliffe et al., 2016) and brain (Berweck et al., 2008; Heinen et al., 1999; Vry et al., 2008). In the spinal cord, inhibitory control of motoneurons from sensory pathways, post-activation depression and presynaptic inhibition within sensory reflex pathways are reduced in adults with spastic CP (Achache et al., 2010; Condliffe et al., 2016). In the brain, both the ipsilateral (Heinen et al., 1999) and contralateral (Vry et al., 2008) silent periods evoked by transcranial magnetic stimulation (TMS) are reduced, suggesting a reduction of intercortical and intracortical inhibition in the motor cortex respectively.

Short-interval intracortical inhibition (SICI), which is mediated by the activation of cortical $GABA_A$-interneurons (Rothwell et al., 2009), is thought to help shape the motor output from the brain in neurologically intact individuals. Specifically, it is modulated prior to and during a sustained voluntary contraction and that modulation is specific to the active muscle (Reynolds and Ashby, 1999). Therefore, it seems plausible that reduced SICI could contribute to the difficulties that people with spastic CP have in activating a single muscle without co-activation of other muscles (Milner-Brown and Penn, 1979; Richards and Malouin, 2013; Tedroff et al., 2006). In fact, SICI is reduced in the motor cortex supplying muscles of the hand in people with unilateral CP secondary to congenital stroke (Berweck et al., 2008).

SICI is measured using paired-pulse TMS in which the motor evoked potential (MEP) resulting from a suprathreshold pulse is conditioned by a preceding subthreshold stimulus. The result is a smaller MEP than would be evoked by the suprathreshold pulse alone. However, both inhibitory and facilitatory interneurons can be activated by the conditioning stimulus and impact the amplitude of the conditioned MEP. As a result, the recruitment profile of SICI throughout a range of conditioning stimulation intensities is U-shaped (Ortu et al., 2008; Ziemann et al.,

1996). In this pilot study, we explored the recruitment profile of SICI in adults with bilateral spastic CP during an active contraction and compared it to the recruitment of SICI observed in neurologically intact (NI) peers.

We examined SICI in the motor cortex supplying the plantarflexors of the leg (soleus) because the lower extremities are consistently impaired in people with bilateral spastic CP whereas upper extremity function is frequently spared (Koman et al., 2004). Specifically, the plantarflexors contribute to gait impairments (Eek et al., 2011) and produce more antagonistic coactivation than dorsiflexors (Elder et al., 2003). Due to difficulties evoking MEPs in soleus at rest, participants performed active contractions throughout testing. This also enabled us to examine the inhibitory circuitry responsible for SICI during muscle activation.

Methods

The Human Research Ethics Board at the University of Alberta approved this study. It was conducted in accordance with the Declaration of Helsinki and all participants provided informed consent. Seven adults with spastic CP and 7 NI control participants of similar age volunteered for this study. No participant had contraindications to TMS (Rossi et al., 2009). In addition, all participants with CP: 1) had spasticity as their dominant motor impairment (as opposed to dystonia, choreoathetosis or ataxia), 2) had not received any recent (within 2 years) botulinum toxin injections in the lower legs, 3) were not taking medication known to impact SICI (Ziemann, 2004), and 4) were able to provide informed consent.

Characterization of Participants

Strength and resistance to passive stretch in the plantarflexors were assessed using the Medical Research Council score (MRC) (Hislop et al., 2014) and the modified Ashworth scale (Bohannon and Smith, 1987). The participants in this study also participated in another study that included a brain MRI (Condliffe et al., 2016). All participants with CP were scanned using a 1.5T Siemens Sonata. Anatomical abnormalities observed on T1-weighted, T2-weighted and fluid-attenuated inversion recovery (FLAIR) sequences were classified as described by Towsley et al. (2011). The severity of any periventricular white matter injury (PVWMI) was rated qualitatively based on cerebral white matter volume loss and T2 hyper-intensities (Table 5-1).

Experimental setup

In the CP group, the more impaired leg, i.e. the leg each participant identified as having a greater negative impact on overall function, was tested. The right leg was studied in the NI group except in 1 participant with a previous right ankle injury. Participants were seated with the hips and knees flexed to 90 degrees and their ankle at neutral. A custom binding held the foot during plantarflexion contractions. Background muscle activity levels and MEPs were recorded using surface electromyography (EMG) recorded from pairs of conductive adhesive hydrogel electrodes (3.81 x 2.24 cm, Covidien Ltd., Dublin, Ireland). Raw analog EMG was preamplified (1k gain, 10-1000 Hz band-pass filtered, AMT-8, Bortec Biomedical Ltd., Calgary, AB) prior to digitizing using Axon hardware (5kHz sampling rate, Digidata 1440A) and software (Axoscope 10.3, Molecular Devices LLC, Sunnyvale, CA). Data were analyzed using Matlab (R2011b, The Mathworks Inc., Natick, MA). EMG signals were further bandpass filtered (10-500Hz, 0 phase-shift) prior to processing.

The maximum voluntary activity (MVA) of the soleus muscle was calculated at the start of the experiment. The average of the highest two trials that were within 10% of each other was defined as the MVA and used to set a target for submaximal (~20% MVA) contractions. The maximum rectified EMG, averaged over 1s, was calculated for each trial with at least 60 s of rest between each trial. In the weakest participants, the electrical noise recorded at rest (1.0-1.5 μV) was a significant proportion of the maximal activity (~10 μV). Therefore, noise was subtracted from measures of voluntary activity for each participant. During the SICI assessments, participants performed submaximal (~20% MVA) contractions aided by visual feedback of the soleus EMG and verbal encouragement.

SICI measurements

Paired-pulse TMS was delivered by a Magstim BiStim stimulator (The Magstim Company Ltd, Carmarthenshire, UK), using a custom batwing coil (P/N 15857: 90-mm wing diameter). This coil facilitates focal stimulation of deeper cortical areas such as the soleus motor representation. The coil was oriented to induce posterior-anterior current in the brain. TMS was delivered at the optimal location for contralateral soleus muscle activation with a minimum distance of 2 cm lateral to vertex. This 2 cm offset was used to minimize current spread to the

other leg motor cortex as per Maegaki et al. (1999). Active motor threshold (aMT) was defined as the lowest stimulation intensity needed to produce a distinguishable MEP in 3/5 trials. The test TMS intensity was set at 1.3 x aMT and the conditioning stimulation was given 3 ms before the test pulse. To determine if the recruitment curve followed a U-shaped profile, we increased the conditioning stimulation from 0.6 to 1.0 aMT in steps of 0.1 aMT. Fewer intensities were used in some participants to avoid fatigue. Blocks of 10 stimuli containing 7 paired-pulses and 3 test-alone stimuli, randomly delivered with 5-10 seconds between stimuli, were performed at each conditioning intensity. In total, an average of 14.4±3.4 unconditioned (test) MEPs were obtained per experiment. To quantify SICI, the average peak-to-peak amplitude of the conditioned MEPs at each intensity was expressed as a percentage of the average unconditioned (test) MEPs.

Data and statistical analysis

Statistical comparisons were performed in SPSS (version 21, IBM, Armonk, NY) using the Mann-Whitney U-test due to our small sample size with the median (range) reported for each group. The conditioning intensity at which maximal SICI was elicited and the maximal SICI for each individual was compared between the CP and NI groups. Additional comparisons of MVA, background activity (%MVA), and test MEP amplitude were also performed to describe the groups and compare testing conditions. The alpha level for significance was set at 0.05.

Results

Participant Characteristics

Participants with CP had bilateral motor impairments with a range of motor dysfunction as assessed by the Gross Motor Function Classification System. The majority of participants with CP (6/7) had evidence of mild to moderate periventricular white matter injury (PVWMI) on both sides of the brain (Fig. 5-1, Table 5-1). One participant also had evidence of a cerebral malformation, polymicrogyria. One participant with CP (CP-7) had normal brain imaging. As expected, the CP group had substantial plantarflexion weakness. This was noted on manual muscle testing with an MRC score of <5 in 5/7 participants with CP and by comparing the MVA between groups. The median MVA in the CP group was 31 μV compared to 178 μV in the NI group ($p=0.002$, Table 5-1).

ID	Sex	Age	Leg	MVA (μV)	Test Intensity (% MSO)	GMFCS	MRC	mAsh	MRI
CP-1	F	21	R	56	100	I	5	0	PVWMI +
CP-5	F	35	L	33	100	IV	1	2	PVWMI ++
CP-7	F	28	L	28	100	II	5	1	Normal
CP-11	F	19	L	11	72	IV	2	1	PVWMI ++/CM
CP-13	M	42	R	31	91	III	2	1	PVWMI +
CP-16	F	57	R	69	52	I	5	0	PVWMI +
CP-17	M	32	R	9	82	II	4	1	PVWMI ++
CP	F: 5 M: 2	32 (19-57)	R: 4 L: 3	31 (9-69)	91 (52-100)				
NI	F: 3 M: 4	33 (22-50)	R: 6 L: 1	178 (141-242)	77 (65-99)				

Table 5-1. Listed for each participant in the CP group is their identification code, gender (Sex, F=female, M=male), age, leg tested, maximum voluntary activation (MVA) in the soleus, test stimulation intensity, Gross Motor Function Classification System level (GMFCS), plantarflexor strength as determined by the Medical Research Council score (MRC), modified Ashworth score (mAsh) of the plantarflexors, and MRI findings. PVWMI = periventricular white matter injury of mild (+) or moderate (+) severity. CM = cerebral malformation. Participant IDs are non-sequential as not all participants who were recruited for the larger study are included in this study. The groups are summarized in the bottom rows as follows: 1) total counts for gender, and tested leg; 2) median (range) for age, MVA and test stimulation intensity.

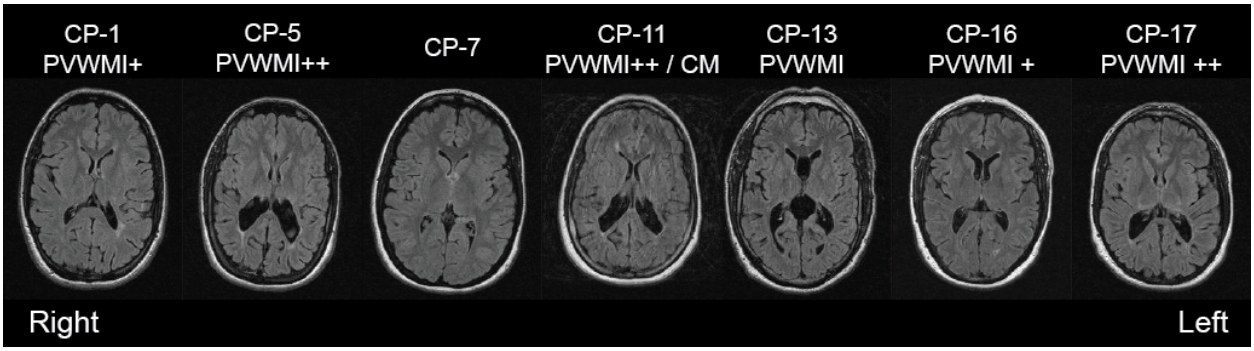

Figure 5-1 MRI Slices. Example slices of axial T2 Flair MRI sequences with a slice through the lateral ventricles are shown for each participant. The group displays a spectrum of brain abnormalities from no abnormalities (CP-7) to moderate periventricular white matter injury (PVWMI) and a cerebral malformation (CM) (CP-2). Data published in part in (Condliffe et al., 2016).

Short-interval intracortical inhibition (SICI)

Test MEPs measured in the soleus muscle were suppressed in response to a prior (3 ms) conditioning stimulus for participants in both the NI and CP groups. Likewise, both groups showed a U-shaped recruitment profile in response to incrementing intensities of conditioning stimulation. Example recruitment curves for a representative NI (NI-1) participant and for two participants in the CP group (CP-13 & CP-1) are displayed in Figure 5-2. The deepest part of the SICI recruitment curve (i.e., maximal SICI) occurred near a conditioning stimulation intensity of 0.8 x aMT.

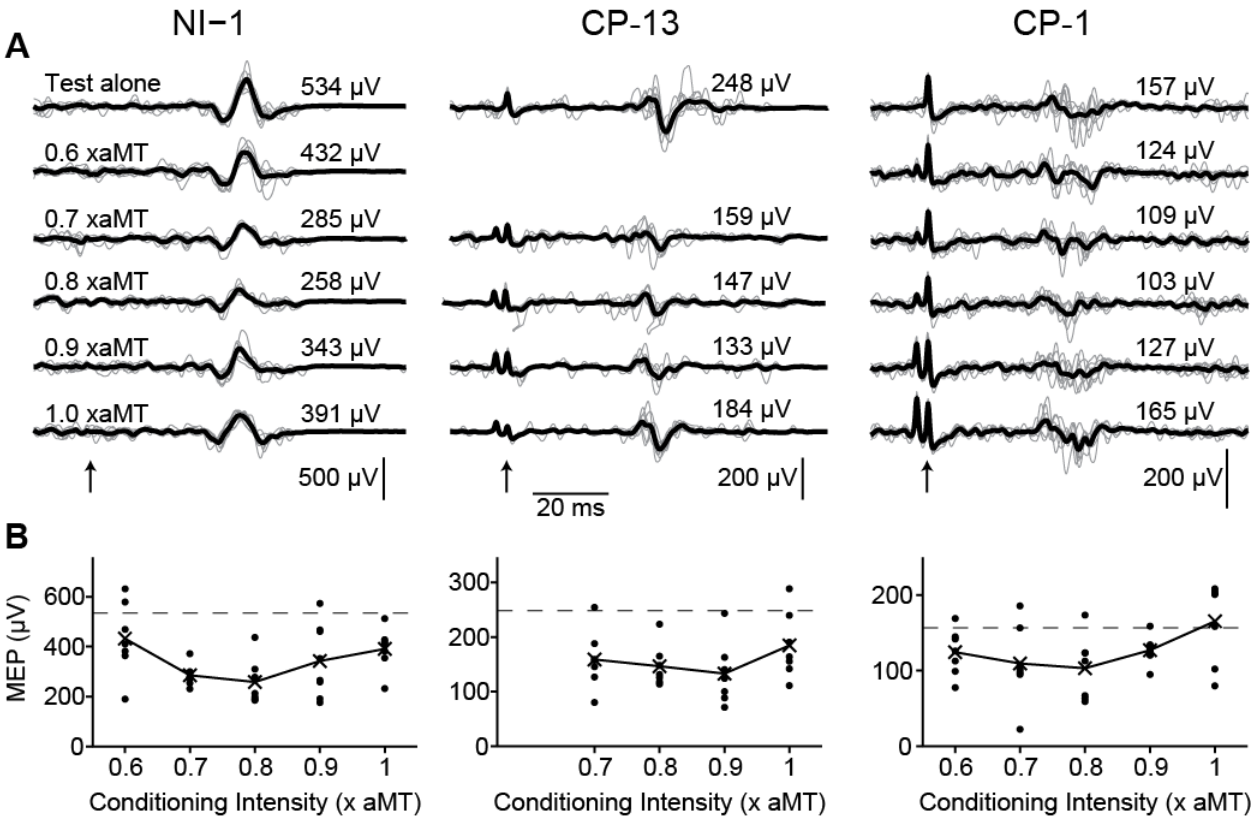

Figure 5-2 Representative Raw Data. A. Responses to test stimuli alone (top traces) and paired-pulses at incrementing conditioning intensities as indicated on left. Arrows mark timing of the test stimulus. Individual trials for each condition are superimposed (gray traces) along with the average MEP amplitude (black trace). The mean peak-to-peak MEP amplitude are indicated above each trace. The y-axis scales are smaller for the participants with CP who are weaker and have smaller MEPs than the NI participant. **B.** The peak-to-peak MEP amplitude (dots) for each individual trial and the mean amplitude (x's) are plotted at each conditioning intensity for the same participants. Dotted horizontal lines indicate the amplitude of MEPs evoked by the test stimulation alone. As maximal SICI was clearly found at the intensities tested, CP-13 was not tested with a conditioning intensity of 0.6 x aMT.

