Descending Motor Pathways and Brainstem Reflexes in Cerebral Palsy

by

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Abstract

This thesis discusses the organization of motor pathways in adults and children with cerebral palsy (CP). Individuals with CP experience impairments in the control of head and neck functions. The cranial nerves that innervate the head and neck pass through the brainstem. Thus, motor impairment could be indicative of dysfunction in brainstem circuitry. While brainstem reflexes are atypical in other neurological disorders in which there is known brainstem damage, the function and structure of brainstem circuitry in CP is less well known. In Chapter 2, we investigated the function of brainstem circuitry in CP by stimulating the trigeminal nerve to evoke a long latency, startle-like reflex in the sternocleidomastoid (SCM) muscle. We propose that this long-latency reflex is mediated by reticulospinal pathways. Individuals with CP showed facilitation of ongoing SCM EMG compared to age- and sex-matched controls, who showed suppression of ongoing SCM EMG. Additionally, injury to the sensorimotor system has shown to influence the development of cortico-reticulospinal circuits to mediate motor function. We also investigated if the modulation of cortically-evoked responses in the SCM was altered following the activation of brainstem circuits by trigeminal afferents. Some participants with CP had abnormally large, long-lasting responses in both the SCM and biceps brachii muscles when trigeminal nerve and cortical stimuli were combined. The enhanced excitatory responses evoked from trigeminal afferents in CP may be produced by heightened excitability of brainstem circuits, resulting in the augmented activation of reticulospinal pathways in response to early brain injury. Enhanced activation of reticulospinal pathways in CP may provide a compensated activation of the spinal cord and/or contribute to impairments in the precise control of head and neck functions.

The sensorimotor regions of the brain are common sites of damage during development. Individuals with CP experience reorganization of sensorimotor pathways resulting in limited limb activity and motor impairment. Current therapies aimed at improving walking have shown promising functional results; however, the degree to which they influence underlying motor systems is unknown. In Chapter 3, we investigated the development of corticospinal pathways and the effects of intensive leg therapy to improve walking function in children with hemiplegic CP aged 8 months to 4 years of age. Motor pathway excitability was measured by applying transcranial magnetic stimulation over the leg representation of each motor cortex. We examined the onset latency and prevalence of motor-evoked potentials (MEPs) in lower limb muscles. Data is presented from an on-going, randomized, controlled trial. It remains unclear *if* and *how* intensive physiotherapy during infancy influences descending motor pathway development, as no consistent changes in MEP prevalence or correlation between MEP prevalence and functional improvements were seen throughout the study. However, we did observe several trends in the data whereby MEPs in leg muscles were more prevalent from the unaffected cortex in children older than 2 years of age, likely as a result of underlying maturation and refinement of corticospinal pathways. MEP onset latencies became progressively shorter with increases in age, suggesting myelination and increased synaptic efficacy of the motor system facilitate fast conduction of action potentials. Although we were unable to deduce the effect of training on MEP prevalence, we show that it is possible to characterize the development of descending motor pathways in children with CP under the age of 4 years.

Preface

The research projects presented in this thesis, as described in Chapter 2 and 3, received research ethics approval from the Health Research Ethics Board at the University Alberta (Pro00053278 and Pro00072587, respectively).

In Chapter 2, Dr. Monica Gorassini and I were responsible for project design, data collection, data analysis and manuscript composition. All neurophysiological experiments were performed in Dr. Gorassini's laboratory. Most participants with cerebral palsy were recruited by Dr. Elizabeth Condliffe who also supplied associated MRI images which were performed at the Peter S. Allan MR Research Center. A version of Chapter 2 will be submitted for publication with Dr. Gorassini as the supervising author.

In Chapter 3, Dr. Jaynie Yang was responsible for project design. The Clinic for Ambulatory Rehabilitation Research and Education (CARRE), under the supervision of Dr. Yang, was responsible for participant recruitment, training of participants, functional data collection and analysis. Neurophysiological experiments were performed in Dr. Gorassini's laboratory with data collection done by Dr. Gorassini, Dr. Yang, the CARRE staff and I. Dr. Gorassini, Dr. Yang and I were responsible for the analysis of the neurophysiology data. Dr. Gorassini and I were responsible for manuscript composition.

Chapter 1 and 4 are original work done by me with supervision from Dr. Gorassini.

For mom and dad - thank you for teaching me how beautiful learning can be.

Acknowledgments

Most importantly, a huge thank you to Dr. Monica Gorassini. You taught me how to ask questions, find answers, hypothesize and wonder. You have given me an environment to challenge myself and develop skills I never knew I could (or would!) learn. Thank you for being incredibly patient throughout my (many) mistakes and questions. I will always be grateful. Thank you, Dr. Jaynie Yang, for your guidance. The care and dedication you devote to your research is admirable. Thank you, Dr. Matthew Prowse, for your guidance and for welcoming me into your clinic. Thank you, Dr. François Roy, for your friendly introduction to neurophysiology at the University of Alberta and for agreeing to be on my examining committee.

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List of Abbreviations

ANOVA	analysis of variance
APPIS	arterial presumed perinatal ischemic stroke
Contra	contralateral
CIMT	constraint-induced movement therapy
CM	cerebral malformation
CP	cerebral palsy
CRN	cochlear root neuron
CS	corticospinal
CST	corticospinal tract
EMG	electromyography
FDI	first dorsal interosseous
FLAIR	fluid-attenuated inversion recovery
fMRI	functional magnetic resonance imaging
GABA	gamma-aminobutyric acid
GMFCS	Gross Motor Function Classification System
GMFM	Gross Motor Function Measure
Ham	hamstrings
ICF	intracortical facilitation
Ipsi	ipsilateral
ISI	interstimulus interval
LICI	long-interval intracortical inhibition
M1	primary motor cortex
MCA	middle cerebral artery
MEP	motor-evoked potential
MRI	magnetic resonance imaging
MSO	maximum stimulator output
MT	motor threshold
NI	neurologically intact (controls)
n.r.PnO	nucleus reticularis pontis oralis
n.r. PnC	nucleus reticularis pontis caudalis
n.r. Gi	nucleus reticularis pontis gigantocellularis
N/A	not available
NT	not tested
PCA	post-conception age
PMRF	pontomedullary reticular formation
Pr5	principal sensory trigeminal nucleus
PVI	periventricular venous infarction
PVWMI	periventricular white matter injury
Quad	quadriceps
r	Pearson's correlation coefficient/ effect size
r^2	coefficient of determination
RAHFT	Rejoyce Arm and Hand Function Test
RCT	randomized controlled trial
r_s	Spearman correlation
RST	reticulospinal tract
rTMS	repetitive TMS
SCM	sternocleidomastoid
SICF	short-interval intracortical facilitation
SICI	short-interval intracortical inhibition
Sol	gastrocnemius-soleus
STN	spinal trigeminal nucleus
TA	tibialis anterior
TCR	trigemino-cervical reflex
tDCS	transcranial direct current stimulation
TES	transcranial electrical stimulation
TMS	transcranial magnetic stimulation
TNS	trigeminal nerve stimulation
TSR	trigeminal spinal reflex

Chapter 1. Introduction

Forward

People with cerebral palsy (CP) experience injury to the brain during the perinatal period which causes motor disorders that persist throughout the lifespan. This thesis will explore the underlying neural pathways in adults and children with CP to gain a better understanding of why and how motor disorders exist. This thesis contains 2 main projects. The first project explores the function of brainstem circuitry in adults with CP. Chapter 1 reviews the pathophysiology of CP, the problems of head and neck functions suggestive of brainstem dysfunction and the organization of brainstem reflexes. To gain a better understanding of the functional integrity of the brainstem, Chapter 2 examines the function of brainstem circuitry and the interaction between brainstem and cortical motor pathways in adults with bilateral CP. Increased brainstem excitability is demonstrated in CP, which may be indicative of altered brainstem circuits involving impairments in the control of head and neck functions. The second project explores the development of corticospinal motor pathways in children with CP. Chapter 1 reviews the development of corticospinal pathways and the influence of intervention applied during critical periods. Chapter 3 examines the organization of descending motor pathways from the motor cortex to leg muscles in children with unilateral perinatal stroke. Although intensive physiotherapy did not influence the prevalence of muscle responses evoked from stimulation of the cortex, increases in age influenced the prevalence and onset latency of muscle responses. To conclude this thesis, in Chapter 4, I will discuss possible pathways that mediate the brainstem circuits in Chapter 2 and future directions for both projects.

Cerebral Palsy

Definition and prevalence

CP is defined as "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 CP are often accompanied by disturbances of sensation, perception, cognition, communication, and behaviour, by epilepsy and by secondary musculoskeletal problems" (Rosenbaum et al., 2007). Twenty-four months of age is defined as the maximum age that an acquired injury to the developing brain may be considered as CP (Shevell et al., 2013).

The prevalence of CP among a cohort of 5-year-old children born between 2008 and 2010 in Northern Alberta is 2.22 per 1000 children (Robertson et al., 2017). Provincial studies have examined the prevalence of CP among cohorts of preterm infants – the most recent report from the Canadian Cerebral Palsy Registry in Quebec showing a rate of 1.84 per 1000 children alive at 9-10 years in 2010 (Oskoui et al., 2013a, Oskoui et al., 2013b). On a global scale, CP is the most common cause of childhood-onset, lifelong physical impairment in Western populations with an estimated prevalence of 2.0-2.5 per 1000 live births (Hirtz et al., 2007). A systematic review and meta-analysis revealed the worldwide prevalence of CP for children born between 1985 and 2004 is 2.11 per 1000 live births (Oskoui et al., 2013a).

Brain lesions and pathogenesis in CP

Regions in the brain affected in children with CP have been identified with Magnetic Resonance Imaging (MRI). MRIs also give insight into the etiology of CP and the neurologic sequela individuals will likely experience. In approximately 90% of children with CP, an underlying cerebral abnormality can be identified with brain imaging (Arnfield et al., 2013, Reid et al., 2014). The most frequent lesion is periventricular white matter injury (PVWMI), followed by diffuse (cortical and subcortical) gray matter injury. Other brain abnormalities include brain malformation and cerebral vascular accident (Krageloh-Mann and Horber, 2007, Towsley et al., 2011). Typical MRIs of PVWMI (Fig. 1-1C&c), grey matter lesions (Fig. 1-1D&d) and brain maldevelopments (Fig. 1-1A&a and B&b), reveal that abnormalities can occur in both hemispheres (Fig 1-1, top row) or mainly in one hemisphere (Fig. 1-1, bottom row). Approximately 13% of children with MRIs have normal imaging results (Towsley et al., 2011). The most common lesions, their etiology and the functional abnormalities individuals with these lesions express will be explored. The tendency of specific lesions to occur during different developmental processes will also be reviewed.

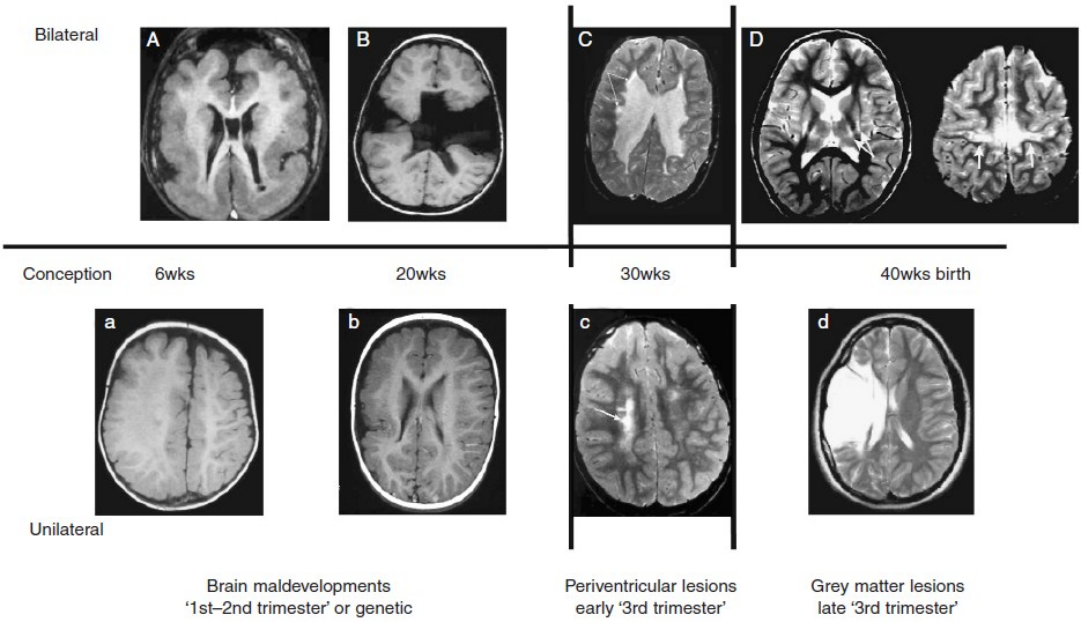

Figure 1-1. Pathogenic patterns of brain injury in CP. Typical MRI examples are given for different time periods in bilateral (top row) or unilateral (bottom row) CP. (Krageloh-Mann and Horber, 2007)

Periventricular white matter injury (PVWMI)

The most frequent lesions identified by MRIs in children with CP are in the PVWM (Krageloh-Mann and Horber, 2007, Towsley et al., 2011) accounting for 46.2% of lesions classified by neuroimaging (Arnfield et al., 2013). The PVWM describes the tracts of axons (including the corticospinal tract [CST] – a major descending pathway from the sensorimotor cortex to spinal motor neurons) located lateral to the lateral ventricles. This location is especially vulnerable because it is the termination point of long blood vessels, particularly branches originating from the middle cerebral artery. Early in development, these vessels are sensitive to changes in cerebral blood flow pressure as they do not mature until the late prenatal period. Thus, injury to the CST commonly occurs because of ischemia and/or inflammation, causing injury or death of pre-myelinating oligodendrocytes and ultimately impaired myelination of axons (Khwaja

and Volpe, 2007). Injury to the CST is responsible for most motor deficits in children with CP, which often results in hemiplegic cerebral palsy (Banker and Larroche, 1962, Staudt et al., 2000, Kirton, 2013). Because the CST is responsible for coordinating fine motor control in the limbs, it is not surprising that lesions to the CST correlate with upper and lower limb impairment, particularly with fine motor control (Staudt et al., 2000).