In both the CP and NI groups, the magnitude of SICI varied with conditioning intensity in the classic U-shape (Fig. 5-3A). The conditioning intensity that produced maximal SICI for each individual was similar at 0.8 x aMT (0.6-0.9 x aMT) in the CP group and 0.8 x aMT (0.7-1.0 x aMT) in the NI group (p=0.53, Fig 5-3B).

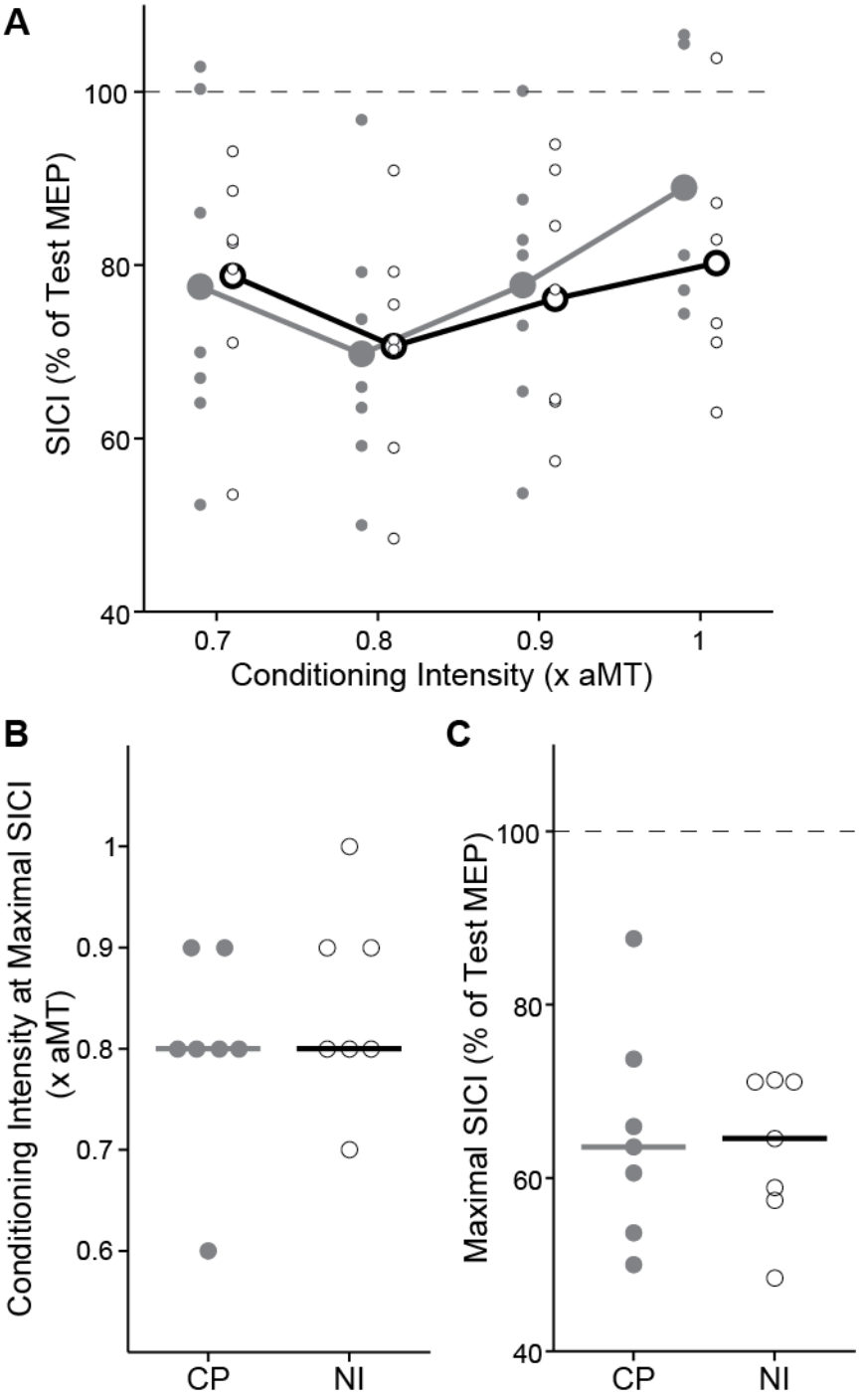

Figure 5-3 Similar SICI between Groups. A. SICI for each participant (small circles) and the mean SICI for each group (large circles) at each conditioning intensity. A slight horizontal skew was added to each group to better visualize overlapping data. Note, not all participants were tested with a conditioning intensity of 1.0 xMT. **B.** The conditioning intensity at which maximal SICI was found for each individual (small circles) and the group median (horizontal lines). **C.** The magnitude of maximal SICI (small circles) for each individual and group median (horizontal lines). In **A, B** & **C** solid grey circles represent the CP group and open black circles represent the NI group.

Of note, the *relative* testing conditions were similar between groups. The level of tonic contraction during the SICI measurements was comparable between the two groups at 19% MVA (17-42%) in the CP group and 20% MVA (17-22%, p=0.70) in the NI group although the absolute levels were smaller in the CP group at 6.3 μV (2.9-13.3 μV) compared to the NI group at 36.1 μV (26.6-47.8 μV p <0.001). The test-pulse intensities were standardized at 1.3 x aMT in both groups, and the median absolute intensity of TMS, as a percentage of maximal stimulator output (%MSO), was trended towards being larger in the CP group at 91% MSO compared to the NI group at 77% MSO but this difference did not reach statistical significance (p=0.44, Table 5-1). Despite the similar intensity of stimulation with respect to aMT, the absolute amplitude of MEPs produced by the test stimuli alone were expectedly smaller in the CP group at 131 μV (58 - 248 μV) compared to the NI group at 613 μV (429 - 820 μV, p=0.002). The smaller absolute test MEP size and background contraction levels make it difficult to compare SICI amplitudes between groups as explored in the Discussion. None-the-less, the magnitude of maximal SICI was similar between groups with a suppression of test MEPs to 64% (50 – 88%) in the CP group and 65% (48-71%) in the NI group (p=0.85, Fig 5-3C).

Discussion

Similar to NI controls, the SICI recruitment curve in participants with CP followed a U-shaped profile that was centered near 0.8 x aMT. We discuss below that this may reflect a similar recruitment profile of cortical inhibitory and excitatory interneurons activated by the conditioning stimulation. Comparisons of absolute magnitude of SICI between the two groups is more problematic given the experimental confounds and this will be discussed as well.

Activation of Similar Cortical Networks by the Conditioning Stimuli

Similar SICI recruitment profiles suggest that similar populations of interneurons were activated by the conditioning stimuli. As mentioned in the Introduction, the U-shaped recruitment profile stems from the recruitment of both inhibitory and facilitatory interneurons. At very low conditioning intensities (e.g. 0.6 x aMT), few of the $GABA_A$ inhibitory interneurons are activated and thus the degree of MEP suppression is low. As the conditioning intensity increases more of the $GABA_A$ inhibitory interneurons producing SICI are activated, as reflected by the

descending arm of the recruitment profile. However, interneurons mediating intracortical facilitation may also be recruited and at higher stimulation intensities their impact likely begins to dominate to produce the ascending arm of the recruitment profile. Overall this produces the U-shaped, SICI recruitment curve (Ni and Chen, 2008) that was observed in both groups (Figure 5-3A).

When damage exists in the descending motor pathways, as in incomplete spinal cord injury, this relationship can be shifted (Roy et al., 2011). With cerebral damage in the CP group it is possible that we may have recruited a different constellation of excitatory and inhibitory interneurons with the conditioning stimulation. However, both groups displayed a similar U-shaped profile in the SICI recruitment curve and both curves peaked at the relative intensity of 0.8 x aMT. This suggests that similar populations of excitatory and inhibitory cortical networks were recruited in both groups.

Background Muscle Activity

While the reproducible and similar suppression of test MEP responses (to 65%) suggests that the SICI network was indeed activated, we cannot be certain that we are comparing the magnitude of SICI obtained under matched conditions. The CP group had profound muscle weakness and reduced responses to TMS of the motor cortex supplying the soleus muscle. In terms of the absolute background muscle activity (EMG in μV), maximal activity in some of the participants with CP would correspond to minimal voluntary effort in the NI participants. Increased cortical excitability (including intracortical facilitation) produced by higher levels of background muscle activity reduces SICI (Garry and Thomson, 2009; Ortu et al., 2008; Ridding et al., 1995). Therefore, we provided a target for the background muscle activity at 20% of each individual's MVA in an attempt to match the level of relative cortical excitability. However, with the median MVA more than 5x smaller in the CP group, and the potential that non-cortical causes of weakness contribute to this (e.g., corticospinal tract damage noted in Chapter 3), we cannot be certain that the cortical excitability at 20% MVA is the same in the CP and NI groups.

Test MEP

Similarly, we attempted to match the type of neurons activated by the test stimulus despite clear differences in the test MEP amplitude. The test MEP evoked by the unconditioned,

supra-threshold stimulus is mediated by the activation of excitatory interneurons and possibly by direct corticospinal axonal activation. These interneurons trans-synaptically activate corticospinal neurons with increasing synaptic delays to produce a series of volleys known as I-waves (e.g., I_1, I_2, I_3, etc) (Di Lazzaro et al., 2012; Nakamura et al., 1997). The later I-waves (e.g., I_3) are particularly susceptible to SICI, but are not activated by low test stimulation intensities that produce very small MEPs (Di Lazzaro et al., 2012). However, while the later I-waves are still activated at high stimulation intensities that produce maximal or near-maximal MEPs, SICI decreases. It is thought that high stimulation intensities activate the excitatory (MEP activating) interneurons located at a greater radius from the stimulating coil than the inhibitory (SICI activating) interneurons activated by the lower conditioning stimulation (Ni and Chen, 2008). Therefore not all of the neurons activated at high stimulation intensities are susceptible to SICI modulation.

The difference in test MEP amplitudes between the CP and NI groups theoretically could indicate recruitment of different populations of cortical neurons between the two groups. Without recording the I-waves more invasively through epidural recordings or a marker of I-wave activity through motor unit recordings (Priori et al., 1993), it is difficult to be certain that similar cortical interneurons (with similar susceptibility to SICI) are activated by a given test stimulus in the two populations. However, we attempted to activate similar cortical networks with the test stimulus by matching its intensity relative to each participant's motor threshold, as suggested by a mechanistic study (Garry and Thomson, 2009). This approach has been used by previous studies in populations with neurological lesions (Berweck et al., 2008; Honaga et al., 2013; Liepert et al., 2000). Multiple I-waves are indeed evoked by stimulation at 1.3 x aMT, such as that used here, and the epidural responses increase at higher intensities demonstrating that 1.3 xMT remains sub-maximal (Di Lazzaro et al., 2001). In addition, the smaller test MEP amplitudes recorded in participants with CP may not be due to the activation of a different population of neurons by the test stimulus. Damage to the descending pathways, as discussed in Chapter 3, and the smaller-sized soleus muscles in CP (Reid et al., 2014), could also cause smaller test MEP amplitudes. Therefore, the difference in the test MEP amplitude may not be indicative of activating different populations of cortical neurons.

Potential Confirmatory Studies

Certainly reproducing the findings in a larger group of participants would increase our confidence that SICI is intact in adults with bilateral spastic CP, particularly because CP is a diverse disorder. However, the similar distribution of the results in both groups (see Fig. 5-3) suggests that this is a true finding and not a result of an underpowered study. Unfortunately, 10x more participants would be needed to prove the two groups are statistically equivalent (Weber and Popova, 2012).

From a mechanistic perspective more thorough assessments could enable stronger interpretation of the findings. Evaluating the recruitment profiles at rest and at a range of levels of background activation would increase our confidence that changes in cortical, or even spinal, excitability associated with voluntary activation, did not contribute to the findings of this study. As discussed in the Introduction, the soleus muscle is a highly relevant muscle to study in adults with bilateral spastic CP; however, it is often difficult to elicit soleus MEPs at rest. Testing SICI in the tibialis anterior or first dorsal interosseous muscle of the hand would facilitate this capability. Not only are MEPs elicited at lower levels background activity, but there are smaller differences in strength between CP and NI groups (Elder et al., 2003; Hussain et al., 2014; Kesar et al., 2012). Therefore it may be possible to explore SICI at matched levels of the absolute and relative background activity as well as at both matched test stimulus intensities and MEP amplitudes. Under these conditions, the comparison of the magnitude of SICI between CP and NI controls would be more valid.

Conclusions

This preliminary study provides the first evidence that recruitment profiles of SICI may not be impaired in adults with bilateral spastic CP. While our population of participants was small, the individuals experience a wide range of motor dysfunction, representative of the population of people with spastic CP. Where possible effort was taken to match experimental conditions to increase the confidence that SICI was indeed similar in both groups. However further studies are needed to make conclusions about the magnitude of SICI in CP.

References

Achache V, Roche N, Lamy JC, Boakye M, Lackmy A, Gastal A, Quentin V, Katz R. Transmission within several spinal pathways in adults with cerebral palsy. Brain 2010;133:1470-1483.

Berweck S, Walther M, Brodbeck V, Wagner N, Koerte I, Henschel V, Juenger H, Staudt M, Mall V. Abnormal motor cortex excitability in congenital stroke. Pediatr Res 2008;63:84-88.

Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther 1987;67:206-207.

Condliffe EG, Jeffery DT, Emery DJ, Gorassini MA. Spinal inhibition and motor function in adults with spastic cerebral palsy. J Physiol 2016.

Di Lazzaro V, Oliviero A, Profice P, Meglio M, Cioni B, Tonali P, Rothwell JC. Descending spinal cord volleys evoked by transcranial magnetic and electrical stimulation of the motor cortex leg area in conscious humans. J Physiol 2001;537:1047-1058.

Di Lazzaro V, Profice P, Ranieri F, Capone F, Dileone M, Oliviero A, Pilato F. I-wave origin and modulation. Brain stimulation 2012;5:512-525.

Eek MN, Tranberg R, Beckung E. Muscle strength and kinetic gait pattern in children with bilateral spastic CP. Gait Posture 2011;33:333-337.

Elder GC, Kirk J, Stewart G, Cook K, Weir D, Marshall A, Leahey L. Contributing factors to muscle weakness in children with cerebral palsy. Dev Med Child Neurol 2003;45:542-550.

Garry MI, Thomson RH. The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. Exp Brain Res 2009;193:267-274.

Heinen F, Kirschner J, Fietzek U, Glocker FX, Mall V, Korinthenberg R. Absence of transcallosal inhibition in adolescents with diplegic cerebral palsy. Muscle Nerve 1999;22:255-257.

Hislop HJ, Avers D, Brown M, Daniels L. Daniels and worthingham's muscle testing : Techniques of manual examination and performance testing. 9th ed. St. Louis, Mo.: Elsevier/Saunders; 2014.

Honaga K, Fujiwara T, Tsuji T, Hase K, Ushiba J, Liu M. State of intracortical inhibitory interneuron activity in patients with chronic stroke. Clin Neurophysiol 2013;124:364-370.

Hussain AW, Onambele GL, Williams AG, Morse CI. Muscle size, activation and coactivation in adults with cerebral palsy. Muscle Nerve 2014;49:76-83.

Kesar TM, Sawaki L, Burdette JH, Cabrera MN, Kolaski K, Smith BP, O'Shea TM, Koman LA, Wittenberg GF. Motor cortical functional geometry in cerebral palsy and its relationship to disability. Clin Neurophysiol 2012;123:1383-1390.

Koman LA, Smith BP, Shilt JS. Cerebral palsy. Lancet 2004;363:1619-1631.

Liepert J, Storch P, Fritsch A, Weiller C. Motor cortex disinhibition in acute stroke. Clin Neurophysiol 2000;111:671-676.

Maegaki Y, Maeoka Y, Ishii S, Eda I, Ohtagaki A, Kitahara T, Suzuki N, Yoshino K, Ieshima A, Koeda T, Takeshita K. Central motor reorganization in cerebral palsy patients with bilateral cerebral lesions. Pediatr Res 1999;45:559-567.

Milner-Brown HS, Penn RD. Pathophysiological mechanisms in cerebral palsy. J Neurol Neurosurg Psychiatry 1979;42:606-618.

Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H. Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. J Physiol 1997;498 (Pt 3):817-823.

Ni Z, Chen R. Short-interval intracortical inhibition: A complex measure. Clin Neurophysiol 2008;119:2175-2176.

Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC. Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. J Physiol 2008;586:5147-5159.

Pandyan AD, Gregoric M, Barnes MP, Wood D, Van Wijck F, Burridge J, Hermens H, Johnson GR. Spasticity: Clinical perceptions, neurological realities and meaningful measurement. Disabil Rehabil 2005;27:2-6.

Priori A, Bertolasi L, Dressler D, Rothwell JC, Day BL, Thompson PD, Marsden CD. Transcranial electric and magnetic stimulation of the leg area of the human motor cortex: Single motor unit and surface EMG responses in the tibialis anterior muscle. Electroencephalogr Clin Neurophysiol 1993;89:131-137.

Reid SL, Pitcher CA, Williams SA, Licari MK, Valentine JP, Shipman PJ, Elliott CM. Does muscle size matter? The relationship between muscle size and strength in children with cerebral palsy. Disabil Rehabil 2014:1-6.

Reid SM, Carlin JB, Reddihough DS. Distribution of motor types in cerebral palsy: How do registry data compare? Dev Med Child Neurol 2011;53:233-238.

Reynolds C, Ashby P. Inhibition in the human motor cortex is reduced just before a voluntary contraction. Neurology 1999;53:730-735.

Richards CL, Malouin F. Cerebral palsy: Definition, assessment and rehabilitation. Handb Clin Neurol 2013;111:183-195.

Ridding MC, Taylor JL, Rothwell JC. The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. J Physiol 1995;487 (Pt 2):541-548.

Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M. A report: The definition and classification of cerebral palsy - april 2006. Dev Med Child Neurol 2007;49:8-14.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008-2039.

Rothwell JC, Day BL, Thompson PD, Kujirai T. Short latency intracortical inhibition: One of the most popular tools in human motor neurophysiology. J Physiol 2009;587:11-12.

Roy FD, Zewdie ET, Gorassini MA. Short-interval intracortical inhibition with incomplete spinal cord injury. Clin Neurophysiol 2011;122:1387-1395.

Shevell MI, Dagenais L, Hall N. The relationship of cerebral palsy subtype and functional motor impairment: A population-based study. Dev Med Child Neurol 2009;51:872-877.

Tedroff K, Knutson LM, Soderberg GL. Synergistic muscle activation during maximum voluntary contractions in children with and without spastic cerebral palsy. Dev Med Child Neurol 2006;48:789-796.

Towsley K, Shevell MI, Dagenais L. Population-based study of neuroimaging findings in children with cerebral palsy. Eur J Paediatr Neurol 2011;15:29-35.

Vry J, Linder-Lucht M, Berweck S, Bonati U, Hodapp M, Uhl M, Faist M, Mall V. Altered cortical inhibitory function in children with spastic diplegia: A TMS study. Exp Brain Res 2008;186:611-618.

Weber R, Popova L. Testing equivalence in communication research: Theory and application. Commun Methods Meas 2012;6:190-213.

Ziemann U. TMS and drugs. Clin Neurophysiol 2004;115:1717-1729.

Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol 1996;496:873-881.

Chapter 6. Discussion

Introduction

Thank you for reading this thesis examining mechanisms of motor impairment in spastic cerebral palsy (or, perhaps more accurately, *a few* mechanisms). You are almost there. Much of the interpretation of our findings is incorporated into each experimental chapter (3-5). The same cohort of participants with CP participated in each of the studies. Here I will discuss how characteristics of this cohort impact the generalization of our findings to the population of people with spastic CP. This is followed by a more thorough discussion of the limitations in our experimental methods. Lastly a few suggestions for work building on the challenges and findings are discussed. This may have been the most fun section to write, though I imagine it will be read by only a few. I hope you find some of the ideas thought provoking or at least interesting.

Generalization of our Findings based on Participant Factors

As described briefly in each of the experimental chapters, the group of participants with spastic CP studied in this thesis represents a diverse cohort but is not representative of the entire population with spastic CP. We did not study children or people who could not provide assent to participate in our studies. Within this thesis, our participants were described based on their body functions and structures. They are also diverse with respect to their activities and participation. Some are employed competitively without any adaptations. Others work or study with some assistance or adaptations to the environment and others have disabilities that preclude them from working. Interestingly the participant with the greatest motor impairments, with help from adaptive technology and a personal support worker, is competitively employed. This diversity is difficult to represent in an academic paper but is likely representative of the population of adults with spastic CP. This section will reflect on the rationale behind inclusion criteria of our participants and the ability to extrapolate our findings from a relatively small population of participants to the general population of adults with spastic CP.

Age

All of the participants are adults. In most cases it would be reasonable to expect the mechanisms shown to be impaired in this thesis to also be impaired in children. However, the

practicalities involved in executing these experiments are easier in adults. It may be ethically problematic to recruit children who can provide assent, but not consent (in many Canadian jurisdictions) for a study that is slightly invasive but does not even potentially produce any benefit to the participant, such as the study involving motor unit recordings. In addition, the degree of voluntary participation required for the TMS studies, particularly providing muscle activation at specific levels, may have been more difficult for children. In fact, this is one of the reasons that a previous study that evaluated responses to TMS during voluntary activity used maximal activity (Vry et al., 2008). The TMS data for most participants was collected in a single experiment in which the participant was an active participant, intermittently matching target contraction levels for up to two-hours. Children may have difficulty with the duration of the experiment and the degree of monotonous and specific tasks required of them.

Another advantage to studying adults is stability of the measured parameters. Many of the electrophysiological measures employed in this thesis develop throughout childhood and into adolescence but are generally considered stable in neurologically-intact adults (Paus et al., 1999; Pitcher et al., 2002). For example, motor threshold and short-interval intracortical inhibition are both higher in children and reach adult levels during adolescence (Croarkin et al., 2014; Garvey et al., 2003; Walther et al., 2009). Whether these electrophysiological measures are also stable in people with spastic CP is unknown. By definition, the brain injuries causing CP are not degenerative (Rosenbaum et al., 2007). However, motor function in adults with spastic CP is not stable and frequently deteriorates [see Haak et al. (2009) for a review].

However, characterizing our adult participants was likely more challenging than it may have been in children. The birth and early childhood medical records, including the records from around the time of diagnosis, were not reviewed for participants born outside of Edmonton. Even when early medical records were available, the understanding of the etiology of spastic CP and medical imaging has evolved since our participants were young. While intracranial ultrasounds are standard for babies known to be at risk for CP and an MRI is now recommended within two years for all people with CP (Ashwal et al., 2004), none of our participants had one at that age. Similarly, the dating of pregnancies was less accurate prior to the use of fetal ultrasounds (Butt et al., 2014), and thus determining if any of our adult participants were born prematurely was not possible in many cases.

Motor Classification

All of our participants had bilateral motor impairments on examination. This was not an explicit inclusion criteria during recruitment. In fact three participants described themselves as having spastic hemiplegia during the initial conversation. Using the Gross Motor Functional Classification System (GMFCS), our participants were almost evenly distributed among levels I-IV (see Table 3.1 in Chapter 3 for details). However, in the general population of people with CP, more people function at GMFCS I (44%) than any other level (10-18%) (Shevell et al., 2009). People with unilateral motor impairments frequently function at higher levels (most at GMFCS I) (Shevell et al., 2009). Our underrepresentation of people with unilateral motor impairments is thus consistent with the underrepresentation of high functioning individuals in our studies.

While we recruited through diverse sources including the CP Association in Alberta and adaptive recreation groups as well as hospital-based clinics, high functioning individuals frequently have less need for specialized medical care or community supports as adults. The use of social media might lead to more representative recruitment. In addition, even high functioning children with spastic CP are usually followed in a specialized medical clinic such as the Physical Medicine Clinic or Tone Management Service for Children here in Edmonton. A time and resource intensive review of historical charts from these clinics would aid in identifying a more complete spectrum of people with spastic CP. However, one could argue that it's more important to understand the mechanisms of impairment in the population of people who utilize additional health care or community resources. In that case, the population of participants in this study is likely representative of that group of adults.

None of our participants had the most severe motor impairments (GMFCS V). This selection bias was again not deliberate and no exclusion criteria were based on the GMFCS. However, our studies required the ability to assent to the study, a minimal level of voluntary motor control, and the ability to follow instructions. People functioning at GMFCS V are less likely to meet these requirements. Additionally, people with greater motor impairments have shorter life-expectancies (Blair et al., 2001; Strauss et al., 2008). While 16% of children with CP function at GMFCS V (Shevell et al., 2009), that proportion would be smaller in adults.

Imaging Findings

Comparing the MRI findings of the CP participants (see Table 3.1 in Chapter 3 for details) with population-based studies of brain imaging revealed a few differences. However, it is also worth noting a few differences in how the classification was completed. Our MRI's were performed on adult participants and interpreted by a radiology resident and a neuroradiologist. The population-based studies usually use MRI's completed in young children and classified by pediatric neurologists or pediatric radiologists (Himmelmann and Uvebrant, 2011; Towsley et al., 2011; Wu et al., 2006). While we chose to describe all major findings within our images, most of the populations-based studies use mutually exclusive categories. While all our participants with abnormal imaging had evidence of bilateral periventricular white matter injury (PVWMI), five had additional findings such as cerebral malformations. When cerebral malformations are present, it would likely be considered the primary finding if mutually exclusive categories were used. The other combinations (PVWMI and cerebrovascular insult or PVWMI and deep grey matter injury) are less clear cut and decisions often require consensus (personal communication, Adam Kirton, pediatric neurologist). It is also possible that some of the PVWMI observed in our adult participants with multiple abnormalities resulted secondary to the other abnormality, perhaps due to Wallerian degeneration.

If participants with multiple abnormalities were assigned to a category other than PVWMI, our population of 16 people is roughly representative of people with spastic CP as described in a recent systematic review (Reid et al., 2014). The distribution of findings for our participants followed by the population estimates for people with bilateral spastic CP in parenthesis are: normal imaging 12.5% (5-19%), PVWMI 56% (24-66%), (deep) gray matter injury 6% (16-35%), focal vascular insult 6% (0-11%), malformation 19% (3-12%). Regarding gray matter injury, our definition of deep gray matter injury, while similar to the Canadian classification scheme available at the onset of this study (Towsley et al., 2011), is slightly different from the broader definition for gray matter injury used to compare multiple population studies by Reid et al. (2014). With that exception, our population does generalize well to the general population of people with bilateral spastic CP.

Participants Taking Neuromodulatory Medication

To be inclusive, we chose not to exclude participants taking neuromodulatory medication. However, all primary findings in this thesis were confirmed when excluding participants taking medication including those medications that can modify the activity of neurons. In addition, the results for each individual taking neuromodulatory medication were inspected relative to the entire group with consideration of the possible drug impact. In no case did the medication clearly alter the findings. For example, CP-11 takes the selective serotonin re-uptake inhibitor sertraline daily. In a single dose, sertraline increases the MEP amplitude in NI adults (Ziemann, 2013). The $MEP_{1.2}$ and MEP_{Peak} for CP-11 were both below the group median. It is possible that without sertraline, the MEP amplitudes would be even lower. However this would not change the findings between our groups. Instead, including a participant taking sertraline increases our type II error, that we would falsely not demonstrate a difference in MEP amplitude between groups. Sertraline does not have an effect on motor thresholds, nor does it impact the magnitude of short-interval intracortical inhibition (Ziemann, 2004). All six participants taking neuromodulatory medications were included in the primary presentation of the data deliberately to maintain an inclusive population. Excluding all participants taking medication for spasticity, for example, might bias our study population toward participants whose spasticity is not problematic and thus be less representative of the total population.

Technical Limitations

Muscle Selection

As mentioned throughout this thesis, impairments in plantarflexors are particularly relevant to motor function in spastic CP. Plantarflexors in particular exhibit: weakness (Engsberg et al., 2000; Wiley and Damiano, 1998), spasticity (Elder et al., 2003), reduced selective motor control (Arpin et al., 2013), and impairments in plantarflexion function that impact gait (Berger et al., 1982; Eek et al., 2011). Of the plantarflexors, in this thesis we focused on the soleus muscles.

The soleus muscle is frequently used in neurophysiological studies of plantarflexors including spinal reflexes (often evaluated by modulation of H-reflexes which are easy to evoke in the resting soleus). While many studies involving TMS targeting the lower extremities, target

the dorsiflexor, tibialis anterior, MEPs have also been reported in the soleus muscle by many groups. The gastrocnemius is another potent plantarflexor and it is also a knee flexor. In general, muscles like the gastrocnemius that act across two joints are thought to be more spastic (National Institute for Health Care Excellence, 2012), and thus potentially more relevant to movement in spastic CP. This theory appears to be based on reductions in spasticity when the gastrocnemius is shortened with the knee flexed (Singh, 2013). A greater proportion of the voluntary plantarflexor force is attributed to the soleus in this position and there is reduced resistance to passive movement. However, flexing the knee does not distinguish between spastic activation of the soleus and gastrocnemius. Spasticity is reduced with the knee flexed, but spastic activation of both muscles, not just the gastrocnemius, is reduced (Perry, 1993). Therefore I would argue that our selection of the soleus muscle is neurophysiologically sound and functionally relevant.