Diffuse (cortical and subcortical) gray matter lesions

Cortical and subcortical (basal ganglia and thalamus) gray matter lesions occur in approximately 14 – 22% of children with CP (Towsley et al., 2011, Reid et al., 2014) and constitute 25.4% of lesions classified by neuroimaging (Arnfield et al., 2013). Injury to grey matter is commonly associated with stroke (ischemic or hemorrhagic), inflammation due to infection, toxins, or hypoglycaemia (Myers, 1972, Kuenzle et al., 1994, Reid et al., 2015). Perinatal ischemic strokes often involve middle cerebral artery lesions which can damage widespread areas of the basal ganglia and multiple cortical motor areas (Kirton, 2013). Decreased volume of cortical grey matter is shown in perinatal ischemic strokes (Li et al., 2012). Grey matter injury is largely associated with severe motor impairments involving all limbs where reduced gross motor function requires mobility aids (GMFCS IV-V; Shevell et al., 2013, Reid et al., 2015).

Brain Malformation

Brain malformations account for 9.3% of all lesions classified by neuroimaging (Arnfield et al., 2013). Interference to processes of cortical neurogenesis where neuronal precursor cells undergo proliferation, migration and organization affects development of the entire brain. These processes can be disrupted by genetic deficits or acquired (viral or

toxic) impairments (Marret et al., 2013). Brain malformation can include abnormal structure of the cortex (polymicrogyria, lissencephaly, pachygyria), displacement of cells (heteritopia, cortical dysplasia) and abnormal connection between brain structures (e.g. agenesis of the corpus callosum), among other conditions (Towsley et al., 2011). Individuals with brain malformations most commonly have spastic hemiplegia, with over half classified as having gross motor function requiring mobility aids (GMFCS III-V; Arnfield et al., 2013).

Timing of brain injury

The vulnerability of brain structures and the types of motor impairments an individual with CP will experience is highly dependent on the post-conceptual age at which brain injury occurs. The brain undergoes different developmental processes in each trimester of pregnancy and thus, different types of injury tend to occur at different times.

In the first (weeks 1-12) and second (weeks 13-27) trimesters, important processes of cortical neurogenesis occur where neuronal precursor cells undergo proliferation, migration and organization in specific brain areas and networks. Disruption of these processes by genetic deficits or acquired (viral or toxic) impairments cause brain maldevelopment (Fig. 1-1A&a and B&b; Krageloh-Mann and Horber, 2007, Arnfield et al., 2013, Marret et al., 2013). In the third trimester (weeks 28-40) and extending until 2 years of age, growth and differentiation of neurons (axonal and dendrite growth, synapse formation, and myelination), refinement of pathways (neural cell apoptosis, redundant synapse elimination) and specialization of circuitry occur (Marret et al., 2013). PVWMI is presumed to occur early in the 3rd trimester before the 36th week (Fig. 1-1C&c), while grey matter lesions are presumed to occur later in the 3rd trimester and/or at birth (Fig. 1-

1D&d; Staudt et al., 2000, Krageloh-Mann and Horber, 2007). The least common types of brain lesions occur after birth, accounting for 1% of all lesions classified by neuroimaging. These lesions are classified as acquired brain injuries rather than congenital lesions (Arnfield et al., 2013).

Whether babies are born preterm (<37 weeks) also greatly influences the type of brain injury and functional motor outcome of individuals with CP. Fifty five % of brain lesions occur in children born preterm, with the majority of lesions occurring in the 3rd trimester (Krageloh-Mann and Horber, 2007, Arnfield et al., 2013). In children with CP born preterm, PVWMI is the most common lesion identified by MRI (Reid et al., 2014) resulting in spastic diplegia with gross motor function that is more severely affected compared to those with PVWMI born at term. Individuals born at term with PVWMI tend to have spastic hemiplegia (Shevell et al., 2013). Children with CP born at term are more likely to have brain lesions of grey matter injury, focal vascular insult and brain malformation (Reid et al., 2014).

Classification of CP

Given the heterogeneity in the timing of brain injury and the areas of the brain that can be affected, there is a wide range of clinical presentations in individuals with CP. Thus, individuals with CP can be described by multiple classification systems, including the topography of motor impairments (the distribution of motor impairments in limbs), the predominant motor abnormality (the characteristics of the movements) or the functional motor abilities (levels of gross motor function) they express. Classification systems for CP are clinically useful to describe the severity of motor impairments, predict

the current and future service needs and evaluate the change in the condition over time (Bax et al., 2005).

Topography of motor impairments

The traditional classification of CP is by the anatomical distribution of motor impartments in limbs (Bax et al., 2005). Hemiplegia, where motor impairments are mainly lateralized to one side of the body, is the most common subtype of CP in populations within Canada and Europe (Himmelmann et al., 2005, Robertson et al., 2017). Vascular insults (i.e. stroke) and damage to the internal capsule (within which descends the CST) are predominantly seen in children with hemiplegia (Mercuri et al., 1999, Reid et al., 2014). In individuals with quadriplegia, impairment is seen in all limbs and the most frequent imaging pattern is grey matter injury (Reid et al., 2014). Diplegia refers to involvement of any 2 limbs; however, by convention, it has taken to refer to involvement of lower limbs more often than upper limbs (Shevell, 2010). Individuals with diplegia have the highest rate of white matter injury (Reid et al., 2014). Triplegia (impairments affecting 3 limbs) and monoplegia (impairments affecting one limb) are less common (Shevell et al., 2009). The lack of specific definitions for each subtype creates discrepancies of how to classify individuals. Also, classification of individuals by motor impairments only in their limbs, fails to account for other common impairments in CP (e.g. truncal posture), giving an incomplete description of the condition in each person (Cans, 2000, Bax et al., 2005).

Predominant motor abnormality

Individuals with CP can be classified by their motor abnormalities as being spastic, dyskinetic or ataxic (Rosenbaum et al., 2007). For individuals that have more than one type of motor disorder, they are classified by the predominant disorder. Spastic CP is the most common motor abnormality in CP, contributing to 88% of children with CP (Reid et al., 2014, Robertson et al., 2017). Spasticity is characterized by an increase in involuntary muscle activation and hyperactive reflexes that can spread to other muscles whose tendon was not stretched (Cans, 2000). PVWMI is most frequent in individuals with spastic CP (Reid et al., 2014). 8-11% of children have the dyskinetic subtype (Shevell et al., 2013, Robertson et al., 2017), which is characterized either as dystonic (reduced muscle activity and increased tone) or choreo-athetotic (increased muscle activity and decreased tone; Cans 2000). Individuals with dyskinetic CP often have deep grey matter injury (Marret et al., 2013). Less than 5% of children with CP have the ataxic subtype (Shevell et al., 2013, Robertson et al., 2017), which is characterized by abnormal coordination of force, rhythm and accuracy (Cans, 2000). Individuals with ataxic CP often have normal imaging or cerebral malformation (Reid et al., 2014).

Functional motor abilities

The gross motor function of individuals with CP up to 18 years of age can be categorized using the Gross Motor Function Classification System (GMFCS). Movements during sitting, standing and walking and the use of mobility aids are used to identify the level of function that best describes a person's abilities (Palisano et al., 1997, Palisano et al., 2008, Richards and Malouin, 2013). The GMFCS has become widely used in the clinic to describe the development of gross motor function and to categorize

participants in experimental studies (Rethlefsen et al., 2010). Gross motor function is classified on a 5-level scale with functional skills described for 5 age groups: less than 2 years of age, 2 to 4 years of age, 4 to 6 years of age, 6 to 12 years of age and 12 to 18 years of age. Forty four % of children and youth are within the most functionally able level, Level I as they can walk without limitations. Fewer than 19% of children and youth are within each of the remaining 4 levels. Individuals in Level II walk with limitations; in Level III, they walk using a hand-held mobility device; in Level IV, they are self-mobile with limitations so they may use powered mobility; and in the least functionally able level, Level V, they are transported in a manual wheelchair (Palisano et al., 2008, Shevell et al., 2009, Rethlefsen et al., 2010). Generally, the GMFCS level observed around 12 years of age does not change and is the level of gross motor function individuals will have throughout their lifetimes (McCormick et al., 2007).

Indications of brainstem dysfunction in CP

Structural abnormalities of the brainstem in CP

The incidence of oral motor dysfunction and head stability problems are increased in individuals with CP (discussed below in *Abnormalities of sensory motor control of the head and neck in CP*). The cranial nerves that innervate muscles, skin and other structures of the face and neck originate in the brainstem. Thus, problems with control of musculature in the head and neck could be indicative of dysfunction in brainstem circuitry – either because the brainstem was *directly* injured at the time of birth, or influenced *indirectly,* whereby it has developed differently in response to injury of other brain structures. Injury has been apparent in some conditions of prenatal stroke where

there is development of abnormal blood vessels surrounding the brainstem or thrombosis of the basilar artery (Govaert, 2009). Abnormalities in the brainstem are apparent in MRI images showing reduced volume (Kulak and Sobaniec, 2007), reduced fractional anisotropy (Mu et al., 2014) and atrophy of the brainstem (Govaert, 2009). Asymmetry in the volume of the medulla oblongata is correlated with upper extremity motor function (Staudt et al., 2000). It is possible that brainstem dysfunction contributes to problems with control of head and neck functions.

Abnormalities of sensory and motor control of the head and neck in CP

Impairments with sensory and motor control of the head and neck are apparent in individuals with CP as an indication of dysfunction in brainstem structures. A large population-based study conducted in areas within the United States and Canada assessed the growth and nutrition of 230 children diagnosed with moderate to severe CP to reveal that 58% of children had feeding problems – 23% of which were classified as severe (Fung et al., 2002). Likewise, a survey conducted from disability registers in London, England found that more than 90% of children with CP had clinically significant oral motor dysfunction and severe feeding problems preceded the diagnosis of CP in 60% of children (Reilly et al., 1996). Individuals with CP show patterns of oral dysfunction, including abnormal tongue movements (specifically thrusting), abnormal jaw function, hyper- and hypoactive gag reflexes and prolonged and exaggerated biting reflexes (Reilly and Skuse, 1992, Reilly et al., 1996, Rogers, 2004). Inadequate, biting, chewing, coordination between cheek and lip musculature and swallowing also occur in children with CP (Griggs et al., 1989, Reilly and Skuse, 1992, Casas et al., 1994, Reilly et al.,

1996, Rogers, 2004). Abnormal swallowing can lead, not only to feeding problems, but also to increased incidence of sialorrhea in CP (Dougherty, 2009).

Children with CP often have postural impairments that contribute to feeding and swallowing problems, including poor head stability (Redstone and West, 2004). Deficits in head stability are apparent in children with CP over a range of body positions and movements. During quiet sitting, children with CP have greater head movements in the sagittal and frontal plains than typically developing children or adults. Even with varying levels of trunk support and with eyes open or closed, children with CP show greater displacement, variability of speed and speed of movement, compared to typically developing children and adults (Saavedra et al., 2010). Head instability is also apparent during whole body movements of standing to squatting and squatting to standing. Typically developing children are able to maintain initial head orientation (on average, angular position of head was 1 degree from the initial orientation) while diplegic children show excessive neck extension when standing up and excessive neck flexion when squatting (on average, angular position of head was 25 degrees from the initial orientation; Dan et al., 2000). Similarly, children with CP also show head instability during walking. When walking at a comfortable speed, the head moves in a sinusoidal trajectory in the sagittal plane. In children with hemiplegic CP, the head trajectory showed more fluctuations during the sinusoidal period and higher amplitude of the sinusoidal path compared to typically developing children and adults (Holt et al., 1999).

Sensory and motor dysfunction of the face and head instability during quiet sitting, whole body movements and during walking give an indication of dysfunction in brainstem circuits that control these movements.

Trigeminal Neural Circuits

One way to examine brainstem circuitry is to examine the trigeminal system. The trigeminal nerve is the 5th cranial nerve and is responsible for sensation and motor functions in the face. The trigeminal nerve exits the brainstem at the level of the pons and branches into 3 peripheral nerves: the ophthalmic nerve, innervating the scalp, forehead, upper eyelid and nose; the maxillary nerve, innervating the lower eyelid, cheek, upper lip, upper teeth and gums; and the mandibular nerve, innervating the lower lip, lower teeth and gums, chin and jaw. All branches convey sensory information, while only the mandibular branch provides motor innervation to muscles of the lower face (Upadhyay et al., 2008, Pazhaniappan, 2017). Reflex tests can help assess the function of nerve circuits and structures involved. Trigeminally-evoked reflexes including the startle reflex, the trigeminal cervical reflex and the trigeminal spinal reflex are initiated with trigeminal activation and have pathways that involve reticular nuclei within the brainstem. The organization of these brainstem nuclei and trigeminal reflex pathways will be explored further.