Active Contractions

Testing of reflex and TMS evoked responses during voluntary contractions adds to the experimental complexity. It is easier to be certain conditions are matched between participants at rest and responses are easier to identify when there is no other EMG activity. However, motor function requires voluntary activity. Therefore neurophysiologic evaluations performed during activity are more likely to be functionally relevant. In addition, many of the responses obtained in this theses (e.g. inhibitory phase of cutaneomuscular reflexes and TMS responses in the soleus) are difficult to obtain at rest. Therefore testing was performed during voluntary contractions.

Care was taken to obtain maximal voluntary activity (MVA) values that were truly representative of an individual's maximum (Gandevia, 2001). Verbal encouragement, visual feedback, and considerable rest between trials were provided. Many participants had multiple 3 second trials, though this time frame was only a guideline. Participants who had difficulty ramping up their contraction could continue until their activity ceased to increase. Targets for all submaximal contractions were set based on the MVA values. While the practice of normalizing to MVA is both common, and more physiologically representative of effort than comparing raw EMG values between individuals, it does introduce a potential source of error into an experiment.

Participants with spastic CP had particular difficulty matching target background activity levels even with visual feedback and verbal encouragement. This was not unexpected given the known impairments in muscle selection, timing and degree of activation. Originally we attempted to perform the experiments at a lower level of background muscle activity (10% MVA). However we found in pilot testing that participants could more consistently maintain a target background activity level at 20% MVA. To ensure that repeated contractions did not result in significant fatigue, participants could rest at any time. In the longer experiment involving TMS, there was no consistent evidence of fatigue. The amplitude of MEPs at the intensity that produced the largest response during characterization of the recruitment curve, and MVA's were similar at the end of the experiment to those obtained during the experiment. However many participants did report mild muscle soreness the next day. Therefore it is difficult to be certain our experimental protocol did not induce any fatigue.

Localization of TMS

All TMS responses in this thesis were evoked using a custom "batwing" coil designed to produce deep, focal stimulation and thus, well suited to selective stimulation of muscles represented within the precentral gyrus adjacent to the interhemispheric fissure. This coil is designed to combine the focality of stimulation produced by a figure-8 coil and the depth of penetration produced by the double-cone coil. Both the thin polyurethane coating of the custom coil and the batwing shape enable the coil to be placed closer to the head than the double-cone coil. These features are advantageous for selectively activating the soleus with reduced activation of the contralateral cortex in neurologically intact individuals given the deep midline motor representation. However in CP, as shown by others and confirmed in our data in Chapter 3, the optimal site for stimulation of leg musculature is further from the midline than in neurologically intact participants (Kesar et al., 2012; Maegaki et al., 1999; Vandermeeren et al., 2009). With the use of fMRI-guided neuronavigation, we could better explore the region of the brain activated by TMS. However, TMS does not produce pin-point stimulation, even with the batwing coil. TMS, particularly at the relatively high stimulation intensities required by many participants with CP, can produce MEPs in other muscles including contralateral muscles. Recall that during the characterization of the stimulus-recruitment curves, MEP amplitudes were still increasing at 100% MSO in 11 of the 13 participants. However, using a paired pulse paradigm to activate

intracortical facilitation along with neuronavigation, may facilitate exploration of the full recruitment curves and the brain area activated by that TMS.

In addition to providing information as to the location of the centre of stimulation, neuronavigation can be used to determine the distance from the coil to the brain. Increased distance of stimulation from the brain decreases the currents induced by the magnetic field and thus increases the motor thresholds (Hess et al., 1986). While it is highly unlikely that the higher motor thresholds found in multiple studies involving participants with CP (including this thesis) (Berweck et al., 2008; Koerte et al., 2011; Vry et al., 2008), result from greater distance between the stimulating coil and the cortex, we cannot eliminate that possibility definitively with the techniques used here.

Similarly, evaluating TMS responses with MEPs recorded with sEMG in the distal musculature does not provide information regarding the localization of the transmitting pathway. By recording short latency responses in single motor units in the reticular formation Fisher et al. (2012) provided evidence that MEP responses recorded over muscles may result, at least in part, by activation of the cortico-reticulospinal pathway. Given the impairments in selective motor control seen in CP can be similar to those seen following lesions to brainstem pathways (Lawrence and Kuypers, 1965), an increased role of brainstem pathways during voluntary movement in CP has been proposed (Cahill-Rowley and Rose, 2014). The latency of the MEP or even estimates of the central motor conduction time (brain to cord) by subtraction of the spinally-evoked responses is too crude a measure to assess the role of brainstem pathways and invasive techniques like recording brainstem responses are not generally feasible in humans. It's possible that a wavelet-based analysis of MEPs (McKay et al., 2013) might reveal meaningful differences that could suggest an increased role of a slightly delayed pathway such as a brainstem pathway. However, differences in the muscle architecture and fibre type distribution that also influence the MEP waveform could confound the interpretation of this data.

Quantification of MEPs

As discussed in Chapter 1 and reviewed by Lieber and Smith (2014), muscles in CP are different than muscles in people without neurological injuries. Any quantification of MEPs recorded in muscle is potentially confounded by these differences. Normalization of MEPs to the maximal response elicited by peripheral nerve stimulation (M_{max}) attempts to account for some

of these differences. All comparisons of raw MEP magnitude were confirmed normalizing to M_{max}. However, as any normalization introduces another potential source of error the absolute (μV) magnitudes are primarily reported.

In addition, quantification of an MEP with peak-to-peak amplitude is arguably less physiologic than quantification of the MEP area. In the soleus muscle, with its high proportion of slow twitch muscle fibres, evoked responses are more likely to be polyphasic. Peak-to-peak amplitude only quantifies the highest and lowest phases of the response and may underestimate an MEP if there is temporal dispersion between the action potentials recorded from the muscle resulting in multiple smaller phases. The MEP area quantifies the entire response. For this reason, we explored quantification of MEPs through the use of MEP area. However, it requires estimation of the MEP onset and offset. Our pilot testing revealed that during active contractions this approach introduced greater variability in the MEP measurement, particularly for small MEPs. This variability could be reduced by quantifying the area of a response generated by averaging multiple (e.g. 10) MEPs. I have one additional concern about applying this technique to our population. We have not studied it formally, but subjectively, the MEP shape seemed less consistent from trial to trial (see Fig 3-3 and Fig 5-2 for examples of overlying MEPs). If this is indeed the case, averaging of the MEPs could lead to a greater loss in amplitude.

Limited Mechanistic Interpretations

It would be great to be able to extrapolate these neurophysiological experiments to cellular or even molecular mechanisms, but alas, we cannot. For example, the active motor thresholds discussed in Chapter 3, reflect the excitability of low threshold interneurons that synapse on cortical motoneurons. However, factors like skull thickness, the integrity of the cortical motoneuron or even the amount of subcutaneous fat overlying the muscle from which the MEP is recorded also impact the active motor threshold. Similarly, we do not know if myelination, axon packing density or an increase in crossing fibers are responsible for reduced levels of fractional anisotropy found with diffusion tractography. In Chapter 4, we cannot identify the precise circuits involved in increased sensory-evoked activation of motoneurons. The use of the more invasive fine-wire recordings of single motor units enables us to provide evidence of decreased IPSP activation and we do not know how the inputs to the motoneuron that normally produce the IPSP have changed or if there are new excitatory inputs. Lastly in

Chapter 5, we unfortunately cannot record directly from the inhibitory interneurons responsible for SICI or stain for the density of $GABA_A$ receptors. Doing so would negate the need to match the unconditioned (test) MEP amplitude and thus make comparison of SICI between people with and without CP much easier. Fortunately animal models that may help address these issues are improving and increasingly represent features (such as involuntary postures) of CP in humans (Clowry et al., 2014). Nevertheless the studies here suggest mechanisms (on the pathway level) that contribute to motor impairments in spastic CP.

Suggestions for Future Work

Participants

Our participants represent a convenience sample and we were fortunate that they share some common clinical characteristics (bilateral motor symptoms) and yet a diverse spectrum of impairment (GMFCS I to IV). Generation and use of a database of people with spastic CP would greatly help with participant recruitment. In addition it would facilitate studies of specific subgroups of participants with CP. With the help of a database it would be easier to explore associations between neurophysiological measures and specific clinical characteristics such as GMFCS level or etiology (e.g. isolated periventricular white matter injury secondary to preterm delivery).

Dominant Motor Pathways

Ipsilateral motor pathways have been studied predominantly in participants with unilateral CP. As discussed in Chapter 2, participants with spastic CP who have ipsilateral motor pathways tend to have lower levels of motor function. Of our participants 4/17 did not have contralateral motor pathways, but did have ipsilateral pathways. Neither their TMS responses (i.e. MEP amplitudes from the ipsilateral pathway) nor their cutaneomuscular reflexes (motor unit evidence of inhibition or $I1_{max}$) were systematically different from the participants with contralateral responses. On the other hand, these 4 participants had a lower MVA ($p=0.029$) and diffusion tractography of their corticospinal tract suggested lower anatomic integrity (FA $p=0.061$) or was untraceable (CP-15). A more thorough characterization of adults with bilateral CP having evidence of only an ipsilateral motor pathway to their more impaired limb would be interesting. I imagine that consistent with people with unilateral CP, and our MVA findings,

motor function would be more impaired in the group with ipsilateral only pathways. Similarly, consistent with people with unilateral CP and our tractography findings, I would hypothesize decreased FA and increased perpendicular diffusivity. It is harder to predict the characterization from the TMS studies. The MEP amplitudes in our four participants stimulated over the ipsilateral cortex are very similar in median and distribution to those stimulated over the contralateral cortex. However as MVA was associated with MEP amplitudes and the ipsilateral group had lower MVA, I would hypothesize reduced MEP amplitudes in responses obtained from the ipsilateral cortex in a larger group.

In addition, while TMS responses were not extensively explored in the ipsilateral cortex for most of the participants with contralateral responses, at least 7 more participants had ipsilateral MEPs. This poses the question, in people with TMS responses in their more impaired limb from both the contralateral and ipsilateral cortices, what is the relative contribution of each pathway? One approach would be to compare the responses to TMS from each cortex. A metric similar to the laterality index which compares the strength of bilateral sensorimotor indices recorded with fMRI could be applied (Johansen-Berg et al., 2002). For example, if the dominant cortex was determined as the cortex from which TMS evoked larger MEPs, I imagine that those individuals whose contralateral cortex was dominant would have greater motor function than those whose ipsilateral cortex was dominant. I also suspect that gross motor function would be highest in those with a clearly dominant contralateral cortex and lowest in those with a clearly dominant ipsilateral cortex consistent with the continuum of motor function observed in those with unilateral spastic CP. However, I would be intrigued to explore whether indices of selective motor control, such as antagonist co-activation during a grasping or fine motor task, were most impaired in those with TMS responses of similar magnitude from both cortices.

Control of Voluntary Activity

During voluntary activity, SICI is thought to contribute to the specificity of signals from the motor cortex. It is decreased prior to initiating voluntary activity and this change is local, only impacting the muscle that will be activated as opposed to antagonists muscles(Reynolds and Ashby, 1999). Given the impairments with timing of muscle activation and co-activation of antagonists, it would be interesting to evaluate the modulation of SICI in people with spastic CP. While we did not observe impairments in SICI in the active soleus, others have shown in people

with unilateral CP that SICI was reduced when measured at rest (Berweck et al., 2008). It is possible that in people with CP, SICI is impaired at rest and that the modulation of SICI with voluntary activity is lost. This could result in the similar levels of SICI that we observed during muscle activity. In fact, a case study involving a man with unilateral CP revealed SICI to the affected hand was not modulated during voluntary activation of that hand (Cincotta et al., 2000). Further experiments comparing SICI at rest and during voluntary contractions in CP could probe the role of SICI in the specificity of signals from the motor cortex (or lack thereof) in people with spastic CP.

Use of Electrophysiology to Customize and Evaluate Interventions

The efficacy of current treatments is highly variable, and research is challenged by the high variability in the clinical presentation of CP (Narayanan, 2012; Novak et al., 2013). The use of physiologic markers to help select specific patients for specific interventions could help ameliorate this problem (Jaspers et al., 2015). In addition, improved understanding of the impairments underlying motor dysfunction could help select interventions with a scientific basis (Nielsen et al., 2015). These ideas are supported by the finding of different neuroplastic changes as measured by transcranial magnetic stimulation (TMS) in response to the same intervention in two subpopulations of people with unilateral CP (Juenger et al., 2013). Does resistance training that results in improved joint kinematics in gait (Kirk et al., 2016) occur along with improved activation of muscles by descending pathways or greater facilitation with voluntary activity? Do any of the TMS metrics explored in chapter 3 predict who will or will not obtain benefit from resistance training or the rate of such benefit? I am also interested to explore if the response to upper extremity therapy differed in those with ipsilateral pathways to those with bilateral or contralateral pathways. In specific, does the TMS pathway predict whether a patient may benefit more from constraint-induced movement therapy or hand-arm *bimanual* intensive therapy (HABIT)?

One way electrophysiology could particularly help with customizing interventions is by guiding the selection of patients for selective dorsal rhizotomy. As discussed in the Translational Perspective section of chapter 4, the ability to walk is a commonly used criteria for selective dorsal rhizotomy. However, while spasticity is consistently reduced following surgery, the impacts on motor function are varied. I wonder if patients with abnormal sensory-evoked

activation of motoneurons, as demonstrated by cutaneomuscular reflexes, would be more likely to have improved motor function following selective dorsal rhizotomy. Our finding of an association between the cutaneomuscular reflex and GMFCS suggest that this may be the case. If so, this reflex may be a useful screening tool in the selection of patients for selective dorsal rhizotomy.

Lastly, very few trials have been done looking for ways to improve motor function in adults with spastic CP. Many studies have clearly demonstrated improvements in response to interventions in others with chronic neurological injuries such as stroke or spinal cord injury. However, given the chronicity of injury and demands of adult life, it's unlikely a brief intervention would be effective unless it was so intensive that many adults with careers would be able to participate. What if we could predict the response to a prolonged (e.g. 1 year) intervention by the neurophysiologic response within the first month? For example, improvements may be noted in MEPs or motoneuron inhibition before they are noted in the strength or quality of movement. Participants with neurophysiologic evidence of plastic changes could then be encouraged to continue and funding agencies to continue paying for the therapy. On the other hand, participants without neurophysiologic changes could then use their available time and funding to shift to other treatment options. The utility of physiologic markers is currently unproven, though given the great variability in responses to interventions, I believe it warrants further investigation.

Concluding Remarks

I hope this chapter has provided some perspectives on the experiments (Chapters 3-5) that form the main body of this thesis exploring motor impairments in spastic CP. While we studied only 17 individuals with CP, the findings of our experiments likely generalize to the population of adults with bilateral spastic CP. However, the findings need to be interpreted with caution. Attempts were made to control experimental factors, but as discussed here, many factors could be improved upon and others are difficult to control in people with spastic CP. Nevertheless, the findings here contribute to our understanding of the mechanisms of motor impairment in spastic CP. They also trigger questions for further research and suggest techniques that can be applied to study differences within people with spastic CP or used in the evaluation

of clinical interventions. Following validation studies, they maybe be able to help direct treatment decisions.

References

Arpin DJ, Stuberg W, Stergiou N, Kurz MJ. Motor control of the lower extremity musculature in children with cerebral palsy. Res Dev Disabil 2013;34:1134-1143.

Ashwal S, Russman BS, Blasco PA, Miller G, Sandler A, Shevell M, Stevenson R, Quality Standards Subcommittee of the American Academy of N, Practice Committee of the Child Neurology S. Practice parameter: Diagnostic assessment of the child with cerebral palsy: Report of the quality standards subcommittee of the american academy of neurology and the practice committee of the child neurology society. Neurology 2004;62:851-863.

Berger W, Quintern J, Dietz V. Pathophysiology of gait in children with cerebral palsy. Electroencephalogr Clin Neurophysiol 1982;53:538-548.

Berweck S, Walther M, Brodbeck V, Wagner N, Koerte I, Henschel V, Juenger H, Staudt M, Mall V. Abnormal motor cortex excitability in congenital stroke. Pediatr Res 2008;63:84-88.

Blair E, Watson L, Badawi N, Stanley FJ. Life expectancy among people with cerebral palsy in western australia. Dev Med Child Neurol 2001;43:508-515.

Butt K, Lim K, Society of O, Gynaecologists of C. Determination of gestational age by ultrasound. J Obstet Gynaecol Can 2014;36:171-183.

Cahill-Rowley K, Rose J. Etiology of impaired selective motor control: Emerging evidence and its implications for research and treatment in cerebral palsy. Dev Med Child Neurol 2014;56:522-528.

Cincotta M, Borgheresi A, Liotta P, Montigiani A, Marin E, Zaccara G, Ziemann U. Reorganization of the motor cortex in a patient with congenital hemiparesis and mirror movements. Neurology 2000;55:129-131.

Clowry GJ, Basuodan R, Chan F. What are the best animal models for testing early intervention in cerebral palsy? Front Neurol 2014;5:258.

Croarkin PE, Nakonezny PA, Lewis CP, Zaccariello MJ, Huxsahl JE, Husain MM, Kennard BD, Emslie GJ, Daskalakis ZJ. Developmental aspects of cortical excitability and inhibition in depressed and healthy youth: An exploratory study. Front Hum Neurosci 2014;8:669.

Eek MN, Tranberg R, Beckung E. Muscle strength and kinetic gait pattern in children with bilateral spastic CP. Gait Posture 2011;33:333-337.

Elder GC, Kirk J, Stewart G, Cook K, Weir D, Marshall A, Leahey L. Contributing factors to muscle weakness in children with cerebral palsy. Dev Med Child Neurol 2003;45:542-550.

Engsberg JR, Ross SA, Olree KS, Park TS. Ankle spasticity and strength in children with spastic diplegic cerebral palsy. Dev Med Child Neurol 2000;42:42-47.

Fisher KM, Zaaimi B, Baker SN. Reticular formation responses to magnetic brain stimulation of primary motor cortex. J Physiol 2012;590:4045-4060.

Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 2001;81:1725-1789.

Garvey MA, Ziemann U, Bartko JJ, Denckla MB, Barker CA, Wassermann EM. Cortical correlates of neuromotor development in healthy children. Clin Neurophysiol 2003;114:1662-1670.

Haak P, Lenski M, Hidecker MJC, Li MIN, Paneth N. Cerebral palsy and aging. Dev Med Child Neurol 2009;51:16-23.

Hess CW, Mills KR, Murray NM. Magnetic stimulation of the human brain: Facilitation of motor responses by voluntary contraction of ipsilateral and contralateral muscles with additional observations on an amputee. Neurosci Lett 1986;71:235-240.

Himmelmann K, Uvebrant P. Function and neuroimaging in cerebral palsy: A population-based study. Dev Med Child Neurol 2011;53:516-521.

Jaspers E, Byblow WD, Feys H, Wenderoth N. The corticospinal tract: A biomarker to categorize upper limb functional potential in unilateral cerebral palsy. Front Pediatr 2015;3:112.

Johansen-Berg H, Dawes H, Guy C, Smith SM, Wade DT, Matthews PM. Correlation between motor improvements and altered fMRI activity after rehabilitative therapy. Brain 2002;125:2731-2742.

Juenger H, Kuhnke N, Braun C, Ummenhofer F, Wilke M, Walther M, Koerte I, Delvendahl I, Jung NH, Berweck S, Staudt M, Mall V. Two types of exercise-induced neuroplasticity in congenital hemiparesis: A transcranial magnetic stimulation, functional MRI, and magnetoencephalography study. Dev Med Child Neurol 2013;55:941-951.

Kesar TM, Sawaki L, Burdette JH, Cabrera MN, Kolaski K, Smith BP, O'Shea TM, Koman LA, Wittenberg GF. Motor cortical functional geometry in cerebral palsy and its relationship to disability. Clin Neurophysiol 2012;123:1383-1390.

Kirk H, Geertsen SS, Lorentzen J, Krarup KB, Bandholm T, Nielsen JB. Explosive resistance training increases rate of force development in ankle dorsiflexors and gait function in adults with cerebral palsy. J Strength Cond Res 2016.

Koerte I, Pelavin P, Kirmess B, Fuchs T, Berweck S, Laubender RP, Borggraefe I, Schroeder S, Danek A, Rummeny C, Reiser M, Kubicki M, Shenton ME, Ertl-Wagner B, Heinen F. Anisotropy of transcallosal motor fibres indicates functional impairment in children with periventricular leukomalacia. Dev Med Child Neurol 2011;53:179-186.

Lawrence DG, Kuypers HG. Pyramidal and non-pyramidal pathways in monkeys: Anatomical and functional correlation. Science 1965;148:973-975.

Lieber RL, Smith LR. Skeletal muscle changes due to cerebral palsy. In: Shepherd RB, editor. Cerebral palsy in infancy: Targeted activity to optimize early growth and development. Edinburgh; New York: Churchill Livingstone/Elsevier; 2014. p. 135-155.

Maegaki Y, Maeoka Y, Ishii S, Eda I, Ohtagaki A, Kitahara T, Suzuki N, Yoshino K, Ieshima A, Koeda T, Takeshita K. Central motor reorganization in cerebral palsy patients with bilateral cerebral lesions. Pediatr Res 1999;45:559-567.

McKay JL, Welch TD, Vidakovic B, Ting LH. Statistically significant contrasts between EMG waveforms revealed using wavelet-based functional anova. J Neurophysiol 2013;109:591-602.

Narayanan UG. Management of children with ambulatory cerebral palsy: An evidence-based review. J Pediatr Orthop 2012;32 Suppl 2:S172-181.

National Institute for Health Care Excellence. Spasticity in children and young people. London: National Institue for Health and Care Excellence; 2012.

Nielsen JB, Willerslev-Olsen M, Christiansen L, Lundbye-Jensen J, Lorentzen J. Science-based neurorehabilitation: Recommendations for neurorehabilitation from basic science. J Mot Behav 2015;47:7-17.

Novak I, McIntyre S, Morgan C, Campbell L, Dark L, Morton N, Stumbles E, Wilson SA, Goldsmith S. A systematic review of interventions for children with cerebral palsy: State of the evidence. Dev Med Child Neurol 2013;55:885-910.

Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, Rapoport JL, Evans AC. Structural maturation of neural pathways in children and adolescents: In vivo study. Science 1999;283:1908-1911.

Perry J. Determinants of muscle function in the spastic lower extremity. Clin Orthop Relat Res 1993:10-26.

Pitcher JB, Ogston KM, Miles TS. Age and sex differences in human motor cortex input-output characteristics. J Physiol 2002;546:605-613.

Reid SM, Dagia CD, Ditchfield MR, Carlin JB, Reddihough DS. Population-based studies of brain imaging patterns in cerebral palsy. Dev Med Child Neurol 2014;56:222-232.

Reynolds C, Ashby P. Inhibition in the human motor cortex is reduced just before a voluntary contraction. Neurology 1999;53:730-735.

Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M. A report: The definition and classification of cerebral palsy - april 2006. Dev Med Child Neurol 2007;49:8-14.

Shevell MI, Dagenais L, Hall N. The relationship of cerebral palsy subtype and functional motor impairment: A population-based study. Dev Med Child Neurol 2009;51:872-877.

Singh D. Nils silfverskiöld (1888–1957) and gastrocnemius contracture. Foot Ankle Surg 2013;19:135-138.

Strauss D, Brooks J, Rosenbloom L, Shavelle R. Life expectancy in cerebral palsy: An update. Dev Med Child Neurol 2008;50:487-493.

Towsley K, Shevell MI, Dagenais L. Population-based study of neuroimaging findings in children with cerebral palsy. Eur J Paediatr Neurol 2011;15:29-35.

Vandermeeren Y, Davare M, Duque J, Olivier E. Reorganization of cortical hand representation in congenital hemiplegia. Eur J Neurosci 2009;29:845-854.

Vry J, Linder-Lucht M, Berweck S, Bonati U, Hodapp M, Uhl M, Faist M, Mall V. Altered cortical inhibitory function in children with spastic diplegia: A TMS study. Exp Brain Res 2008;186:611-618.

Walther M, Berweck S, Schessl J, Linder-Lucht M, Fietzek UM, Glocker FX, Heinen F, Mall V. Maturation of inhibitory and excitatory motor cortex pathways in children. Brain Dev 2009;31:562-567.

Wiley ME, Damiano DL. Lower-extremity strength profiles in spastic cerebral palsy. Dev Med Child Neurol 1998;40:100-107.

Wu YW, Lindan CE, Henning LH, Yoshida CK, Fullerton HJ, Ferriero DM, Barkovich AJ, Croen LA. Neuroimaging abnormalities in infants with congenital hemiparesis. Pediatr Neurol 2006;35:191-196.

Ziemann U. TMS and drugs. Clin Neurophysiol 2004;115:1717-1729.

Ziemann U. Pharmaco-transcranial magnetic stimulation studies of motor excitability. Handb Clin Neurol 2013;116:387-397.

Bibliography

Achache V, Roche N, Lamy JC, Boakye M, Lackmy A, Gastal A, Quentin V, Katz R. Transmission within several spinal pathways in adults with cerebral palsy. Brain 2010;133:1470-1483.

Aisen ML, Kerkovich D, Mast J, Mulroy S, Wren TA, Kay RM, Rethlefsen SA. Cerebral palsy: Clinical care and neurological rehabilitation. Lancet neurology 2011;10:844-852.

Aniss AM, Gandevia SC, Burke D. Reflex responses in active muscles elicited by stimulation of low-threshold afferents from the human foot. J Neurophysiol 1992;67:1375-1384.

Arpin DJ, Stuberg W, Stergiou N, Kurz MJ. Motor control of the lower extremity musculature in children with cerebral palsy. Res Dev Disabil 2013;34:1134-1143.

Ashwal S, Russman BS, Blasco PA, Miller G, Sandler A, Shevell M, Stevenson R, Quality Standards Subcommittee of the American Academy of N, Practice Committee of the Child Neurology S. Practice parameter: Diagnostic assessment of the child with cerebral palsy: Report of the quality standards subcommittee of the american academy of neurology and the practice committee of the child neurology society. Neurology 2004;62:851-863.

Aymard C, Katz R, Lafitte C, Lo E, Penicaud A, Pradat-Diehl P, Raoul S. Presynaptic inhibition and homosynaptic depression: A comparison between lower and upper limbs in normal human subjects and patients with hemiplegia. Brain 2000;123 (Pt 8):1688-1702.

Baek SO, Jang SH, Lee E, Kim S, Hah JO, Park YH, Lee JM, Son SM. Cst recovery in pediatric hemiplegic patients: Diffusion tensor tractography study. Neurosci Lett 2013;557 Pt B:79-83.

Baker SN. The primate reticulospinal tract, hand function and functional recovery. J Physiol 2011;589:5603-5612.

Barber L, Hastings-Ison T, Baker R, Barrett R, Lichtwark G. Medial gastrocnemius muscle volume and fascicle length in children aged 2 to 5 years with cerebral palsy. Dev Med Child Neurol 2011;53:543-548.

Barrett RS, Lichtwark GA. Gross muscle morphology and structure in spastic cerebral palsy: A systematic review. Dev Med Child Neurol 2010;52:794-804.

Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 2002;15:435-455.

Berbrayer D, Ashby P. Reciprocal inhibition in cerebral palsy. Neurology 1990;40:653-656.

Berger W, Quintern J, Dietz V. Pathophysiology of gait in children with cerebral palsy. Electroencephalogr Clin Neurophysiol 1982;53:538-548.

Berweck S, Walther M, Brodbeck V, Wagner N, Koerte I, Henschel V, Juenger H, Staudt M, Mall V. Abnormal motor cortex excitability in congenital stroke. Pediatr Res 2008;63:84-88.

Blair E, Watson L, Badawi N, Stanley FJ. Life expectancy among people with cerebral palsy in western australia. Dev Med Child Neurol 2001;43:508-515.

Bohannon RW, Smith MB. Interrater reliability of a modified Ashworth scale of muscle spasticity. Phys Ther 1987;67:206-207.

Boulenguez P, Liabeuf S, Bos R, Bras H, Jean-Xavier C, Brocard C, Stil A, Darbon P, Cattaert D, Delpire E, Marsala M, Vinay L. Down-regulation of the potassium-chloride cotransporter kcc2 contributes to spasticity after spinal cord injury. Nat Med 2010;16:302-307.

Brouwer B, Smits E. Corticospinal input onto motor neurons projecting to ankle muscles in individuals with cerebral palsy. Dev Med Child Neurol 1996;38:787-796.

Butt K, Lim K, Society of O, Gynaecologists of C. Determination of gestational age by ultrasound. J Obstet Gynaecol Can 2014;36:171-183.

Cahill-Rowley K, Rose J. Etiology of impaired selective motor control: Emerging evidence and its implications for research and treatment in cerebral palsy. Dev Med Child Neurol 2014;56:522-528.

Campbell SK, Hedeker D. Validity of the test of infant motor performance for discriminating among infants with varying risk for poor motor outcome. J Pediatr 2001;139:546-551.

Carr LJ. Development and reorganization of descending motor pathways in children with hemiplegic cerebral palsy. Acta Paediatr Suppl 1996;416:53-57.

Carr LJ, Harrison LM, Evans AL, Stephens JA. Patterns of central motor reorganization in hemiplegic cerebral palsy. Brain 1993;116 (Pt 5):1223-1247.

Centers for Disease Control and Prevention [CDC]. Economic costs associated with mental retardation, cerebral palsy, hearing loss, and vision impairment--united states, 2003. MMWR Morb Mortal Wkly Rep 2004;53:57-59.