Reticular nuclei within the brainstem

The pontomedullary reticular formation (PMRF) in the brainstem is comprised of interconnected nuclei with cytoarchitecture that is well-conserved across mammalian species including the rat, cat, opossum and macaque monkey (Beran and Martin, 1971, Peterson et al., 1979, Jones and Yang, 1985, Sakai et al., 2009). The nuclear subdivisions include the nucleus reticularis (n.r.) pontis oralis (PnO), n.r. pontis caudalis (PnC), n.r. gigantocellularis (Gi) and n.r. ventralis. These nuclei send projections to all levels of the

spinal cord via the medial and lateral reticulospinal tracts (RST; Peterson, 1979, Peterson et al., 1979, Sakai et al., 2009, Baker, 2011, Wilson and Peterson, 2011). The medial RST originates primarily from the n.r. Gi to innervate motor neurons and interneurons mainly in the ipsilateral spinal cord, while the lateral RST originates from the n.r. Gi, and n.r. PnC with a large bilateral terminal field in the spinal cord (Peterson, 1979, Peterson et al., 1979, Wilson and Peterson, 2011).

Startle reflex

Facial and skeletal muscles respond to sudden acoustic, tactile or vestibular stimuli to convey a typical body position of eyes closed, neck flexed in dorsal direction, shoulders elevated, flexed elbows, clenched hands and knees flexed (Yeomans et al., 2002). When multiple stimuli are applied synchronously, whole-body movements and amplitudes of muscle responses in animals are larger, compared to when stimuli are experienced individually (Li and Yeomans, 1999). Similarly, in humans, blink reflexes are larger and faster when acoustic and trigeminal stimulation are applied together compared to when stimuli are applied separately (Plant and Hammond, 1989). Summation of startle responses suggests the convergence of startle information within a specific area in the brain. Neurons that integrate this information have been found in the n.r. PnC. The n.r. PnC receives projections from cochlear, vestibular and trigeminal nuclei of rats (Yeomans et al., 2002), and via the lateral RST, projects to motor neurons and interneurons in the spinal cord to innervate musculature throughout the body (Fig. 1-2; Davis et al., 1982, Yeomans et al., 2002, Wilson and Peterson, 2011, Valls-Sole,

2012). Lesions of the n.r. PnC block the startle response evoked from acoustic, vestibular, or tactile stimulation (Yeomans et al., 2002).

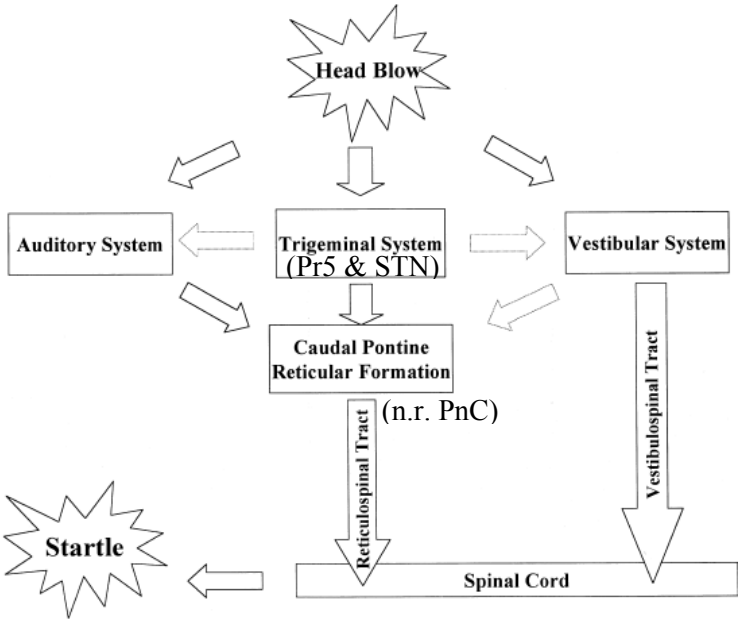

Figure 1-2. Circuits of the startle reflex. Acoustic, trigeminal, and vestibular stimuli converge in the nucleus reticularis pontis caudalis (n.r. PnC). The reticulospinal tract, which originates in the n.r. PnC, projects to motorneurons and interneurons in the spinal cord. Pr5 = principle sensory trigeminal nucleus, STN = spinal trigeminal nucleus.
(modified from Yeomans et al., 2002)

Trigeminal input to the startle pathway is triggered when mechanoreceptors respond to tactile stimulation of the face, which sends information to the principle sensory trigeminal nucleus (Pr5) and the spinal trigeminal nucleus in the brainstem. Electrophysiological studies have revealed direct, monosynaptic, glutamatergic connections from the Pr5 and the spinal trigeminal nucleus to the n.r. PnC (Fig. 1-2; Schmid et al., 2003). Stimulation of trigeminal nuclei evokes whole body movements in rats (Scott et al., 1999). The relationship between the acoustic startle reflex with the trigeminal cervical reflex and the trigeminal spinal reflex (both described below) will be explored further in Chapter 4.

Trigeminal cervical reflex (TCR)

Short latency TCR

The trigemino-cervical reflex (TCR) has a pathway that travels through the brainstem, beginning with activation of the trigeminal nerve which synapses in the spinal trigeminal nucleus in the brainstem (Fig. 1-3). Axons from the trigeminal nucleus project to the spinal accessory nucleus, where the spinal accessory nerve (cranial nerve XI) projects to the sternocleidomastoid (SCM) neck muscle (Fig. 1-3; Di Lazzaro et al., 1996). Stimulation of the infraorbital or supraorbital branch of the trigeminal nerve evokes muscle responses in the SCM, splenius and trapezius (Di Lazzaro et al., 1995, Di Lazzaro et al., 1996). Surface electromyography (EMG) recordings show responses in the SCM with short onset latencies of 12.9 ms (Di Lazzaro et al., 1995).

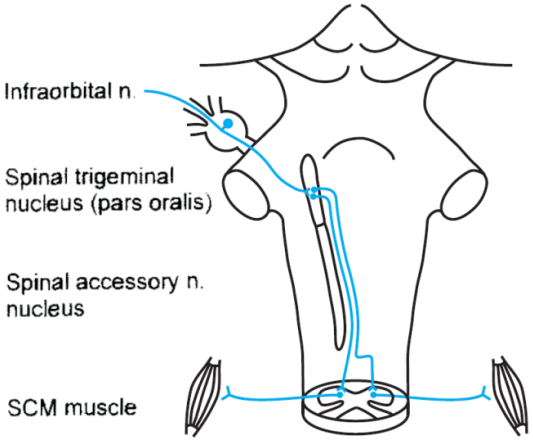

Figure 1-3. Trigemino-cervical reflex pathway. The trigeminal nerve (cranial nerve V) synapses in the spinal trigeminal nucleus. Axons from the trigeminal nucleus then project to the spinal accessory nucleus where the spinal accessory nerve (cranial nerve XI) innervates the sternocleidomastoid (SCM) neck muscle.
(Di Lazzaro et al., 1996)

The TCR, in addition to other reflexes with pathways that travel through the brainstem, is used to identify and locate lesions within the brainstem. Individuals with brainstem injuries have shown abnormalities in TCR responses (Di Lazzaro et al., 1996, Serrao et al., 2011, Magnano et al., 2014). For example, short latency reflexes are altered in individuals with multiple sclerosis that have lesions in the medulla oblongata where TCR responses in the SCM have reduced amplitude, delayed onset, and asymmetric

responses between the right and left SCM (Magnano et al., 2014). TCR reflexes can be absent altogether in individuals with progressive supranuclear palsy, a Parkinson's-type condition with significant neurodegeneration of the brainstem (Serrao et al., 2011).

Long latency TCR

In addition to evoking short latency responses, high intensity stimulation of the supraorbital branch of the trigeminal nerve also evokes long latency responses in neck muscles. A combination of responses occur simultaneously with an onset of 35-41 ms, where voluntarily active anterior neck muscles (SCM) show EMG suppression (Sartucci et al., 1986, Nakashima et al., 1989) and posterior neck muscles (semispinalis capitis) show EMG facilitation (Ertekin et al., 2001, Serrao et al., 2003). The anatomical pathway of the long-latency component of the TCR is not known but is thought to be similar, but distinct from, the startle reflex. This pathway begins with activation of the n.r. PnC which receives afferents from the Pr5 and the spinal trigeminal nucleus in the brainstem (Yeomans et al., 2002, Serrao et al., 2003). Motor activation is then propagated through the RST, which projects to motor neurons innervating the neck, shoulder, arm, and trunk (Peterson, 1979, Peterson et al., 1979, Sakai et al., 2009). The combined outcome of suppressed anterior neck muscles and facilitated posterior neck muscles allows the head to be withdrawn from facial stimuli – known as the head withdrawal reflex (Sartucci et al., 1986, Ertekin et al., 1996, 2001). Long latency responses of the TCR have longer onset latencies and reduced amplitudes in individuals with Parkinson's and multiple system atrophy – conditions that can lead to neurodegeneration of the brainstem (Perrotta et al., 2005, Serrao et al., 2011).

Trigeminal spinal reflex (TSR)

Long latency trigeminally-evoked reflexes seen in neck muscles also extend to proximal arm muscles. In uninjured controls, a bilateral EMG facilitation of the biceps can be evoked at 40ms, a similar latency to which posterior (41 ms) neck muscles are activated from high intensity stimulation of the supraorbital nerve. The biceps component of this reflex is known as the trigeminal spinal reflex (TSR), as it excites the motor neurons innervating the biceps in C5 –C6 spinal segments. The long latency TCR and the TSR possibly share a similar polysynaptic brainstem pathway where afferents from the trigeminal nuclei activate the n.r. PnC, from which the RST projects to motor neurons in the cervical spinal cord (Serrao et al., 2003). Functionally, the TCR and TSR, both initiated by trigeminal stimulation, coordinate neck and limb muscles, allowing the head to be withdrawn and arms to be raised in a protective position as a startle-like response (Davis et al., 1982, Serrao et al., 2003). Similar to the long-latency TCR responses in neck muscles, the TSR has longer onset latencies and reduced amplitudes in individuals with neurodegeneration of the brainstem from Parkinson's and multiple system atrophy (Perrotta et al., 2005, Serrao et al., 2011).

Convergence of trigeminal and cortical circuits in the brainstem

Brainstem nuclei receive input from multiple central and peripheral neural structures. The characteristics of the connections between the trigeminal nuclei and PMRF nuclei have been investigated in rats where the Pr5 and spinal trigeminal nucleus project to the n.r. PnC and PnO. *In vitro* anterograde tracers placed in the Pr5 in rats, labeled fibers terminating in the n.r. PnC. Electrophysiological studies concluded that this was a monosynaptic glutamatergic connection (Schmid et al., 2003). Similarly, *in vitro*

retrograde tracers placed in the n.r. PnC and n.r. PnO of rats revealed labelled cell groups in multiple trigeminal nuclei, including the Pr5 and spinal trigeminal nuclei pars oralis and pars interpolaris (Shammah-Lagnado et al., 1987).

In addition to trigeminal afferents, brainstem nuclei also receive cortical inputs. *In vivo* retrograde tracings in the PnC of cats showed bilateral projections from premotor and motor cortices (Rho et al., 1997). Single unit recording from the PMRF in an anesthetized monkey revealed short and long latency responses following transcranial magnetic stimulation (TMS) applied to the primary motor cortex (Fisher et al., 2012). The brainstem receives trigeminal afferents and corticoreticular projections, creating a potential site for integration of motor information/commands. Further investigation of this interaction is discussed in Chapter 2 with trigeminal nerve stimulation and cortical stimulation.

Corticospinal tract development and plasticity in children with CP

Transcranial magnetic stimulation (TMS) to activate the motor cortex

The use of magnetic stimulation to activate neural structures non-invasively was developed by Barker et al in 1985. Magnetic stimulation consists of a capacitor from which current of 5000 amp or more is discharged through an inductor coil, which creates a magnetic field with a pulse duration of 1 ms (Jalinous, 2001). When applied over the primary motor cortex (M1), the magnetic field can penetrate through the skull and induce a perpendicular electrical current within brain tissue (Malmivuo and Plonsey, 1995, Hallett, 2007). The rate of change of the magnetic field (~30 kT/s) is proportional to the induced current in the tissue (~ 15 mA/cm^2; Jalinous, 2001). Electrical currents are

propagated readily through neurons, given their electrical characteristics; however, this is dependent on their size, shape, and orientation (Malmivuo and Plonsey, 1995). The electrical current preferentially activates horizontally aligned axons that then transynaptically activate fast-conducting corticospinal neurons. This produces multiple volleys down the CST which then activate spinal interneurons and motoneurons. The resulting motor-evoked potential (MEP) in a muscle can be measured using Electromyography (EMG; Hallett, 2007, Di Lazzaro and Ziemann, 2013).

Typical corticospinal tract development

The CST, a major signaling pathway from the sensorimotor cortex to spinal motor neurons, is essential for modulating fine motor movements in distal limb muscles (Clowry, 2007, Lemon, 2008). The CST undergoes development in the prenatal and postnatal periods. By 24 weeks post-conceptional age (PCA), axons of the CST reach the spinal cord and progressively innervate the grey matter as shown by markers for axonal growth (Eyre et al., 2000). Functional monosynaptic connections between the brain and spinal neurons are established prior to birth (Eyre et al., 2000, Eyre et al., 2001). MEPs can be evoked in ipsilateral and contralateral upper limb muscles as early as 26 weeks PCA (as studied in preterm babies) where amplitudes and thresholds are similar. Ipsilateral MEP onset latencies are shorter, which is consistent with shorter ipsilateral pathway length (Eyre et al., 2001). Although functional synapses between the CST terminals and spinal neurons are present, the capability of the CST to control movement does not develop until later in infancy.

After CST terminals contact their spinal targets, activity-dependent processes help to establish the mature pattern of topographic and connectional specificity (Martin et al.,

2007). Less active pathways are withdrawn and more active pathways are strengthened, through a process called activity-dependent pruning (Eyre et al., 2001). In monkeys, the majority of axons within the CST are eliminated early in development, leaving only 13% as ipsilateral projections (Lacroix et al., 2004, Rosenzweig et al., 2009). Similarly, in humans, 8-15% of ipsilateral CST axons remain, leaving the spinal cord innervated mainly by crossing corticospinal connections (Eyre et al., 2001, Clowry, 2007, Staudt, 2010). Terminal branches from the unpruned CST continue to grow within the spinal grey matter (Eyre et al., 2001, Staudt, 2010). In kittens, clusters of presynaptic boutons containing synaptic vesicles, in which neurotransmitters are stored, are formed on CST terminals, creating more functionally effective connections with spinal neurons (Li and Martin, 2002, Meng et al., 2004).

Overlapping with the time period of activity-dependent processes, myelination of axons within the CST refine the corticospinal system further. Myelination largely occurs during the 3rd trimester to 2-3 years of age (Brody et al., 1987, Kinney et al., 1988, ten Donkelaar et al., 2004). Myelin forms in a proximal to distal manner, descending from the brain to the lumbar spinal cord (Stanfield, 1992, ten Donkelaar et al., 2004, Purger et al., 2016), where myelination of large diameter axons of the CST occurs first (Stanfield, 1992, Purger et al., 2016). Over the first year of life, ipsilateral MEPs decrease in amplitude, while contralateral MEPs increase in amplitude and decrease in threshold (Nezu et al., 1997, Eyre et al., 2001) – results that are reflective of the underlying maturation of the CST.

A later phase of CST development is refinement of the motor cortex. When kittens reach 2 months of age (approximately 2 years in children), further development of

the cortical motor map occurs where limb muscles begin to be represented in a topographic arrangement on the motor cortex (Martin, 2005). Overall, changes throughout infancy establish a functionally effective CST, allowing development of motor control.

Organization of the corticospinal tract in hemiplegic CP

Unilateral brain injury

The CST and sensorimotor cortex are common sites of damage in the event of prenatal and perinatal brain injury (Khwaja and Volpe, 2007). Injury on one side of the brain during development not only affects the CST from the injured hemisphere, but also from the non-directly injured hemisphere as well (Friel et al., 2013) – ultimately causing reorganization of the entire corticospinal system. The CST from the injured hemisphere is less competitive in establishing and maintaining synaptic connections with spinal neurons compared to the uninjured hemisphere (Martin et al., 1999, Martin et al., 2007). As a result, the uninjured hemisphere dominates innervation to both sides of the spinal cord and brainstem, which includes the typical contralateral projections and aberrant ipsilateral projections (Farmer et al., 1991, Carr et al., 1993, Eyre, 2007). Furthermore, reduced activity in the injured hemisphere encourages increased withdrawal of the remaining contralateral CST, which is displaced by more active ipsilateral projections from the uninjured cortex (Eyre, 2007).

After the perinatal period, TMS applied to the undamaged motor cortex in children with hemiplegic CP evokes MEPs in ipsilateral and contralateral muscles with similar latencies and thresholds (Farmer et al., 1991, Staudt et al., 2002, Eyre, 2007). In contrast, ipsilateral responses are not readily evoked in typically developing children

(Fig. 1-4A&B) or in people who have experienced unilateral cortical injury in adulthood (refs in Eyre 2007, Farmer 1991) unless high levels of both muscle contraction and stimulation intensity are applied (Ziemann et al., 1999, Tazoe and Perez, 2014). A comparison of EMG traces between 2 children can be seen in figure 1-4 where a typically developing child has MEPs only in the hand contralateral to the simulated cortex (Fig. 1-4A) and a child with hemiplegic CP has MEPs in both ipsilateral and contralateral hands with similar onset latencies (Fig. 1-4C&D; Farmer et al., 1991). The presence of ipsilateral pathways following damage to the developing CST reflect that ipsilateral pathways are not "unmasked" due to injury, but rather develop following a disruption in the *development* of CST (Martin et al., 2007).

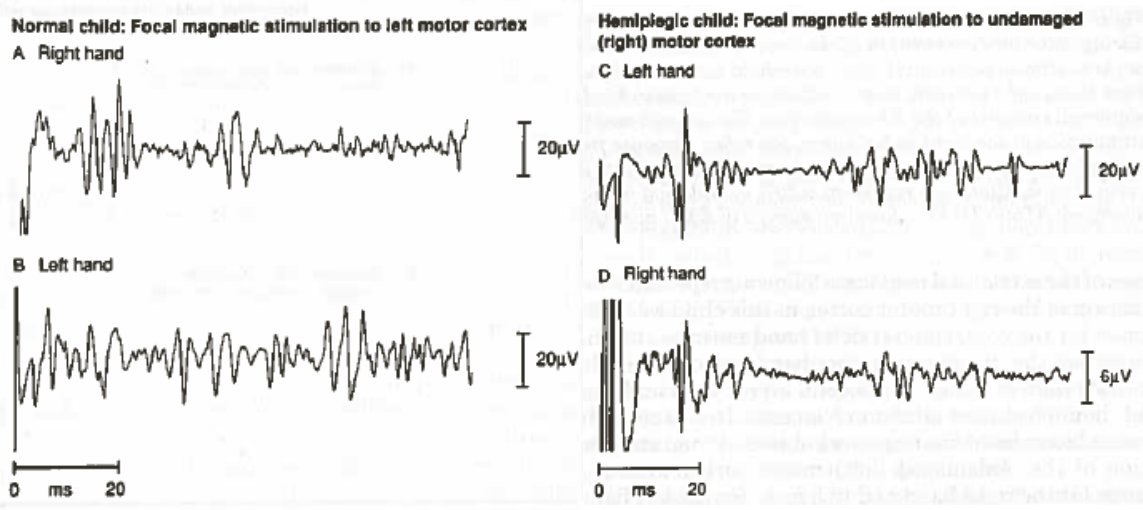

Figure 1-4. EMG traces from the first dorsal interosseous (FDI) muscle. TMS applied to the left motor cortex in a typically developing child evoked a muscle response (at approximately 15 ms) in the contralateral FDI (A), but not the ipsilateral FDI (B). Stimulation of the uninjured motor cortex in a child with hemiplegic CP evoked a muscle response (at approximately 15 ms) in both the contralateral (C) and ipsilateral (D) FDI. Both children at 2 years of age.
(Farmer et al., 1991)

Functional implications of unilateral brain injury

Signs of hemiplegia are often not expressed until 2 years of age (Basu, 2014); however, the development of hemiplegia can be predicted by earlier indications of absent or abnormal general movements between 9 and 13 weeks after birth (Prechtl et al., 1997, Basu, 2014). At older ages, asymmetrical movements prior to 12 months are common symptoms of unilateral CP (Wu et al., 2006).

There is debate over whether persistent ipsilateral projections from the uninjured hemisphere in CP are maladaptive. Ipsilateral pathways to the hand are correlated with poor hand function in children (Carr et al., 1993, Holmstrom et al., 2010); however, functionally effective and ineffective therapies in cats have shown otherwise. Constraint-induced movement therapy (CIMT) restrains the unaffected limb to force the use of the affected limb. CIMT combined with reach-training which evoke improvements in reaching and grasping in cats showed no differences in ipsilateral CST axon territories in the spinal cord, compared to cats that received CIMT alone and had less effective functional improvements (Friel et al., 2012). In cats, repetitive stimulation of the motor cortex can make specific pathways more active and thus more competitive in establishing and maintaining spinal connections. Functional benefits are shown following either stimulation of the injured cortex (to strengthen the remaining contralateral projections from the injured cortex which act to attenuate aberrant ipsilateral pathways from the uninjured cortex; Salimi et al., 2008) or stimulation of the uninjured cortex (to strengthen aberrant ipsilateral pathways to the affected limb; Carmel and Martin, 2014). It remains unclear *if* and *how* ipsilateral projections from the undamaged hemisphere in CP contribute to functional motor recovery.

The development of lower limb motor pathways in CP

Lower limb function

Lower limb impairments are common throughout development in individuals with CP. The earliest signs of impairment in infants with hemiplegic CP can be observed with absent or abnormal general movements (Prechtl et al., 1997, Basu, 2014) and asymmetric crawling (Bottos et al., 1995). The majority of children with hemiplegia achieve the ability to walk at GMFCS levels I (walking without limitations) to III (walking using a hand-held mobility device; Shevell et al., 2009). The most common abnormal gait patterns experienced by individuals with hemiplegic CP include drop foot in the swing phase, tight heel cord in the stance phase and restricted motion of the knee, hip and ankle throughout the step cycle (Winters et al., 1987, Dobson et al., 2011, Gross et al., 2015). Asymmetric walking has been quantified by unequal pressure distribution, loading and propulsion between the unaffected and affected feet (Femery et al., 2002). A variety of factors contribute to gait abnormalities in individuals with hemi CP. Most reported, are lower limb weakness (Elder et al., 2003, Hussain et al., 2014) and co-contraction of antagonist muscles in the legs – both problems extending into adulthood (Brouwer and Smits, 1996, Berger, 1998, Tedroff et al., 2008, Gross et al., 2015). Delayed initiation and relaxation of leg muscles (Downing et al., 2009) and increased muscle stiffness (Willerslev-Olsen et al., 2014) also make coordination of multiple muscle groups during walking extremely difficult.

Structure of lower limb motor pathways

Few studies characterizing motor pathways to leg muscles have been conducted. In uninjured youth aged 9 to 14 years, contralateral MEPs in the tibialis anterior can be evoked easily during relaxation, yet voluntary contraction is required to elicit MEPs in the ipsilateral muscle (Maegaki et al., 1999). Ipsilateral MEPs have smaller amplitudes and slightly longer onset latencies in comparison with contralateral MEPs (Maegaki et al., 1999). A distinct pattern of CST innervation of specific spinal motor neuron pools is suggested, given that flexor muscles (tibialis anterior) are more readily activated by TMS, compared to extensor muscles (soleus; Brouwer and Ashby, 1991).

Children and youth with hemiplegic CP experience altered motor pathway organization extending from the cortex to spinal motor neurons. Following unilateral brain injury, a remapping of body representation on the undamaged cortex occurs, causing a shift in cortical maps. It is possible to have overlapping upper and lower limb representations (Wittenberg, 2009) and an overall lateral shift of cortical areas for leg muscles (Maegaki et al., 1999). Injury resulting in lower limb dysfunction primarily affects the medial and posterior CST within the internal capsule - where pathways to lower limbs are predominantly located (Bouza et al., 1994, Staudt et al., 2000). In contrast to uninjured development, adults with CP have a loss of distinct CST innervation of specific motoneuron pools as equal activation of flexors and extensors from TMS exists (Brouwer and Ashby, 1991, Brouwer and Smits, 1996). Such changes in CST recruitment of different motoneuron pools may cause functional problems with voluntary contraction and muscle activation.

Although not explored in the leg, it is possible that motor responses in the leg in children with unilateral CP are similar to that in the upper limb. In children aged 2 years of age and older, ipsilateral MEPs in hand muscles evoked from the uninjured cortex resulted in onsets, thresholds and amplitudes similar to contralateral MEPs, unlike uninjured individuals who had ipsi MEPs with higher thresholds and amplitudes to contra MEPs by 18 months of age (Farmer et al., 1991, Eyre et al., 2001, Staudt et al., 2002, Lotze et al., 2009). These findings suggest a bilateral innervation of hand muscles due to axonal branching of the descending CST from the uninjured motor cortex – an organization that could possibly be present in the leg and foot as well.

Intervention in children with CP

Impact of activity-based therapies on function

Early intervention for the lower limb is mainly passive, including orthosis (Wingstrand et al., 2014) and botulinum toxin injections into spastic muscles - the most prevalent medical procedure applied in children with CP (Dobson et al., 2011, McLellan et al., 2012). Within the last 10 years, there is greater emphasis of interventions that promote self-initiated activity where children learn from trial and error (Shepherd, 2014). Activity-based therapies have been functionally beneficial in children with CP. CIMT has been adapted and applied to the upper limb in children with hemiplegic CP. Restraint of the uninjured limb causes forced-use of the most impaired limb (Taub et al., 1999, Taub et al., 2004). CIMT has produced improvement in the use of the more affected hand (Taub et al., 2004, Eliasson et al., 2005) where new motor behaviours developed after 3 weeks of therapy and sustained over a 6 month follow-up period (Taub et al., 2004). An alternative approach of bimanual therapy has shown improved dexterity and coordination

of both hands (Gordon et al., 2007). Fewer activity-based interventions have been applied in the lower limb. Muscle strengthening in various leg muscle groups has produced increased muscle strength, step length, improved gross motor function (Damiano et al., 1995, Damiano and Abel, 1998) and increased muscle volume (McNee et al., 2009). Intensive treadmill walking in children with CP resulted in improved ankle dorsiflexion (Phillips et al., 2007, Willerslev-Olsen et al., 2015) and heel strike during walking (Willerslev-Olsen et al., 2015). Similarly, a body weight supported system to allow for unconstrained movements improved gross motor function and increased rates of motor development (Prosser et al., 2012).