Chakrabarty S, Shulman B, Martin JH. Activity-dependent codevelopment of the corticospinal system and target interneurons in the cervical spinal cord. J Neurosci 2009;29:8816-8827.

Chan G, Miller F. Assessment and treatment of children with cerebral palsy. Orthop Clin North Am 2014;45:313-325.

Chaturvedi SK, Rai Y, Chourasia A, Goel P, Paliwal VK, Garg RK, Rathore RK, Pandey CM, Gupta RK. Comparative assessment of therapeutic response to physiotherapy with or without botulinum toxin injection using diffusion tensor tractography and clinical scores in term diplegic cerebral palsy children. Brain Dev 2013;35:647-653.

Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Evidence from epidural recordings. Exp Brain Res 1999;128:539-542.

Chen R, Yung D, Li JY. Organization of ipsilateral excitatory and inhibitory pathways in the human motor cortex. J Neurophysiol 2003;89:1256-1264.

Cincotta M, Borgheresi A, Liotta P, Montigiani A, Marin E, Zaccara G, Ziemann U. Reorganization of the motor cortex in a patient with congenital hemiparesis and mirror movements. Neurology 2000;55:129-131.

Clowry GJ, Basuodan R, Chan F. What are the best animal models for testing early intervention in cerebral palsy? Front Neurol 2014;5:258.

Concha L, Gross DW, Wheatley BM, Beaulieu C. Diffusion tensor imaging of time-dependent axonal and myelin degradation after corpus callosotomy in epilepsy patients. Neuroimage 2006;32:1090-1099.

Condliffe EG, Jeffery DT, Emery DJ, Gorassini MA. Spinal inhibition and motor function in adults with spastic cerebral palsy. J Physiol 2016.

Cote MP, Gandhi S, Zambrotta M, Houle JD. Exercise modulates chloride homeostasis after spinal cord injury. J Neurosci 2014;34:8976-8987.

Crenna P. Spasticity and 'spastic' gait in children with cerebral palsy. Neurosci Biobehav Rev 1998;22:571-578.

Croarkin PE, Nakonezny PA, Lewis CP, Zaccariello MJ, Huxsahl JE, Husain MM, Kennard BD, Emslie GJ, Daskalakis ZJ. Developmental aspects of cortical excitability and inhibition in depressed and healthy youth: An exploratory study. Front Hum Neurosci 2014;8:669.

Crone C. Reciprocal inhibition in man. Dan Med Bull 1993;40:571-581.

Crone C, Johnsen LL, Biering-Sorensen F, Nielsen JB. Appearance of reciprocal facilitation of ankle extensors from ankle flexors in patients with stroke or spinal cord injury. Brain 2003;126:495-507.

Crone C, Nielsen J, Petersen N, Ballegaard M, Hultborn H. Disynaptic reciprocal inhibition of ankle extensors in spastic patients. Brain 1994;117 (Pt 5):1161-1168.

Damiano DL, Martellotta TL, Sullivan DJ, Granata KP, Abel MF. Muscle force production and functional performance in spastic cerebral palsy: Relationship of cocontraction. Arch Phys Med Rehabil 2000;81:895-900.

Davey NJ, Smith HC, Savic G, Maskill DW, Ellaway PH, Frankel HL. Comparison of input-output patterns in the corticospinal system of normal subjects and incomplete spinal cord injured patients. Exp Brain Res 1999;127:382-390.

Davey NJ, Smith HC, Wells E, Maskill DW, Savic G, Ellaway PH, Frankel HL. Responses of thenar muscles to transcranial magnetic stimulation of the motor cortex in patients with incomplete spinal cord injury. J Neurol Neurosurg Psychiatry 1998;65:80-87.

Di Lazzaro V, Oliviero A, Profice P, Meglio M, Cioni B, Tonali P, Rothwell JC. Descending spinal cord volleys evoked by transcranial magnetic and electrical stimulation of the motor cortex leg area in conscious humans. J Physiol 2001;537:1047-1058.

Di Lazzaro V, Profice P, Ranieri F, Capone F, Dileone M, Oliviero A, Pilato F. I-wave origin and modulation. Brain stimulation 2012;5:512-525.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol 1998;508 (Pt 2):625-633.

Di Lazzaro V, Ziemann U. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. Front Neural Circuits 2013;7:18.

Di Lazzaro V, Ziemann U, Lemon RN. State of the art: Physiology of transcranial motor cortex stimulation. Brain stimulation 2008;1:345-362.

Dobson F, Morris ME, Baker R, Graham HK. Gait classification in children with cerebral palsy: A systematic review. Gait Posture 2007;25:140-152.

Dougherty NJ. A review of cerebral palsy for the oral health professional. Dent Clin North Am 2009;53:329-338.

Downing AL, Ganley KJ, Fay DR, Abbas JJ. Temporal characteristics of lower extremity moment generation in children with cerebral palsy. Muscle Nerve 2009;39:800-809.

Drobyshevsky A, Derrick M, Wyrwicz AM, Ji X, Englof I, Ullman LM, Zelaya ME, Northington FJ, Tan S. White matter injury correlates with hypertonia in an animal model of cerebral palsy. J Cereb Blood Flow Metab 2007;27:270-281.

Duque J, Thonnard JL, Vandermeeren Y, Sebire G, Cosnard G, Olivier E. Correlation between impaired dexterity and corticospinal tract dysgenesis in congenital hemiplegia. Brain 2003;126:732-747.

Edwards JD, Meehan SK, Linsdell MA, Borich MR, Anbarani K, Jones PW, Ferris J, Boyd LA. Changes in thresholds for intracortical excitability in chronic stroke: More than just altered intracortical inhibition. Restorative neurology and neuroscience 2013;31:693-705.

Eek MN, Tranberg R, Beckung E. Muscle strength and kinetic gait pattern in children with bilateral spastic CP. Gait Posture 2011;33:333-337.

Elbert T, Candia V, Altenmuller E, Rau H, Sterr A, Rockstroh B, Pantev C, Taub E. Alteration of digital representations in somatosensory cortex in focal hand dystonia. Neuroreport 1998;9:3571-3575.

Elder GC, Kirk J, Stewart G, Cook K, Weir D, Marshall A, Leahey L. Contributing factors to muscle weakness in children with cerebral palsy. Dev Med Child Neurol 2003;45:542-550.

Eliasson AC, Krumlinde-Sundholm L, Rosblad B, Beckung E, Arner M, Ohrvall AM, Rosenbaum P. The manual ability classification system (macs) for children with cerebral palsy: Scale development and evidence of validity and reliability. Dev Med Child Neurol 2006;48:549-554.

Engsberg JR, Ross SA, Olree KS, Park TS. Ankle spasticity and strength in children with spastic diplegic cerebral palsy. Dev Med Child Neurol 2000;42:42-47.

Evans AL, Harrison LM, Stephens JA. Cutaneomuscular reflexes recorded from the first dorsal interosseous muscle of children with cerebral palsy. Dev Med Child Neurol 1991;33:541-551.

Eyre JA. Corticospinal tract development and its plasticity after perinatal injury. Neurosci Biobehav Rev 2007;31:1136-1149.

Eyre JA, Smith M, Dabydeen L, Clowry GJ, Petacchi E, Battini R, Guzzetta A, Cioni G. Is hemiplegic cerebral palsy equivalent to amblyopia of the corticospinal system? Ann Neurol 2007;62:493-503.

Eyre JA, Taylor JP, Villagra F, Smith M, Miller S. Evidence of activity-dependent withdrawal of corticospinal projections during human development. Neurology 2001;57:1543-1554.

Faist M, Mazevet D, Dietz V, Pierrot-Deseilligny E. A quantitative assessment of presynaptic inhibition of ia afferents in spastics. Differences in hemiplegics and paraplegics. Brain 1994;117 (Pt 6):1449-1455.

Farmer SF, Harrison LM, Ingram DA, Stephens JA. Plasticity of central motor pathways in children with hemiplegic cerebral palsy. Neurology 1991;41:1505-1510.

Fisher KM, Zaaimi B, Baker SN. Reticular formation responses to magnetic brain stimulation of primary motor cortex. J Physiol 2012;590:4045-4060.

Fowler EG, Staudt LA, Greenberg MB, Oppenheim WL. Selective control assessment of the lower extremity (scale): Development, validation, and interrater reliability of a clinical tool for patients with cerebral palsy. Dev Med Child Neurol 2009;51:607-614.

Friel KM, Martin JH. Bilateral activity-dependent interactions in the developing corticospinal system. J Neurosci 2007;27:11083-11090.

Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 2001;81:1725-1789.

Garry MI, Thomson RH. The effect of test TMS intensity on short-interval intracortical inhibition in different excitability states. Exp Brain Res 2009;193:267-274.

Garvey MA, Ziemann U, Bartko JJ, Denckla MB, Barker CA, Wassermann EM. Cortical correlates of neuromotor development in healthy children. Clin Neurophysiol 2003;114:1662-1670.

Gibbs J, Harrison LM, Stephens JA, Evans AL. Cutaneomuscular reflex responses recorded from the lower limb in children and adolescents with cerebral palsy. Dev Med Child Neurol 1999;41:456-464.

Glenn OA, Henry RG, Berman JI, Chang PC, Miller SP, Vigneron DB, Barkovich AJ. DTI-based three-dimensional tractography detects differences in the pyramidal tracts of infants and children with congenital hemiparesis. J Magn Reson Imaging 2003;18:641-648.

Glenn OA, Ludeman NA, Berman JI, Wu YW, Lu Y, Bartha AI, Vigneron DB, Chung SW, Ferriero DM, Barkovich AJ, Henry RG. Diffusion tensor MR imaging tractography of the pyramidal tracts correlates with clinical motor function in children with congenital hemiparesis. AJNR Am J Neuroradiol 2007;28:1796-1802.

Gordon AM, Duff SV. Relation between clinical measures and fine manipulative control in children with hemiplegic cerebral palsy. Dev Med Child Neurol 1999;41:586-591.

Graham HK, Harvey A, Rodda J, Nattrass GR, Pirpiris M. The functional mobility scale (FMS). J Pediatr Orthop 2004;24:514-520.

Grunt S, Fieggen AG, Vermeulen RJ, Becher JG, Langerak NG. Selection criteria for selective dorsal rhizotomy in children with spastic cerebral palsy: A systematic review of the literature. Dev Med Child Neurol 2014;56:302-312.

Guo X, Xiang J, Mun-Bryce S, Bryce M, Huang S, Huo X, Wang Y, Rose D, Degrauw T, Gartner K, Song T, Schmit J, Vargus-Adams J. Aberrant high-gamma oscillations in the somatosensory cortex of children with cerebral palsy: A meg study. Brain Dev 2012;34:576-583.

Haak P, Lenski M, Hidecker MJC, Li MIN, Paneth N. Cerebral palsy and aging. Dev Med Child Neurol 2009;51:16-23.

Hallett M. Transcranial magnetic stimulation: A primer. Neuron 2007;55:187-199.

Heinen F, Kirschner J, Fietzek U, Glocker FX, Mall V, Korinthenberg R. Absence of transcallosal inhibition in adolescents with diplegic cerebral palsy. Muscle Nerve 1999;22:255-257.

Herskind A, Ritterband-Rosenbaum A, Willerslev-Olsen M, Lorentzen J, Hanson L, Lichtwark G, Nielsen JB. Muscle growth is reduced in 15-month-old children with cerebral palsy. Dev Med Child Neurol 2015.

Hess CW, Mills KR, Murray NM. Magnetic stimulation of the human brain: Facilitation of motor responses by voluntary contraction of ipsilateral and contralateral muscles with additional observations on an amputee. Neurosci Lett 1986;71:235-240.

Hicks TP, Onodera S. The mammalian red nucleus and its role in motor systems, including the emergence of bipedalism and language. Prog Neurobiol 2012;96:165-175.

Hidecker MJ, Paneth N, Rosenbaum PL, Kent RD, Lillie J, Eulenberg JB, Chester K, Jr., Johnson B, Michalsen L, Evatt M, Taylor K. Developing and validating the communication function classification system for individuals with cerebral palsy. Dev Med Child Neurol 2011;53:704-710.

Himmelmann K, Uvebrant P. Function and neuroimaging in cerebral palsy: A population-based study. Dev Med Child Neurol 2011;53:516-521.

Hirtz D. Classification of cerebral palsy: Collaborative study perspective. Dev Med Child Neurol 2007;49:23-23.

Hislop HJ, Avers D, Brown M, Daniels L. Daniels and worthingham's muscle testing : Techniques of manual examination and performance testing. 9th ed. St. Louis, Mo.: Elsevier/Saunders; 2014.

Hodapp M, Vry J, Mall V, Faist M. Changes in soleus h-reflex modulation after treadmill training in children with cerebral palsy. Brain 2009;132:37-44.

Holmstrom L, Lennartsson F, Eliasson AC, Flodmark O, Clark C, Tedroff K, Forssberg H, Vollmer B. Diffusion MRI in corticofugal fibers correlates with hand function in unilateral cerebral palsy. Neurology 2011;77:775-783.

Holmstrom L, Vollmer B, Tedroff K, Islam M, Persson JK, Kits A, Forssberg H, Eliasson AC. Hand function in relation to brain lesions and corticomotor-projection pattern in children with unilateral cerebral palsy. Dev Med Child Neurol 2010;52:145-152.

Honaga K, Fujiwara T, Tsuji T, Hase K, Ushiba J, Liu M. State of intracortical inhibitory interneuron activity in patients with chronic stroke. Clin Neurophysiol 2013;124:364-370.

Hoon AH, Jr. Neuroimaging in cerebral palsy: Patterns of brain dysgenesis and injury. J Child Neurol 2005;20:936-939.

Hultborn H, Illert M, Nielsen J, Paul A, Ballegaard M, Wiese H. On the mechanism of the post-activation depression of the h-reflex in human subjects. Exp Brain Res 1996;108:450-462.

Hussain AW, Onambele GL, Williams AG, Morse CI. Muscle size, activation and coactivation in adults with cerebral palsy. Muscle Nerve 2014;49:76-83.

Jantzie LL, Corbett CJ, Firl DJ, Robinson S. Postnatal erythropoietin mitigates impaired cerebral cortical development following subplate loss from prenatal hypoxia-ischemia. Cereb Cortex 2015;25:2683-2695.

Jaspers E, Byblow WD, Feys H, Wenderoth N. The corticospinal tract: A biomarker to categorize upper limb functional potential in unilateral cerebral palsy. Front Pediatr 2015;3:112.

Johansen-Berg H, Dawes H, Guy C, Smith SM, Wade DT, Matthews PM. Correlation between motor improvements and altered fMRI activity after rehabilitative therapy. Brain 2002;125:2731-2742.

Johansen-Berg H, Rushworth MF. Using diffusion imaging to study human connectional anatomy. Annu Rev Neurosci 2009;32:75-94.

Jones CA, Yang JF. Reflex behavior during walking in incomplete spinal-cord-injured subjects. Exp Neurol 1994;128:239-248.

Juenger H, Kuhnke N, Braun C, Ummenhofer F, Wilke M, Walther M, Koerte I, Delvendahl I, Jung NH, Berweck S, Staudt M, Mall V. Two types of exercise-induced neuroplasticity in congenital hemiparesis: A transcranial magnetic stimulation, functional MRI, and magnetoencephalography study. Dev Med Child Neurol 2013;55:941-951.

Katz R, Pierrot-Deseilligny E. Recurrent inhibition in humans. Prog Neurobiol 1999;57:325-355.

Kesar TM, Sawaki L, Burdette JH, Cabrera MN, Kolaski K, Smith BP, O'Shea TM, Koman LA, Wittenberg GF. Motor cortical functional geometry in cerebral palsy and its relationship to disability. Clin Neurophysiol 2012;123:1383-1390.

Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. Arch Dis Child Fetal Neonatal Ed 2008;93:F153-161.

Kim JH, Kwon YM, Son SM. Motor function outcomes of pediatric patients with hemiplegic cerebral palsy after rehabilitation treatment: A diffusion tensor imaging study. Neural Regen Res 2015;10:624-630.

Kirk H, Geertsen SS, Lorentzen J, Krarup KB, Bandholm T, Nielsen JB. Explosive resistance training increases rate of force development in ankle dorsiflexors and gait function in adults with cerebral palsy. J Strength Cond Res 2016.

Kirton A, deVeber G. Cerebral palsy secondary to perinatal ischemic stroke. Clin Perinatol 2006;33:367-386.

Kirton A, Deveber G. Life after perinatal stroke. Stroke 2013;44:3265-3271.

Koerte I, Pelavin P, Kirmess B, Fuchs T, Berweck S, Laubender RP, Borggraefe I, Schroeder S, Danek A, Rummeny C, Reiser M, Kubicki M, Shenton ME, Ertl-Wagner B, Heinen F. Anisotropy of transcallosal motor fibres indicates functional impairment in children with periventricular leukomalacia. Dev Med Child Neurol 2011;53:179-186.

Koman LA, Smith BP, Shilt JS. Cerebral palsy. Lancet 2004;363:1619-1631.

Krageloh-Mann I, Horber V. The role of magnetic resonance imaging in elucidating the pathogenesis of cerebral palsy: A systematic review. Dev Med Child Neurol 2007;49:144-151.

Kruse M, Michelsen SI, Flachs EM, Bronnum-Hansen H, Madsen M, Uldall P. Lifetime costs of cerebral palsy. Dev Med Child Neurol 2009;51:622-628.

Kuhnke N, Juenger H, Walther M, Berweck S, Mall V, Staudt M. Do patients with congenital hemiparesis and ipsilateral corticospinal projections respond differently to constraint-induced movement therapy? Dev Med Child Neurol 2008;50:898-903.

Kuhtz-Buschbeck JP, Sundholm LK, Eliasson AC, Forssberg H. Quantitative assessment of mirror movements in children and adolescents with hemiplegic cerebral palsy. Dev Med Child Neurol 2000;42:728-736.

Kurz MJ, Becker KM, Heinrichs-Graham E, Wilson TW. Children with cerebral palsy have uncharacteristic somatosensory cortical oscillations after stimulation of the hand mechanoreceptors. Neuroscience 2015;305:67-75.

Kwon JY, Chang WH, Chang HJ, Yi SH, Kim MY, Kim EH, Kim YH. Changes in diffusion tensor tractographic findings associated with constraint-induced movement therapy in young children with cerebral palsy. Clin Neurophysiol 2014;125:2397-2403.

Lamy JC, Wargon I, Mazevet D, Ghanim Z, Pradat-Diehl P, Katz R. Impaired efficacy of spinal presynaptic mechanisms in spastic stroke patients. Brain 2009;132:734-748.

Lawrence DG, Kuypers HG. Pyramidal and non-pyramidal pathways in monkeys: Anatomical and functional correlation. Science 1965;148:973-975.

Lebel C, Gee M, Camicioli R, Wieler M, Martin W, Beaulieu C. Diffusion tensor imaging of white matter tract evolution over the lifespan. Neuroimage 2012;60:340-352.

Lee JD, Park HJ, Park ES, Oh MK, Park B, Rha DW, Cho SR, Kim EY, Park JY, Kim CH, Kim DG, Park CI. Motor pathway injury in patients with periventricular leucomalacia and spastic diplegia. Brain 2011;134:1199-1210.

Lee JJ, Lee DR, Shin YK, Lee NG, Han BS, You SJ. Comparative neuroimaging in children with cerebral palsy using fMRI and a novel eeg-based brain mapping during a motor task--a preliminary investigation. NeuroRehabilitation 2013;32:279-285.

Leemans A, Jeurissen G, Sijbers J, Jones DK. Exploredti: A graphical toobox for processing, analyzing, and visualizing diffusion MR data. 17th Annual Meeting of Intl Soc Mag Reson Med. Hawaii, USA2009. p. 3537.

Lennartsson F, Holmstrom L, Eliasson AC, Flodmark O, Forssberg H, Tournier JD, Vollmer B. Advanced fiber tracking in early acquired brain injury causing cerebral palsy. AJNR Am J Neuroradiol 2015;36:181-187.

Leonard CT, Moritani T. H-reflex testing to determine the neural basis of movement disorders of neurologically impaired individuals. Electromyogr Clin Neurophysiol 1992;32:341-349.

Leonard CT, Sandholdt DY, McMillan JA, Queen S. Short- and long-latency contributions to reciprocal inhibition during various levels of muscle contraction of individuals with cerebral palsy. J Child Neurol 2006;21:240-246.

Li Y, Gorassini MA, Bennett DJ. Role of persistent sodium and calcium currents in motoneuron firing and spasticity in chronic spinal rats. J Neurophysiol 2004;91:767-783.

Lieber RL, Smith LR. Skeletal muscle changes due to cerebral palsy. In: Shepherd RB, editor. Cerebral palsy in infancy: Targeted activity to optimize early growth and development. Edinburgh; New York: Churchill Livingstone/Elsevier; 2014. p. 135-155.

Liepert J, Storch P, Fritsch A, Weiller C. Motor cortex disinhibition in acute stroke. Clin Neurophysiol 2000;111:671-676.

Lotze M, Sauseng P, Staudt M. Functional relevance of ipsilateral motor activation in congenital hemiparesis as tested by fMRI-navigated TMS. Exp Neurol 2009;217:440-443.

Mackey A, Stinear C, Stott S, Byblow WD. Upper limb function and cortical organization in youth with unilateral cerebral palsy. Front Neurol 2014;5:117.

Maegaki Y, Maeoka Y, Ishii S, Eda I, Ohtagaki A, Kitahara T, Suzuki N, Yoshino K, Ieshima A, Koeda T, Takeshita K. Central motor reorganization in cerebral palsy patients with bilateral cerebral lesions. Pediatr Res 1999;45:559-567.

Maertens de Noordhout A, Pepin JL, Gerard P, Delwaide PJ. Facilitation of responses to motor cortex stimulation: Effects of isometric voluntary contraction. Ann Neurol 1992;32:365-370.

Manella KJ, Field-Fote EC. Modulatory effects of locomotor training on extensor spasticity in individuals with motor-incomplete spinal cord injury. Restorative neurology and neuroscience 2013;31:633-646.

Marque P, Pierrot-Deseilligny E, Simonetta-Moreau M. Evidence for excitation of the human lower limb motoneurones by group ii muscle afferents. Exp Brain Res 1996;109:357-360.

Marque P, Simonetta-Moreau M, Maupas E, Roques CF. Facilitation of transmission in heteronymous group ii pathways in spastic hemiplegic patients. J Neurol Neurosurg Psychiatry 2001;70:36-42.

Marret S, Vanhulle C, Laquerriere A. Pathophysiology of cerebral palsy. Handb Clin Neurol 2013;111:169-176.

Martin JH, Chakrabarty S, Friel KM. Harnessing activity-dependent plasticity to repair the damaged corticospinal tract in an animal model of cerebral palsy. Dev Med Child Neurol 2011;53 Suppl 4:9-13.

Matsuyama K, Mori F, Nakajima K, Drew T, Aoki M, Mori S. Locomotor role of the corticoreticular-reticulospinal-spinal interneuronal system. Prog Brain Res 2004;143:239-249.

McKay JL, Welch TD, Vidakovic B, Ting LH. Statistically significant contrasts between EMG waveforms revealed using wavelet-based functional anova. J Neurophysiol 2013;109:591-602.

Meyns P, Van Gestel L, Leunissen I, De Cock P, Sunaert S, Feys H, Duysens J, Desloovere K, Ortibus E. Macrostructural and microstructural brain lesions relate to gait pathology in children with cerebral palsy. Neurorehabil Neural Repair 2016.

Mills KR. Magnetic brain stimulation: A tool to explore the action of the motor cortex on single human spinal motoneurones. Trends Neurosci 1991;14:401-405.

Milner-Brown HS, Penn RD. Pathophysiological mechanisms in cerebral palsy. J Neurol Neurosurg Psychiatry 1979;42:606-618.

Mockford M, Caulton JM. The pathophysiological basis of weakness in children with cerebral palsy. Pediatr Phys Ther 2010;22:222-233.

Morita H, Olivier E, Baumgarten J, Petersen NT, Christensen LO, Nielsen JB. Differential changes in corticospinal and ia input to tibialis anterior and soleus motor neurones during voluntary contraction in man. Acta Physiol Scand 2000;170:65-76.

Murray KC, Stephens MJ, Rank M, D'Amico J, Gorassini MA, Bennett DJ. Polysynaptic excitatory postsynaptic potentials that trigger spasms after spinal cord injury in rats are inhibited by 5-ht1b and 5-ht1f receptors. J Neurophysiol 2011;106:925-943.

Nadler MA, Harrison LM, Stephens JA. Cutaneomuscular reflexes following stroke: A 2-year longitudinal study. J Neurol Sci 2004;217:195-203.

Nakamura H, Kitagawa H, Kawaguchi Y, Tsuji H. Intracortical facilitation and inhibition after transcranial magnetic stimulation in conscious humans. J Physiol 1997;498 (Pt 3):817-823.

Narayanan UG. Management of children with ambulatory cerebral palsy: An evidence-based review. J Pediatr Orthop 2012;32 Suppl 2:S172-181.

National Institute for Health Care Excellence. Spasticity in children and young people. London: National Institue for Health and Care Excellence; 2012.

Nelson KB. Can we prevent cerebral palsy? N Engl J Med 2003;349:1765-1769.

Netz J, Lammers T, Homberg V. Reorganization of motor output in the non-affected hemisphere after stroke. Brain 1997;120 (Pt 9):1579-1586.

Newman CJ, O'Regan M, Hensey O. Sleep disorders in children with cerebral palsy. Dev Med Child Neurol 2006;48:564-568.

Nezu A, Kimura S, Takeshita S, Tanaka M. Functional recovery in hemiplegic cerebral palsy: Ipsilateral electromyographic responses to focal transcranial magnetic stimulation. Brain Dev 1999;21:162-165.

Ni Z, Chen R. Short-interval intracortical inhibition: A complex measure. Clin Neurophysiol 2008;119:2175-2176.

Nielsen J, Petersen N, Fedirchuk B. Evidence suggesting a transcortical pathway from cutaneous foot afferents to tibialis anterior motoneurones in man. J Physiol 1997;501 (Pt 2):473-484.

Nielsen JB. How we walk: Central control of muscle activity during human walking. Neuroscientist 2003;9:195-204.

Nielsen JB, Willerslev-Olsen M, Christiansen L, Lundbye-Jensen J, Lorentzen J. Science-based neurorehabilitation: Recommendations for neurorehabilitation from basic science. J Mot Behav 2015;47:7-17.

Noble JJ, Charles-Edwards GD, Keevil SF, Lewis AP, Gough M, Shortland AP. Intramuscular fat in ambulant young adults with bilateral spastic cerebral palsy. BMC musculoskeletal disorders 2014;15:236.

Norton JA, Bennett DJ, Knash ME, Murray KC, Gorassini MA. Changes in sensory-evoked synaptic activation of motoneurons after spinal cord injury in man. Brain 2008a;131:1478-1491.

Norton JA, Thompson AK, Chan KM, Wilman A, Stein RB. Persistent mirror movements for over sixty years: The underlying mechanisms in a cerebral palsy patient. Clin Neurophysiol 2008b;119:80-87.

Novak I, Hines M, Goldsmith S, Barclay R. Clinical prognostic messages from a systematic review on cerebral palsy. Pediatrics 2012;130:e1285-1312.

Novak I, McIntyre S, Morgan C, Campbell L, Dark L, Morton N, Stumbles E, Wilson SA, Goldsmith S. A systematic review of interventions for children with cerebral palsy: State of the evidence. Dev Med Child Neurol 2013;55:885-910.

O'Sullivan MC, Miller S, Ramesh V, Conway E, Gilfillan K, McDonough S, Eyre JA. Abnormal development of biceps brachii phasic stretch reflex and persistence of short latency heteronymous reflexes from biceps to triceps brachii in spastic cerebral palsy. Brain 1998;121 (Pt 12):2381-2395.

Ogawa S, Lee TM, Kay AR, Tank DW. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci U S A 1990;87:9868-9872.

Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC. Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. J Physiol 2008;586:5147-5159.

Oskoui M, Coutinho F, Dykeman J, Jette N, Pringsheim T. An update on the prevalence of cerebral palsy: A systematic review and meta-analysis. Dev Med Child Neurol 2013;55:509-519.

Ostensjo S, Carlberg EB, Vollestad NK. Motor impairments in young children with cerebral palsy: Relationship to gross motor function and everyday activities. Dev Med Child Neurol 2004;46:580-589.

Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Dev Med Child Neurol 1997;39:214-223.

Palisano RJ, Rosenbaum P, Bartlett D, Livingston MH. Content validity of the expanded and revised gross motor function classification system. Dev Med Child Neurol 2008;50:744-750.

Pandyan AD, Gregoric M, Barnes MP, Wood D, Van Wijck F, Burridge J, Hermens H, Johnson GR. Spasticity: Clinical perceptions, neurological realities and meaningful measurement. Disabil Rehabil 2005;27:2-6.

Paus T, Zijdenbos A, Worsley K, Collins DL, Blumenthal J, Giedd JN, Rapoport JL, Evans AC. Structural maturation of neural pathways in children and adolescents: In vivo study. Science 1999;283:1908-1911.

Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain 1937;60:389-443.

Penn RD, Savoy SM, Corcos D, Latash M, Gottlieb G, Parke B, Kroin JS. Intrathecal baclofen for severe spinal spasticity. N Engl J Med 1989;320:1517-1521.

Perry J. Determinants of muscle function in the spastic lower extremity. Clin Orthop Relat Res 1993:10-26.

Petersen TH, Farmer SF, Kliim-Due M, Nielsen JB. Failure of normal development of central drive to ankle dorsiflexors relates to gait deficits in children with cerebral palsy. J Neurophysiol 2013;109:625-639.

Pierrot-Deseilligny E, Burke DJ. Contribution of spinal pathways to the pathophysiology of movement disorders. The circuitry of the human spinal cord: Neuroplasticity and corticospinal mechanisms. Cambridge: Cambridge University Press; 2012. p. 565-579.