Impact of activity-based therapies on motor pathways

In addition to influencing functional outcome, activity-based therapies also impact underlying motor pathway development. Mechanisms have most investigated most thoroughly in a cats, where inactivation of the primary motor region during postnatal weeks 3-7 resulted in a loss of motor map limb representation on the primary motor cortex, aberrant CST terminations on spinal motor neurons and decreased spinal cord interneuron numbers. Such drastic motor system damage resulted in the inability of kittens to reach or grasp food (Martin et al., 2011, Friel et al., 2012). Constraint of the unaffected leg, combined with active training of the impaired limb, restored previously impaired CST connections with ventral regions of the spinal cord, restored the motor cortical map and increased the number of spinal cholinergic interneurons (Friel et al., 2012). Similarly, in children and young adults with CP, CIMT combined with training of the impaired hand, showed an increase in motor cortex excitability of contralateral responses from the injured cortex (Fig. 1-5a1-2&b1-2; similar to typical motor pathway

organization) and a decrease in excitability of ipsilateral responses from the uninjured cortex (Fig. 1-5a3-4&b3-4; motor pathway organization associated with poor motor function) as shown with TMS and fMRI (Juenger et al., 2013). In children who received bimanual training, an increase in cortical map size (Bleyenheuft et al., 2015, Friel et al., 2016), and amplitude of MEPs evoked from the affected cortex was seen (Friel et al., 2016). In the lower limb, intensive treadmill walking in children and youth with CP increased the corticospinal transmission to the tibialis anterior, especially in children younger than 10 years of age (Willerslev-Olsen et al., 2015). This evidence suggests that underlying motor systems benefit positively from activity-based therapies and help improve movement control.

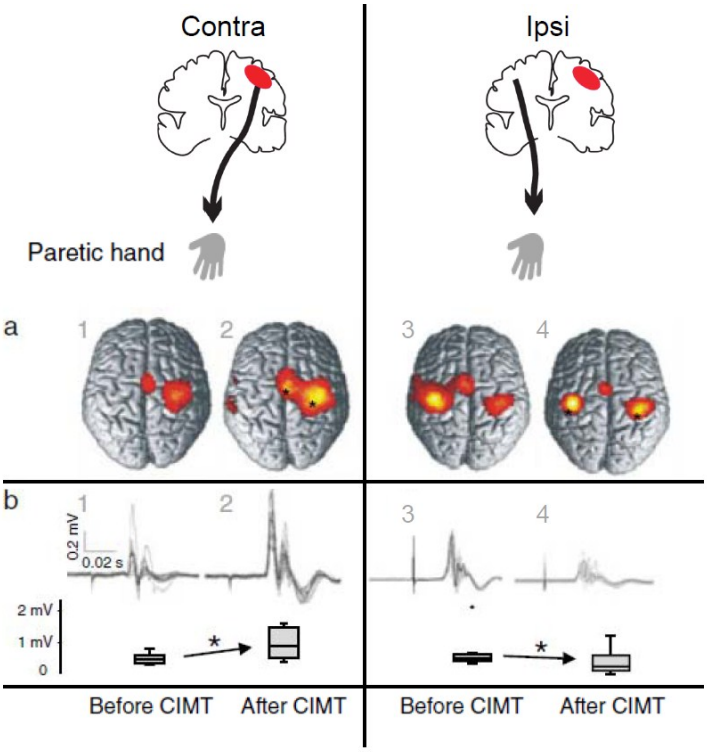

Figure 1-5. Neuroplastic changes in children with unilateral brain injury after 12 days of constraint-induced movement therapy. In children with contralateral projections from the injured cortex to the paretic hand ('Contra' on left), fMRI revealed an increase of activation in the primary sensorimotor cortex and the supplementary motor area (a1-a2) and TMS revealed an increase of MEP amplitude in the flexor pollicis brevis muscle (b1-b2). In children with ipsilateral projections from the uninjured cortex to the paretic hand ('Ipsi', on right), there was a decrease in both activation of the primary sensorimotor cortex (a3-a4) and in the amplitude of MEPs (b3-b4). (Modified from Juenger et al., 2013)

The critical period for intervention in CP

In animals who have received inactivation of the primary motor region, the age at which an intervention is applied largely influences corticospinal circuitry and functional motor outcomes. For example, in cats with unilateral impairments of reaching and grasping in the forelimb, constraint of the unimpaired limb combined with reach training of the impaired limb has had differing outcomes. If applied during postnatal weeks 8-13, the motor cortex map of the previously injured cortex and its contralateral CST were restored, cholinergic spinal interneurons contralateral to the injured cortex were increased and limb function was restored. In contrast, cholinergic spinal interneurons and limb function were not restored when the same intervention was applied at postnatal weeks 20-24 (Friel et al., 2012). Similarly, electrical stimulation of the CST from the injured motor cortex applied during postnatal weeks 7-10, in an attempt to activate injured pathways, improved CST connectivity from the injured cortex and limb function (Salimi et al., 2008). These studies reveal that interventions applied before postnatal week 10 in cats is optimal. By postnatal week 10, important developmental processes of the CST occurs including activity depending pruning, initial processes of myelination and cortical map development (as described in *Typical CST development*). Thus, interventions during these developmental processes is applied during a "critical period" where the competitive advantage of the previously impaired CST is augmented, thereby promoting proper development.

Few studies in children with CP demonstrate a possible critical period where the nervous system is most receptive to interventions. Body weight-supported treadmill training has shown greater improvements in gross motor function when applied in

younger (as young as 1.7 years of age) children with CP compared to older (up to 12 years of age) children with CP (Richards et al., 1997, Schindl et al., 2000, Begnoche and Pitetti, 2007, Cherng et al., 2007, Meyer-Heim et al., 2007, Mattern-Baxter et al., 2009, Yang et al., 2013). In the upper limb, there is no evidence that bimanual therapy or CIMT is more beneficial when applied at a certain age. Systematic reviews have demonstrated positive results when CIMT or bimanual training is applied in children and adolescence where there is no relation between age and functional benefit (Charles and Gordon, 2005, Gordon, 2011, Chiu and Ada, 2016). Although the age at which interventions would be most advantageous has yet to be identified in children, interventions initiated as early as possible are supported (Herskind et al., 2015). Considering the findings in kittens (Friel et al., 2012), the period when the CST is in the process of refining functional connections with spinal motor neurons constitutes a critical period. During these processes, development of the CST is largely shaped by motor activity, making interventions especially efficient. Altogether, the first 2 years of life may be considered a critical period for motor development in children (Basu, 2014, Herskind et al., 2015). TMS measures and the critical period of CST development will be explored further in Chapter 3.

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Chapter 2. Brainstem Reflexes in Adults with Cerebral Palsy

Introduction

Impairments in the control of head and neck functions in persons with cerebral palsy (CP) can include increased incidence of sialorrhea, bruxism, feeding problems and head instability (Dan et al., 2000, Dougherty, 2009, Saavedra et al., 2010, Sewell et al., 2014). In measurements of postural control, children with mild to moderate CP exhibit greater flexion and extension movements of the head during quiet sitting (Saavedra et al., 2010), greater neck flexion during squatting and greater neck extension during straightening up movements (Dan et al., 2000) compared to typically developing children. Because the cranial nerves that innervate the head and neck pass through the brainstem, deficits in head and neck control can be indicative of dysfunction in brainstem circuitry. In CP, the brainstem could be *directly* injured at the time of birth (Govaert, 2009), or affected *indirectly* by developing differently in response to injury of other brain structures. Although cortical, sub-cortical and cerebellar structures have been well studied in CP with magnetic resonance imaging (MRI) and/or electrophysiology (Korzeniewski et al., 2008), the function and structure of brainstem pathways and their interaction with damaged, descending cortical pathways are less well known.

Reflexes evoked from trigeminal afferent stimulation (cranial nerve V) have been used to assess brainstem damage in several neurological disorders (Di Lazzaro et al., 1996, Serrao et al., 2011, Magnano et al., 2014, Cengiz et al., 2017). In humans, the short-latency component of the trigemino-*cervical* reflex (TCR) is thought to have a similar pathway to that in the cat (Manni et al., 1975, Nishimura et al., 1992) whereby afferents from the trigeminal nerve synapse in the spinal trigeminal nucleus (pars oralis)

in the pons (Di Lazzaro et al., 1995). From here, projection neurons then innervate the spinal accessory nucleus in the cervical spinal cord (C1-C5), which gives rise to the spinal accessory nerve (cranial nerve XI) that innervates the sternocleidomastoid (SCM), an anterior neck muscle (Di Lazzaro et al., 1996). The short-latency TCR is reduced in amplitude, has a delayed onset and asymmetric activation in SCM muscles of patients with brainstem damage from multiple sclerosis (Di Lazzaro et al., 1996, Magnano et al., 2014).

The longer-latency component of the TCR also exhibits delayed onset latencies and absent or reduced amplitudes in individuals with supranuclear palsy and multi-system atrophy – conditions that can lead to neurodegeneration of the brainstem (Perrotta et al., 2005, Serrao et al., 2011). The anatomical pathway of the long-latency component of the TCR is not known; however, is thought to be similar, but distinct from, the startle reflex. This pathway is thought to involve afferent projections from the principal sensory (Pr5) or spinal trigeminal nucleus to the nucleus reticularis pontis caudalis (n.r. PnC) (Yeomans et al., 2002, Schmid et al., 2003), the latter giving rise to the reticulospinal tract (RST) which projects to motoneurons innervating the neck, shoulder and arm (Peterson, 1979, Peterson et al., 1979, Sakai et al., 2009). A similar pathway is thought to comprise the trigemino-*spinal* reflex (TSR) where trigeminal nerve stimulation produces long-latency responses in more distal muscles, like the biceps brachii (innervated from spinal root segments C5 to C6), given the more distal innervation of the cervical spinal cord by the RST (Serrao et al., 2003).

To assess the function of brainstem circuitry in CP, we compared the *long-latency* component of the TCR and TSR between individuals with CP and age/sex-matched

uninjured controls because it may have more direct implications on motor function compared to short-latency pathways (Siebner et al., 1999, Serrao et al., 2003). For example, in uninjured controls, stimulation of the supraorbital branch of the trigeminal nerve produces a long-latency TCR where pre-contracted anterior neck muscles, like the SCM, show EMG suppression (Nakashima et al., 1989) and posterior neck muscles show EMG facilitation (Serrao et al., 2003). The combined suppression of anterior neck muscles and facilitated posterior neck muscles allows the head to be withdrawn from facial stimuli – known as the head withdrawal reflex (Schmid et al., 2003, Serrao et al., 2003). Because participants with CP demonstrate reduced activation of long-latency inhibitory reflexes in limb muscles (Evans et al., 1991, Gibbs et al., 1999, Condliffe et al., 2016), we hypothesized there would be a similar reduction in the inhibitory component of the TCR and TSR, reflective of brainstem injury or altered function.

Brainstem circuits can also be activated by cortical inputs. In the monkey, transcranial magnetic stimulation (TMS) to the motor cortex produced both short-latency excitation and long-latency inhibition of neurons in the ponto-medullary reticular formation (Fisher et al., 2012), which gives rise to the RST (Sakai et al., 2009, Baker, 2011). Therefore, we also examined if the modulation of cortically-evoked responses in the SCM is altered following the activation of brainstem circuits by trigeminal afferents due to the abnormal development of cortico-reticulospinal circuits in response to early brain injury. We hypothesized that motor-evoked potentials (MEPs) in the SCM would show greater modulation (either increased or decreased in size) when conditioned with trigeminal stimulation in participants with CP compared to controls – this may reflect altered activation of cortico-reticulospinal circuits in CP.

Methods

Participants

This study was approved by the Heath Research Ethics Board at the University of Alberta (Pro00053278). Written informed consent was obtained from all participants. Inclusion criteria for participants with CP were: confirmed diagnosis of bilateral CP from medical records, cognitive ability to follow experimental instructions and the ability to perform sustained contractions of arm and neck muscles for up to 20 seconds. Exclusion criteria included botulinum toxin injections in the neck or upper arm in the last 6 months, trigeminal neuralgia, or contraindications to TMS (Rossi et al., 2009). Sixteen participants with CP were recruited to the study, with 2 participants withdrawing because they could not tolerate the TMS. Characteristics of the remaining 14 participants with CP are shown in Table 2-1 listing the Gross Motor Function Classification System (GMFCS) score (Palisano et al., 2008) and ReJoyce Arm and Hand Function Test (RAHFT) score (Prochazka and Kowalczewski, 2015) – the latter being an automated test that quantifies the performance of a variety of arm and hand motor tasks which are scored as a percentage of the maximal displacement of a joystick. In addition, the age (mean 37.6, 19 - 53 range), sex (6 females, 8 males) and location of muscles tested (right or left) are listed. The classification of anatomical abnormalities relevant for CP (Towsley et al., 2011), as measured from magnetic resonance imaging (MRI) scans using fluid-attenuated inversion recovery (FLAIR), are listed for 8 of the participants with CP (for details see Condliffe et al., 2016). Fifteen adults (8 females, 7 males) with no known neurological conditions and with a mean age of 37.4 (23 - 53 range) were recruited as neurologically-

intact control participants (NI controls) and were sex- and age-matched (within 6 years) to the CP participants.