Pihko E, Nevalainen P, Vaalto S, Laaksonen K, Maenpaa H, Valanne L, Lauronen L. Reactivity of sensorimotor oscillations is altered in children with hemiplegic cerebral palsy: A magnetoencephalographic study. Hum Brain Mapp 2014;35:4105-4117.

Pinter MJ, Burke RE, O'Donovan MJ, Dum RP. Supraspinal facilitation of cutaneous polysynaptic epsps in cat medical gastrocnemius motoneurons. Exp Brain Res 1982;45:133-143.

Pitcher JB, Ogston KM, Miles TS. Age and sex differences in human motor cortex input-output characteristics. J Physiol 2002;546:605-613.

Poon DM, Hui-Chan CW. Hyperactive stretch reflexes, co-contraction, and muscle weakness in children with cerebral palsy. Dev Med Child Neurol 2009;51:128-135.

Powers RK, Turker KS. Deciphering the contribution of intrinsic and synaptic currents to the effects of transient synaptic inputs on human motor unit discharge. Clin Neurophysiol 2010;121:1643-1654.

Prechtl HF, Einspieler C, Cioni G, Bos AF, Ferrari F, Sontheimer D. An early marker for neurological deficits after perinatal brain lesions. Lancet 1997;349:1361-1363.

Priori A, Bertolasi L, Dressler D, Rothwell JC, Day BL, Thompson PD, Marsden CD. Transcranial electric and magnetic stimulation of the leg area of the human motor cortex: Single motor unit and surface EMG responses in the tibialis anterior muscle. Electroencephalogr Clin Neurophysiol 1993;89:131-137.

Reid SL, Pitcher CA, Williams SA, Licari MK, Valentine JP, Shipman PJ, Elliott CM. Does muscle size matter? The relationship between muscle size and strength in children with cerebral palsy. Disabil Rehabil 2014a:1-6.

Reid SM, Carlin JB, Reddihough DS. Distribution of motor types in cerebral palsy: How do registry data compare? Dev Med Child Neurol 2011;53:233-238.

Reid SM, Dagia CD, Ditchfield MR, Carlin JB, Reddihough DS. Population-based studies of brain imaging patterns in cerebral palsy. Dev Med Child Neurol 2014b;56:222-232.

Reynolds C, Ashby P. Inhibition in the human motor cortex is reduced just before a voluntary contraction. Neurology 1999;53:730-735.

Rha DW, Chang WH, Kim J, Sim EG, Park ES. Comparing quantitative tractography metrics of motor and sensory pathways in children with periventricular leukomalacia and different levels of gross motor function. Neuroradiology 2012;54:615-621.

Richards CL, Malouin F. Cerebral palsy: Definition, assessment and rehabilitation. Handb Clin Neurol 2013;111:183-195.

Ridding MC, Taylor JL, Rothwell JC. The effect of voluntary contraction on cortico-cortical inhibition in human motor cortex. J Physiol 1995;487 (Pt 2):541-548.

Rogasch NC, Burne JA, Turker KS. Comparison of the inhibitory response to tendon and cutaneous afferent stimulation in the human lower limb. J Neurophysiol 2012;107:564-572.

Rose J, Martin JG, Torburn L, Rinsky LA, Gamble JG. Electromyographic differentiation of diplegic cerebral palsy from idiopathic toe walking: Involuntary coactivation of the quadriceps and gastrocnemius. J Pediatr Orthop 1999;19:677-682.

Rose J, McGill KC. Neuromuscular activation and motor-unit firing characteristics in cerebral palsy. Dev Med Child Neurol 2005;47:329-336.

Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M. A report: The definition and classification of cerebral palsy - april 2006. Dev Med Child Neurol 2007;49:8-14.

Ross SA, Engsberg JR. Relation between spasticity and strength in individuals with spastic diplegic cerebral palsy. Dev Med Child Neurol 2002;44:148-157.

Ross SA, Engsberg JR. Relationships between spasticity, strength, gait, and the gmfm-66 in persons with spastic diplegia cerebral palsy. Arch Phys Med Rehabil 2007;88:1114-1120.

Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008-2039.

Rothwell JC, Day BL, Thompson PD, Kujirai T. Short latency intracortical inhibition: One of the most popular tools in human motor neurophysiology. J Physiol 2009;587:11-12.

Rowlandson PH, Stephens JA. Cutaneous reflex responses recorded in children with various neurological disorders. Dev Med Child Neurol 1985;27:434-447.

Roy FD, Zewdie ET, Gorassini MA. Short-interval intracortical inhibition with incomplete spinal cord injury. Clin Neurophysiol 2011;122:1387-1395.

Ruber T, Schlaug G, Lindenberg R. Compensatory role of the cortico-rubro-spinal tract in motor recovery after stroke. Neurology 2012;79:515-522.

Sanger TD, Chen D, Delgado MR, Gaebler-Spira D, Hallett M, Mink JW. Definition and classification of negative motor signs in childhood. Pediatrics 2006;118:2159-2167.

Scheck SM, Boyd RN, Rose SE. New insights into the pathology of white matter tracts in cerebral palsy from diffusion magnetic resonance imaging: A systematic review. Dev Med Child Neurol 2012;54:684-696.

Scheck SM, Pannek K, Fiori S, Boyd RN, Rose SE. Quantitative comparison of cortical and deep grey matter in pathological subtypes of unilateral cerebral palsy. Dev Med Child Neurol 2014;56:968-975.

Sewell MD, Eastwood DM, Wimalasundera N. Managing common symptoms of cerebral palsy in children. BMJ 2014;349:g5474.

Shevell MI, Dagenais L, Hall N. The relationship of cerebral palsy subtype and functional motor impairment: A population-based study. Dev Med Child Neurol 2009;51:872-877.

Singh D. Nils silfverskiöld (1888–1957) and gastrocnemius contracture. Foot Ankle Surg 2013;19:135-138.

Smith LR, Chambers HG, Subramaniam S, Lieber RL. Transcriptional abnormalities of hamstring muscle contractures in children with cerebral palsy. PLoS One 2012;7:e40686.

Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 2002;17:1429-1436.

Stackhouse SK, Binder-Macleod SA, Lee SC. Voluntary muscle activation, contractile properties, and fatigability in children with and without cerebral palsy. Muscle Nerve 2005;31:594-601.

Staudt M, Grodd W, Gerloff C, Erb M, Stitz J, Krageloh-Mann I. Two types of ipsilateral reorganization in congenital hemiparesis: A TMS and fMRI study. Brain 2002;125:2222-2237.

Staudt M, Krageloh-Mann I, Holthausen H, Gerloff C, Grodd W. Searching for motor functions in dysgenic cortex: A clinical transcranial magnetic stimulation and functional magnetic resonance imaging study. J Neurosurg 2004;101:69-77.

Staudt M, Niemann G, Grodd W, Krageloh-Mann I. The pyramidal tract in congenital hemiparesis: Relationship between morphology and function in periventricular lesions. Neuropediatrics 2000;31:257-264.

Stoeckel MC, Binkofski F. The role of ipsilateral primary motor cortex in movement control and recovery from brain damage. Exp Neurol 2010;221:13-17.

Strauss D, Brooks J, Rosenbloom L, Shavelle R. Life expectancy in cerebral palsy: An update. Dev Med Child Neurol 2008;50:487-493.

Sukal-Moulton T, Krosschell KJ, Gaebler-Spira DJ, Dewald JP. Motor impairments related to brain injury timing in early hemiparesis. Part ii: Abnormal upper extremity joint torque synergies. Neurorehabil Neural Repair 2014;28:24-35.

Tedroff K, Knutson LM, Soderberg GL. Synergistic muscle activation during maximum voluntary contractions in children with and without spastic cerebral palsy. Dev Med Child Neurol 2006;48:789-796.

Theologis T. Lever arm dysfunction in cerebral palsy gait. J Child Orthop 2013;7:379-382.

Towsley K, Shevell MI, Dagenais L. Population-based study of neuroimaging findings in children with cerebral palsy. Eur J Paediatr Neurol 2011;15:29-35.

Trinh A, Wong P, Fahey MC, Brown J, Churchyard A, Strauss BJ, Ebeling PR, Fuller PJ, Milat F. Musculoskeletal and endocrine health in adults with cerebral palsy: New opportunities for intervention. J Clin Endocrinol Metab 2016;101:1190-1197.

Trivedi R, Agarwal S, Shah V, Goyel P, Paliwal VK, Rathore RK, Gupta RK. Correlation of quantitative sensorimotor tractography with clinical grade of cerebral palsy. Neuroradiology 2010;52:759-765.

Trivedi R, Gupta RK, Shah V, Tripathi M, Rathore RK, Kumar M, Pandey CM, Narayana PA. Treatment-induced plasticity in cerebral palsy: A diffusion tensor imaging study. Pediatr Neurol 2008;39:341-349.

Tsao H, Pannek K, Boyd RN, Rose SE. Changes in the integrity of thalamocortical connections are associated with sensorimotor deficits in children with congenital hemiplegia. Brain Struct Funct 2015;220:307-318.

Tsao H, Pannek K, Fiori S, Boyd RN, Rose S. Reduced integrity of sensorimotor projections traversing the posterior limb of the internal capsule in children with congenital hemiparesis. Res Dev Disabil 2014;35:250-260.

Turker KS, Powers RK. Effects of large excitatory and inhibitory inputs on motoneuron discharge rate and probability. J Neurophysiol 1999;82:829-840.

Turker KS, Powers RK. Black box revisited: A technique for estimating postsynaptic potentials in neurons. Trends Neurosci 2005;28:379-386.

Turton A, Wroe S, Trepte N, Fraser C, Lemon RN. Contralateral and ipsilateral EMG responses to transcranial magnetic stimulation during recovery of arm and hand function after stroke. Electroencephalogr Clin Neurophysiol 1996;101:316-328.

Ugawa Y, Terao Y, Hanajima R, Sakai K, Kanazawa I. Facilitatory effect of tonic voluntary contraction on responses to motor cortex stimulation. Electroencephalogr Clin Neurophysiol 1995;97:451-454.

van der Aa NE, Verhage CH, Groenendaal F, Vermeulen RJ, de Bode S, van Nieuwenhuizen O, de Vries LS. Neonatal neuroimaging predicts recruitment of contralesional corticospinal tracts following perinatal brain injury. Dev Med Child Neurol 2013;55:707-712.

Vandermeeren Y, Bastings E, Fadiga L, Olivier E. Long-latency motor evoked potentials in congenital hemiplegia. Clin Neurophysiol 2003a;114:1808-1818.

Vandermeeren Y, Davare M, Duque J, Olivier E. Reorganization of cortical hand representation in congenital hemiplegia. Eur J Neurosci 2009;29:845-854.

Vandermeeren Y, Sebire G, Grandin CB, Thonnard JL, Schlogel X, De Volder AG. Functional reorganization of brain in children affected with congenital hemiplegia: fMRI study. Neuroimage 2003b;20:289-301.

Vry J, Linder-Lucht M, Berweck S, Bonati U, Hodapp M, Uhl M, Faist M, Mall V. Altered cortical inhibitory function in children with spastic diplegia: A TMS study. Exp Brain Res 2008;186:611-618.

Wakana S, Jiang H, Nagae-Poetscher LM, van Zijl PC, Mori S. Fiber tract-based atlas of human white matter anatomy. Radiology 2004;230:77-87.

Walther M, Berweck S, Schessl J, Linder-Lucht M, Fietzek UM, Glocker FX, Heinen F, Mall V. Maturation of inhibitory and excitatory motor cortex pathways in children. Brain Dev 2009a;31:562-567.

Walther M, Juenger H, Kuhnke N, Wilke M, Brodbeck V, Berweck S, Staudt M, Mall V. Motor cortex plasticity in ischemic perinatal stroke: A transcranial magnetic stimulation and functional MRI study. Pediatr Neurol 2009b;41:171-178.

Weber R, Popova L. Testing equivalence in communication research: Theory and application. Commun Methods Meas 2012;6:190-213.

Wiley ME, Damiano DL. Lower-extremity strength profiles in spastic cerebral palsy. Dev Med Child Neurol 1998;40:100-107.

Willerslev-Olsen M, Andersen JB, Sinkjaer T, Nielsen JB. Sensory feedback to ankle plantar flexors is not exaggerated during gait in spastic hemiplegic children with cerebral palsy. J Neurophysiol 2014;111:746-754.

Wren TA, Rethlefsen S, Kay RM. Prevalence of specific gait abnormalities in children with cerebral palsy: Influence of cerebral palsy subtype, age, and previous surgery. J Pediatr Orthop 2005;25:79-83.

Wu YW, Lindan CE, Henning LH, Yoshida CK, Fullerton HJ, Ferriero DM, Barkovich AJ, Croen LA. Neuroimaging abnormalities in infants with congenital hemiparesis. Pediatr Neurol 2006;35:191-196.

Yang HS, Kwon HG, Hong JH, Hong CP, Jang SH. The rubrospinal tract in the human brain: Diffusion tensor imaging study. Neurosci Lett 2011;504:45-48.

Yang JF, Fung J, Edamura M, Blunt R, Stein RB, Barbeau H. H-reflex modulation during walking in spastic paretic subjects. Can J Neurol Sci 1991;18:443-452.

Yeo SS, Chang MC, Kwon YH, Jung YJ, Jang SH. Corticoreticular pathway in the human brain: Diffusion tensor tractography study. Neurosci Lett 2012;508:9-12.

Yokochi K, Hosoe A, Shimabukuro S, Kodama K. Gross motor patterns in children with cerebral palsy and spastic diplegia. Pediatr Neurol 1990;6:245-250.

Yoshida S, Hayakawa K, Oishi K, Mori S, Kanda T, Yamori Y, Yoshida N, Hirota H, Iwami M, Okano S, Matsushita H. Athetotic and spastic cerebral palsy: Anatomic characterization based on diffusion-tensor imaging. Radiology 2011;260:511-520.

Yoshida S, Hayakawa K, Yamamoto A, Okano S, Kanda T, Yamori Y, Yoshida N, Hirota H. Quantitative diffusion tensor tractography of the motor and sensory tract in children with cerebral palsy. Dev Med Child Neurol 2010;52:935-940.

Zaaimi B, Edgley SA, Soteropoulos DS, Baker SN. Changes in descending motor pathway connectivity after corticospinal tract lesion in macaque monkey. Brain 2012;135:2277-2289.

Ziemann U. TMS and drugs. Clin Neurophysiol 2004;115:1717-1729.

Ziemann U. Pharmaco-transcranial magnetic stimulation studies of motor excitability. Handb Clin Neurol 2013;116:387-397.

Ziemann U, Ishii K, Borgheresi A, Yaseen Z, Battaglia F, Hallett M, Cincotta M, Wassermann EM. Dissociation of the pathways mediating ipsilateral and contralateral motor-evoked potentials in human hand and arm muscles. J Physiol 1999;518 (Pt 3):895-906.

Ziemann U, Rothwell JC, Ridding MC. Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol 1996;496:873-881.