ID	Sex	Age	MRI	Side	GMFCS	RAHFT
CP-2	M	35	PVWMI++/CM	R	I	52.6
CP-4	F	36	N/A	L	II	97.3
CP-5	F	38	PVWMI ++	L	IV	69.5
CP-6	M	53	PVWMI ++	L	III	66.6
CP-10	M	31	PVWMI+/CM	R	III	95.3
CP-12	M	28	PVWMI +	R	IV	16.3
CP-13	M	46	PVWMI +	L	III	92.9
CP-14	F	43	Normal	L	III	NT
CP-17	M	35	PVWMI ++	R	II	92.2
CP-18	M	46	N/A	R	I	98.7
CP-19	F	19	N/A	R	I	94.2
CP-20	F	39	N/A	L	IV	83.2
CP-21	M	42	N/A	L	II	97.6
CP-22	F	36	N/A	L	III	23.7

Table 2-1. Characteristics of CP participants. Columns list the participant's study ID, sex, age (years), MRI classification (MRI), the side of the body from which muscles were recorded (Side), Gross Motor Functional Classification System (GMFCS) score and the Rejoyce Arm and Hand Function Test (RAHFT) score (maximum 100%). The same ID codes for participants who also took part in the Condliffe et al., 2016 study were used along with the listed MRI classification. PVWMI = periventricular white matter injury, CM = cerebral malformation, + = mild, ++ = moderate. NT = not tested as participant could not grasp REJOYCE handle to perform test. N/A = not available.

Trigemino-cervical and trigemino-spinal reflexes

Trigemino-cervical reflexes (TCRs) and trigemino-spinal reflexes (TSRs) were evoked in the SCM and biceps muscles, respectively, in response to stimulation of the mandibular branch of the trigeminal nerve. Stimulation intensities were set relative to the motor threshold (MT) evoked in the masseter muscle rather than based on perception or reflex thresholds (Rossi et al., 1989, Di Lazzaro et al., 1995, Siebner et al., 1999, Serrao

et al., 2003, Nardone et al., 2015) to objectively standardize the stimulation intensities between the two groups.

General EMG Set-Up

In participants with CP, TCRs were evoked on the side of the body with the most prominent motor impairment, whereas in NI participants, the side of the body with the least dominant hand was tested. After cleaning the skin, pairs of conductive adhesive hydrogel electrodes (Covidien Ltd., Dublin, Ireland) were placed over the masseter (2.24 x 2.24 cm) and SCM (3.81 x 2.24 cm) muscles in a belly-to-bone configuration (Di Lazzaro et al., 1996) and over the belly of the biceps muscle (3.81 x 2.24 cm) with a spacing of 1 cm. Surface EMG signals were amplified by 1000 using a NeuroLog Pre-amplifier (NL844-4Ch AC, Digitimer, Welwyn Garden City, UK), set to 10 Hz high pass, digitized and sampled at 5kHz using Axon Instruments hardware (Digidata 1440A) and software (Axoscope 10.3, Molecular Devices LLC, Sunnyvale, USA).

Nerve Stimulation

The mandibular branch of the trigeminal nerve was stimulated by the cathode that was placed just below the proximal zygomatic arch 3 cm anterior to the tragus, consistent with the location of the motor nerve (masseteric) to the masseter muscle (Borschel et al., 2012). The anode was placed over the mastoid process behind the ear using 2.24 x 2.24 cm hydrogel electrodes. Surface electrical stimulation was applied using a DS7A constant-current stimulator (NL703, Digitimer, Welwyn Garden City, UK) with single-pulse stimulation (0.5 ms pulse width) at 0.8, 1.2 and 2.0 x MT applied every 500 ms for a total of 200 trials. Pilot experiments identified 200 trials to be optimal in evoking clear

reflexes with smooth EMG, where 100 trials produced indistinct reflexes hindered by variable EMG and 300 trials producing reflexes reduced in amplitude likely due to fatigue of the participant to sustain a contraction. The motor threshold for the participants with CP (8.6 ± 2.7 mA) was higher than for controls (6.3 ± 1.3 mA, $p = 0.008$), consistent with reduced excitability of motor axons in this population (Klein et al., 2015).

Background Contraction

The TCR was evoked in the SCM while participants produced a moderate (approximately 50 μV) isometric contraction that could be sustained for a period of 20 s or more. Visual feedback of the rectified and smoothed EMG was provided on an oscilloscope to keep contraction levels steady, while participants pushed their chin against the experimenter's hand with their head facing forward. For reflexes evoked in the biceps muscle (TSR), the upper arm rested horizontally on a platform with the elbow at 90° and the wrist contracting against a mechanical stop to produce a steady contraction of 10% of maximum that was maintained with visual feedback of the rectified and smoothed EMG.

Conditioning cortically-evoked responses with trigeminal nerve stimulation

TMS was applied to the contralateral cortex supplying the SCM muscle using a MagStim 200 stimulator (The Magstim Company Ltd, Carmarthenshire, UK) and a 70 mm figure-of-eight coil. The location which produced the most consistent MEP in the contralateral SCM was identified during a small contraction of the contralateral biceps at 10% of maximum, typically located 3 to 4 cm lateral of vertex, with an onset latency of 11.4 ± 1.4 ms (CP) and 8.3 ± 2.2 ms (NI controls, $p = 0.02$). The stimulation location and

latency of MEP activation is consistent with a cortical, rather than peripheral, activation of the SCM muscle (Thompson et al., 1997). An MEP amplitude of approximately half maximum was evoked with an average peak-to-peak amplitude of the test MEP of 369.9 ± 151.4 μV in the participants with CP and 390.4 ± 245.2 μV in controls ($p = 0.88$).

Five pulses of trigeminal nerve stimulation (200 Hz at 1 xMT) were applied to the mandibular branch of the trigeminal nerve at 20, 50 and 100 ms before the TMS pulse, based on brainstem reflex studies (Siebner et al., 1999, Fisher et al., 2004). Long inter-stimulus intervals (ISIs) were used to assess long-latency pathways and multiple pulses of trigeminal nerve stimulation were used to provide a large barrage of afferent inputs (Zewdie et al., 2014). Ten trigeminal alone, 10 TMS alone (test) and 8 TMS + trigeminal (conditioning) stimuli at each ISI were randomly applied every 8 seconds so that participants could rest between each stimulation. Conditioning SCM MEPs with trigeminal nerve stimulation was investigated in 7 participants with CP and in 10 NI controls.

Data Processing

Long-latency component of the TCR and TSR

Analysis of SCM and biceps EMG was performed offline using custom Matlab (version r2015b) programs. For a given intensity, the surface EMG trace for each stimulation trial (200 in total) was digitally bandpass filtered (5 to 800 Hz), rectified and then averaged together to give a mean TCR/TSR EMG. The mean background EMG (dotted lines in Fig. 2-2) was calculated by averaging the mean EMG in the 100-ms period before the stimulation. The long-latency component of the SCM TCR was measured after the short-latency response was complete (Di Lazzaro et al., 1995), the

former starting typically at 30 ms or later. To measure the long-latency TCR, the average amplitude of the mean EMG that fell below (Fig. 2-2A) or above (lower trace, Fig. 2-2B) the mean background EMG for at least 10 ms or more was calculated (marked by horizontal lines in Fig. 2-2). On average, the duration of the TCR was 50.7 ± 20.5 ms in the participants with CP and 27.8 ± 13.8 ms in the NI controls ($p = 0.004$), with both appearing to have similar onset latencies (CP: 39.5 ± 8.1 ms, NI: 42.0 ± 19.4 ms, $p = 0.68$). Six NI controls and 4 CP participants did not display SCM TCRs at 0.8 and 1.2xMT. In these cases, the window selected for the 2 xMT trials was used to measure the TCR. Two NI controls and 1 CP participant did not display SCM TCRs at any stimulation intensity so their data was not included. The same analysis protocol was used for the biceps TSR where only 1.2 xMT was tested in 15 participants of each group.

To compare TCR/TSR values across participants having different amplitudes of background EMG, the mean background was subtracted from the mean reflex EMG and then divided by the mean background EMG [(mean reflex – mean background) / mean background x100%] similar to Nakashima et al., 1989. Thus, 0% represents a mean reflex amplitude that is equivalent to the mean background, positive values > 0% represent a mean reflex that is greater than the mean background and negative values < 0% represent a mean reflex that is smaller than the mean background (Fig. 2-2C for SCM TCR, Fig. 2-9 for biceps TSR) as per Condliffe et al. (2016).

Conditioned SCM MEPs

The SCM MEPs that were conditioned by a prior trigeminal nerve stimulation were compared to the test alone SCM MEPs. In some cases, the preceding trigeminal nerve stimulation produced a small reflex response or artifact that occurred within the same time window as the test MEP. Thus, to quantify the modulation of the conditioned MEP that was beyond the amount produced by the trigeminal nerve stimulation alone, the rectified and averaged EMG from the nerve stimulation alone trials was subtracted from the rectified and averaged conditioned MEP trace for all ISIs (Fig. 2-1) as per Poon et al. (2008). To do this, the nerve stimulation alone trials were shifted in time, relative to the test TMS pulse to match the time the reflex was evoked during the MEP conditioning trials (e.g., shifted by 20, 50 or 100 ms to the left). The mean EMG measured during the window of the subtracted, conditioned MEP was then expressed as a percentage of the mean EMG during the test alone MEP [(conditioned MEP–test MEP)/ test MEP x 100%].

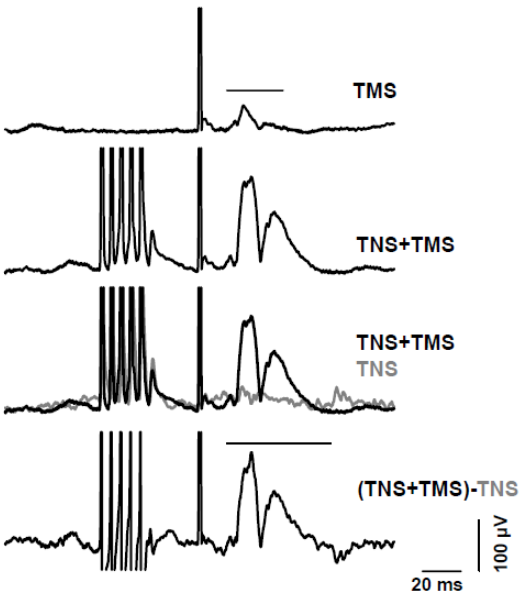

Figure 2-1. Method for measuring the conditioned MEP in the SCM muscle. The trigeminal nerve stimulation (TNS) alone trial was subtracted from the conditioned MEP. Top trace: average of 10 rectified EMG traces following TMS to the contralateral motor cortex. Time of TMS pulse is noted by large stimulus artefact. Second trace: Average of 8 rectified EMG traces where the first of 5 TNS pulses at 1.0 xMT precede the TMS by 50 ms (TNS + TMS). Third trace: Averaged rectified EMG from TNS stimulation alone (gray trace) trial superimposed on the TNS + TMS average. Fourth trace: Subtraction of the TNS trace from the TNS + TMS trace to reveal modulation of the MEP that is greater than the effect from the TNS alone. The mean EMG measured during the window of the TMS MEP (noted by horizontal line) was expressed as a percentage of the mean EMG during the window of the test MEP [(conditioned MEP – test MEP)/ test MEP x 100%] to give a measure of the modulation of the conditioned MEP.

Statistics

Statistical analysis was performed using Sigmaplot 11.0 software and Matlab (version r2015b). Because of the small sample sizes ($n < 20$) and the unequal variance in the data between the two groups, separate Mann-Whitney U-tests were used to compare the SCM TCR *between* the CP and NI groups at the three different stimulation intensities used (0.8, 1.2 and 2.0 xMT, Fig. 2-2C) or to compare the conditioned MEPs at the three different ISIs tested (20, 50 and 100 ms, Fig. 2-6). To account for multiple comparisons, a Bonferroni correction was applied with significance set to $p = 0.017$ (i.e., 0.05/3). To estimate the strength of the difference in the TCR between the two groups, we calculated the effect size (r) according to the equation $r = z/\sqrt{n}$ (Rosenthal, 1991) where z is the distance between the raw value and the population mean (unit of standard deviation), n is the pooled sample size and 0.2 and 0.5 are considered small to medium effect sizes, respectively. To compare the difference in the conditioned MEPs across the different ISIs *within* a group (CP or NI), a Kruskal-Wallis one-way ANOVA was used with post-hoc Mann-Whitney U-tests (Bonferroni correction of $p = 0.017$ for Fig. 2-6). A Spearman correlation (r_s) was used to measure the relationship between the SCM TCR and GMFCS or RHAFT scores in the participants with CP.

Results

SCM TCR

In NI controls, single-pulse stimulation to the mandibular branch of the trigeminal nerve at 1.2 xMT produced a TCR in the SCM muscle where the mean voluntary background EMG was suppressed at a long latency. This is shown for a single participant

in Figure 2-2A (top trace) where the mean EMG fell below background from 49 to 87 ms after the trigeminal nerve stimulation. The amount of EMG suppression during the TCR typically increased at the higher stimulation intensity of 2.0 xMT (bottom trace, Fig. 2-2A). The reverse pattern of SCM TCR activation was observed in most participants with CP where the long-latency TCR became more faciliatory as the stimulation intensity increased (compare top and bottom traces, Fig. 2-2B). This trend is revealed when plotting the mean amplitude of the SCM TCR (see Data Analysis in Methods for calculation) at the three stimulation intensities tested (Fig. 2-2C). The median TCR for the NI group became more suppressed (< 0%) compared to the background EMG as the stimulation intensity increased, whereas the median TCR became more facilitatory (> 0%) in participants with CP. The difference in the median TCR between the two groups was significant at 2.0 xMT (Mann-Whitney U test, $p = 0.004$), having a moderate effect size ($r = 0.58$) compared to the smaller effect size at the 0.8 xMT ($r = 0.42$) and 1.2 xMT ($r = 0.44$) stimulation intensities.

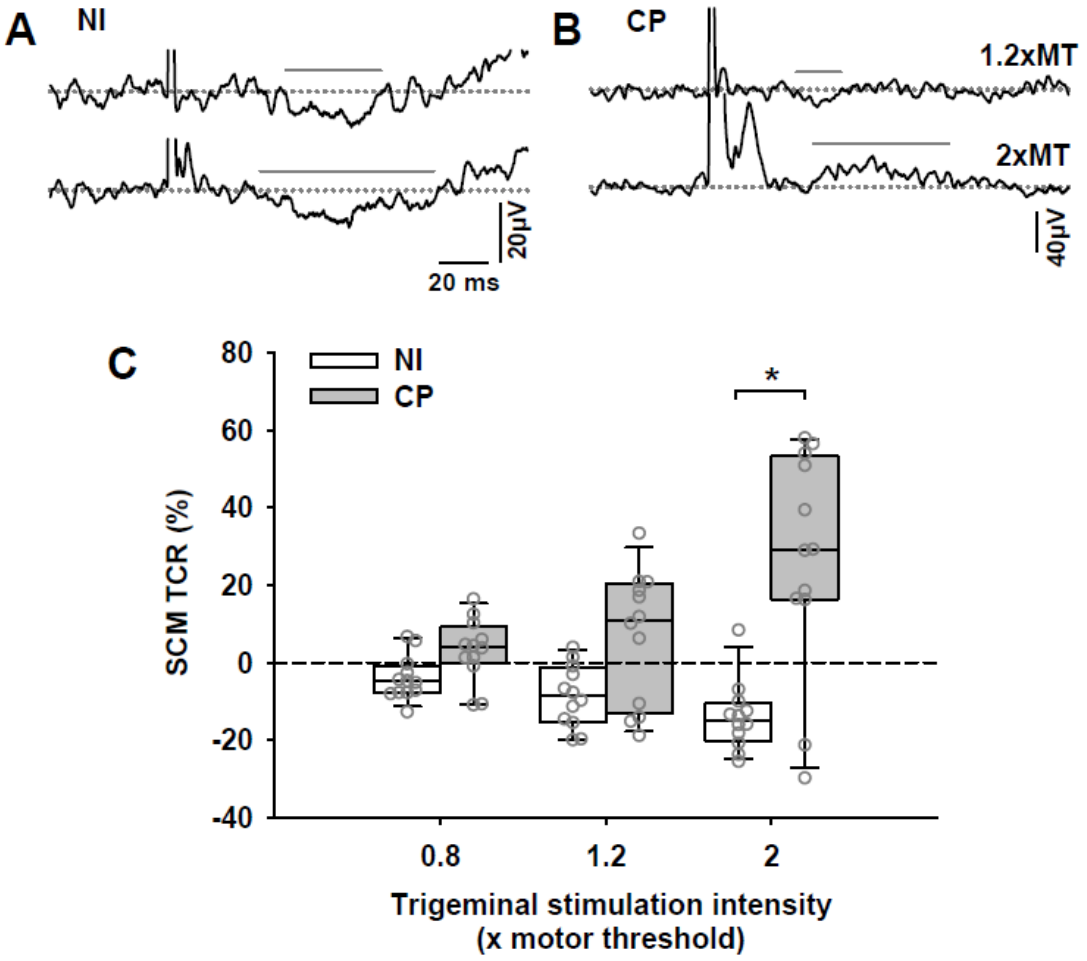

Figure 2-2. Trigemino-cervical reflexes (TCR) in the SCM. EMG traces from an NI control participant (A) and a participant with CP (B). Rectified and averaged EMG from 200 stimuli at 1.2 xMT (top traces) and 2 xMT (bottom traces). Dotted lines indicate the mean background EMG measured 100 ms before the stimulation. Grey solid lines indicate the window that was used to measure the TCR where the mean EMG deviated above or below the mean background EMG for > 10 ms. C) Magnitude of mean TCR EMG expressed as an increase or decrease from the mean background EMG [(TCR EMG – Background EMG)/ Background EMG x 100%)]; NI controls (white bars); CP participants (grey bars); individual values are shown with grey circles. Data from 1 CP and 1 NI control participant was removed due to contamination of the EMG signal from the heart and from movement artifact, respectively. The solid line within the box plot represents the median, the 25th and 75th percentiles by the box bounds, and 95th and 5th percentiles by the whiskers. * = $p < 0.017$

Muscle activation and SCM TCR

On average, participants with CP tended to have slightly lower mean background EMG (48.5 ± 31.5 µV), compared to the NI controls (58.7 ± 31.6 µV, p = 0.10). Thus, to ensure that the differences in the amplitude and direction (facilitation vs suppression) of the SCM TCR between the two groups were not simply due to differences in background EMG, the amplitude of the mean TCR was plotted against its corresponding mean background EMG value (Fig. 2-3). For background contraction levels < 70 µV, SCM TCR values from the participants with CP (white symbols) were mainly located above the line of unity, unlike that for NI controls (solid symbols), indicating that at matched levels of background EMG, the TCR was typically facilitatory in the CP participants and inhibitory in the NI controls. For SCM TCRs measured at a background EMG >70 µV, the mean TCR was suppressed, compared to the background EMG in both groups. Of note, the two participants with CP having inhibitory SCM TCRs at these higher levels of background EMG (CP 14 and 4) had motor function scores or MRI classification that was similar to the NI controls (Table 2-1).

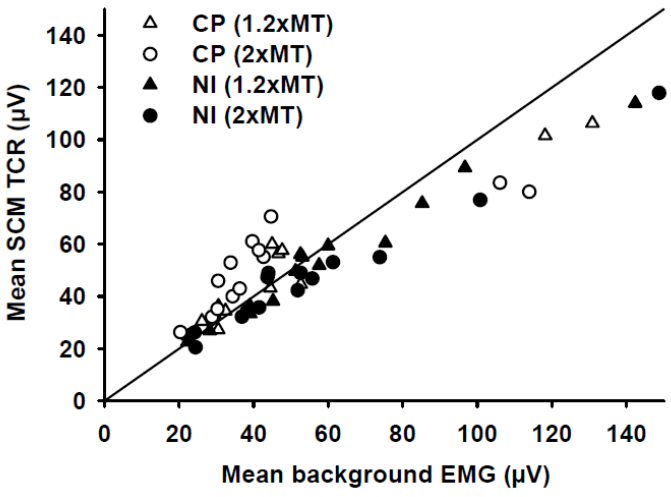

Figure 2-3. Mean SCM TCR EMG vs mean background EMG. Data from NI controls (black symbols) and participants with CP (white symbols) following trigeminal nerve stimulation at 1.2 xMT (triangles) and 2 xMT (circles). The solid line indicates no reflex response (i.e. mean TCR EMG = mean background EMG); data below and above the line of unity indicate an inhibitory and facilitatory TCR respectively.

Relationship between TCR and clinical measures

At 2.0 x MT, there was a large range of TCR values in the participants with CP (Fig. 2-2C). Because the two CP participants with TCR values that were similar to the NI controls had good motor function or normal brain imaging, we measured the relationship between the amplitude of the SCM TCR and motor function or MRI classification (when available). Interestingly, the amplitude of the TCR was not related to gross motor function as assessed by the GMFCS (r_s = -0.14, p = 0.63), nor was it related to arm and hand function as assessed by the RHAFT (r_s = 0.06, p = 0.85, data not shown), likely because these scales do not specifically assess neck function. However, qualitatively, there appeared to be a relationship between the amplitude of the TCR and the severity of the MRI classification (Fig. 2-4A, see legend for details). Participants with normal imaging or mild periventricular white matter injury (PVWMI +) had lower SCM TCR values compared to participants having moderate PVWMI (++) and a combination of both PVWMI and cerebral malformations (Fig. 2-4B).

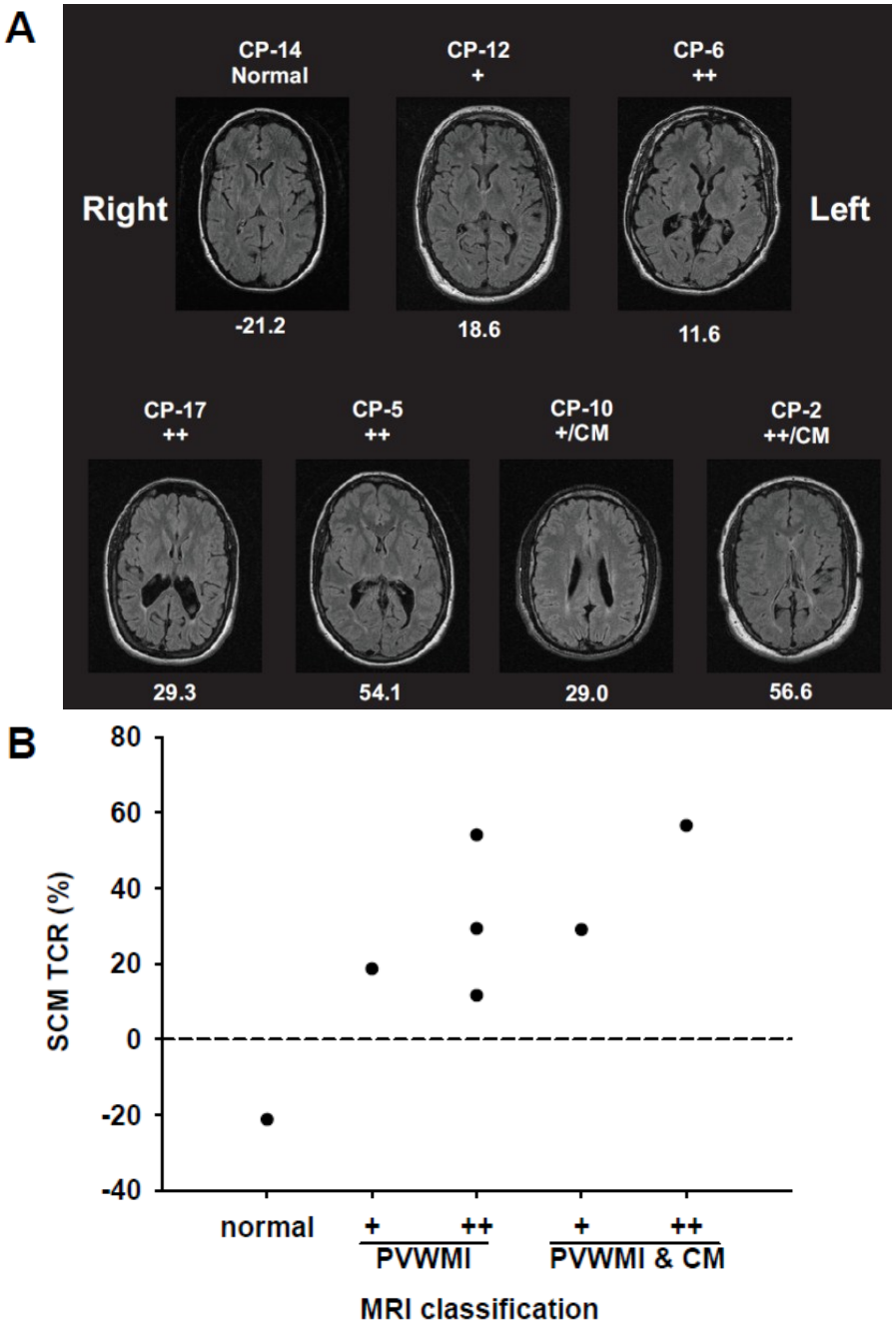

Figure 2-4. Relationship between SCM TCR and MRI classification in participants with CP. A) Axial FLAIR images showing anatomical features in 7 participants with CP (taken from Condliffe et al., 2016). Participant ID code and MRI classification are listed above each image with the SCM TCR value below (trigeminal nerve stimulation at 2 xMT). B) TCR value plotted against corresponding MRI classification from *A.* MRI classifications arranged in estimated order of increasing severity from left to right. PVWMI = periventricular white matter injury; + = mild; ++ = moderate; CM = cerebral malformation. Data from CP-13 was not used due to contamination of the SCM TCR by signals from the heart beat.

Interaction between descending and trigeminal afferent pathways

Because the motor cortex is influenced by afferent inputs from the trigeminal nerve (Richardson and Cody, 1977, Van Loven et al., 2001) and has outputs to brainstem structures also innervated by trigeminal nerve afferents (Rho et al., 1997, Fisher et al., 2012, Fregosi et al., 2017), we examined if the interaction between trigeminal nerve and motor cortex stimulation was altered in CP where damage to the CSTs is common (Korzeniewski et al., 2008). To produce a robust volley of afferent inputs, 5 pulses of trigeminal nerve stimulation were applied at 1.0 x MT at three ISIs (20, 50 and 100 ms) in both groups. As shown for a representative NI control (Fig. 2-5A) and CP participant (Fig. 2-5B), the short-latency MEPs recorded in the SCM muscle were facilitated when the first TNS pulse preceded the TMS by 50 ms, compared to being slightly inhibited at the 20 and 100 ISIs.

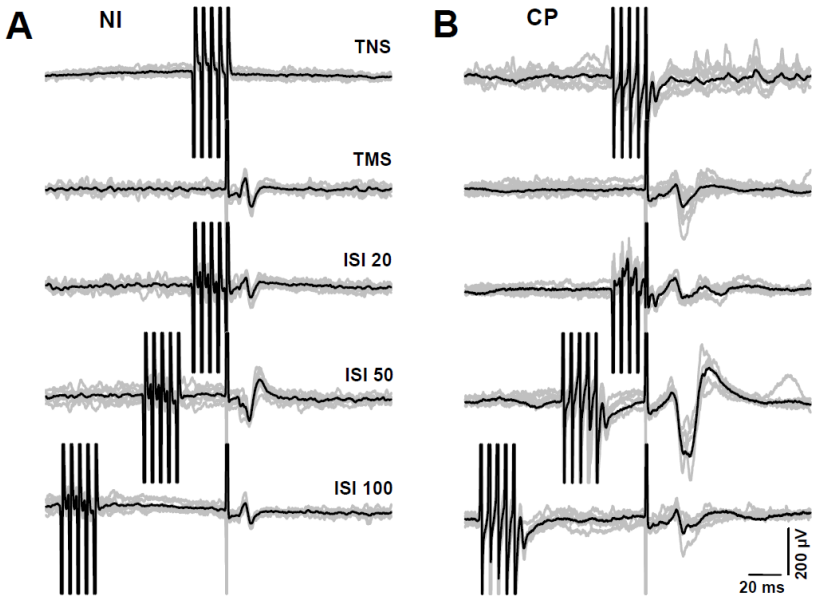

Figure 2-5. Modulation of SCM MEPs by trigeminal afferents. Unrectified EMG traces from a control participant (A) and a participant with CP (B). Grey lines represent individual unrectified EMG traces, black lines the average EMG trace. Top trace: trigeminal nerve stimulation (TNS) alone with 5 pulses, 200 Hz at 1.0 xMT, 10 trials; second trace: TMS applied to contralateral cortex, 10 trials; third to fifth trace: TNS delivered 20, 50 and 100 ms before TMS respectively, 8 trials each.

When comparing the modulation of the conditioned MEP with respect to the test MEP (see Conditioned SCM MEPs in Methods for calculation), a similar pattern of MEP modulation was observed in the NI and CP groups (Fig. 2-6), with similar medians between the two groups at the different ISIs (Mann-Whitney U, p all > 0.22). A Kruskal-Wallis one-way ANOVA revealed a statistically significant effect for ISI in the CP group only ($p = 0.003$), with post-hoc Mann-Whitney U-tests showing that the median MEP modulation was more facilitatory at the 50 ms compared to 20 ms ($p = 0.005$) and 100 ms ($p = 0.001$) ISIs.

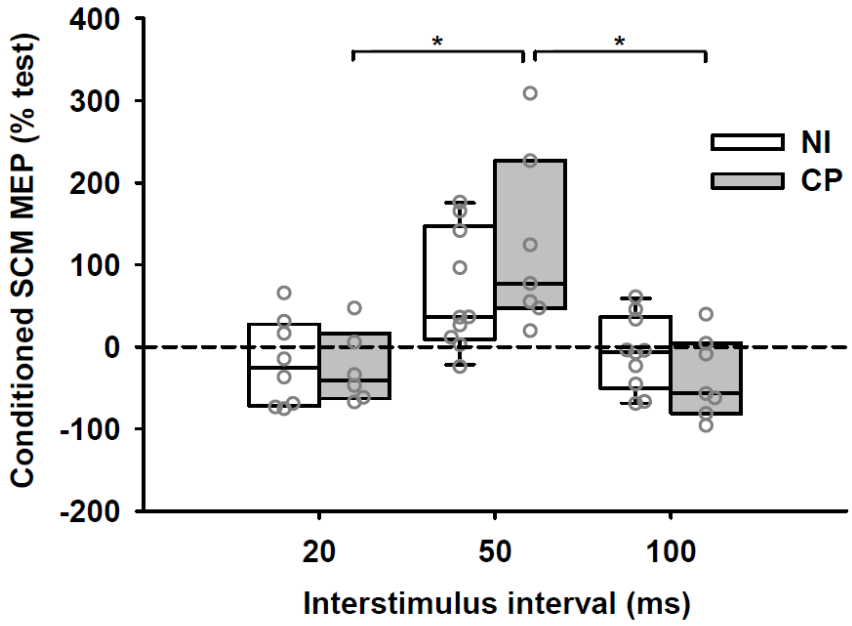

Figure 2-6. TNS-conditioned SCM MEPs in NI controls and participants with CP. Size of the conditioned short-latency MEP from a prior TNS (5 pulses, 1 xMT) applied 20, 50, and 100ms before the TMS for both NI controls (white bars) and participants with CP (grey bars). Conditioned MEP expressed as [(conditioned MEP - test MEP)/ test MEP x 100%]. Two NI controls and 1 CP did not receive the 20 ms ISI trial. The solid line within the box plot represents the median, the 25th and 75th percentiles by the box bounds, and 95th and 5th percentiles by the whiskers. * = $p < 0.017$

Long-lasting facilitation of SCM MEPs in CP

In 3 of the 7 CP participants tested, conditioning the SCM MEP with a prior trigeminal nerve stimulation produced a large (> 400 μV), long-lasting excitation that continued beyond the duration of the unconditioned MEP, especially at the 20 and 50-ms ISIs (Fig. 2-7). In these examples, trigeminal nerve stimulation (TNS) or TMS delivered alone produced little to no response in the SCM muscle (top two traces in Fig. 2-7). However, when the two stimuli were combined, a large evoked response was produced that lasted until ≈ 150 ms after the TMS pulse. Large evoked responses did not occur at the 100 ms ISI as shown for CP21 and CP22 (bottom traces). Moreover, a long-lasting response was also produced in the opposite SCM that was ipsilateral to the TMS (data for the 20 ms ISI is shown for CP10, bottom trace). Interestingly, a short-latency MEP was not produced in the ipsilateral SCM, but only a long-latency response with a similar onset time to the contralateral response. Long-lasting, long-latency responses were not evoked in the SCM muscle of the NI controls (0/10 tested).

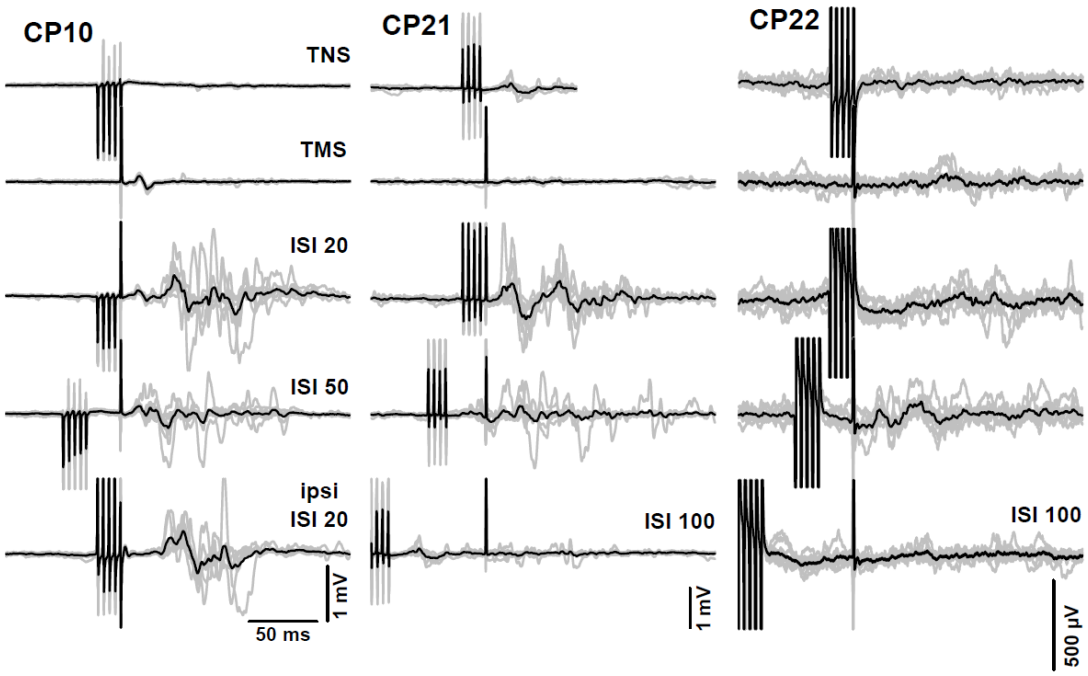

Figure 2-7. Long-lasting facilitation from combined TNS and TMS in 3 participants with CP. Same set up as Figure 2-5 showing long-lasting responses in the SCM muscle in response to combined TNS and TMS. The TNS alone trace for CP-21 was taken from the 100 ms ISI trial. Note the bottom panel for CP10 displays EMG traces from the SCM muscle ipsilateral (ipsi) to the TMS with an ISI of 20 ms. Responses from TNS or TMS given alone were absent in the ipsi SCM (not shown).

Potentiation of MEPs in distal muscles in CP

In addition to producing a large evoked response in the neck muscles, the combined trigeminal nerve stimulation and TMS also produced a large response in a more distal muscle (biceps) in the same 3 participants with CP (Fig. 2-8). This was particularly evident in participant CP22, where MEPs in the biceps were evoked only after the combined activation of a sub-threshold trigeminal nerve stimulation and TMS (Fig. 2-8, right column). Example data is shown for the 50 ms ISI in all three participants, which was the most common interval that produced a facilitation of the biceps MEP.

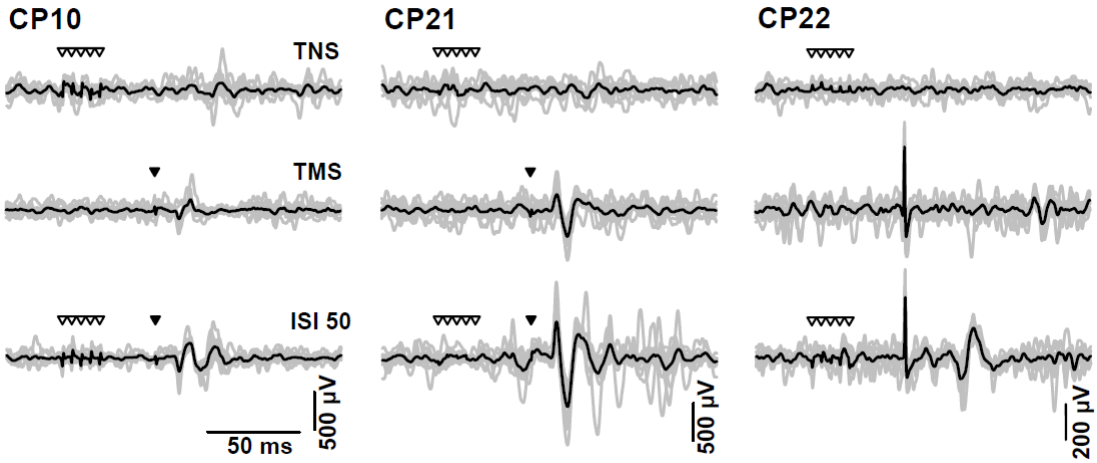

Figure 2-8. Facilitation of biceps MEP from TNS in the same 3 participants with CP. Top traces: individual (grey lines) and average (black line) traces of unrectified EMG recorded in biceps muscle in response to TNS (5 pulses, 200 Hz, 1.0 xMT). The time of TNS is indicated by the white triangles. Middle traces: Biceps MEPs evoked from TMS applied to the contralateral cortex. The time of stimulation is indicated by black triangle for CP-10 and CP-21 and by large stimulation artifact for CP-22. Bottom traces: Facilitation of biceps MEP in response to TNS applied 50 ms before the TMS pulse. Similar format as Figure 2-7.

TSR in biceps

Given the large potentiation of biceps MEPs from trigeminal nerve stimulation in some of the participants with CP, we examined if there were differences in the occurrence and sign (inhibitory or excitatory) of long-latency TSRs in the biceps muscles between the two groups. Surprisingly, there were no systematic differences in the activation of the TSR between the two groups. Appreciable responses (lasting > 10 ms) were seen in 4 of 15 NI controls tested and in 5 of 15 of participants with CP tested. Both EMG suppression (top traces in Fig. 2-9A&B) and facilitation (bottom traces, Fig. 2-9A&B) were observed in both groups, as shown from the mean amplitude of the biceps TSR in Fig. 2-9C, calculated as per the SCM TCR. The average onset was 38.1 ± 7.1 ms

and 33.2 ± 6.5 ms in CP and NI participants, respectively, and the average duration of the biceps TSR was 21.6 ± 4.9 ms in CP and 26.6 ± 8.6 ms in NI controls (p all > 0.32).

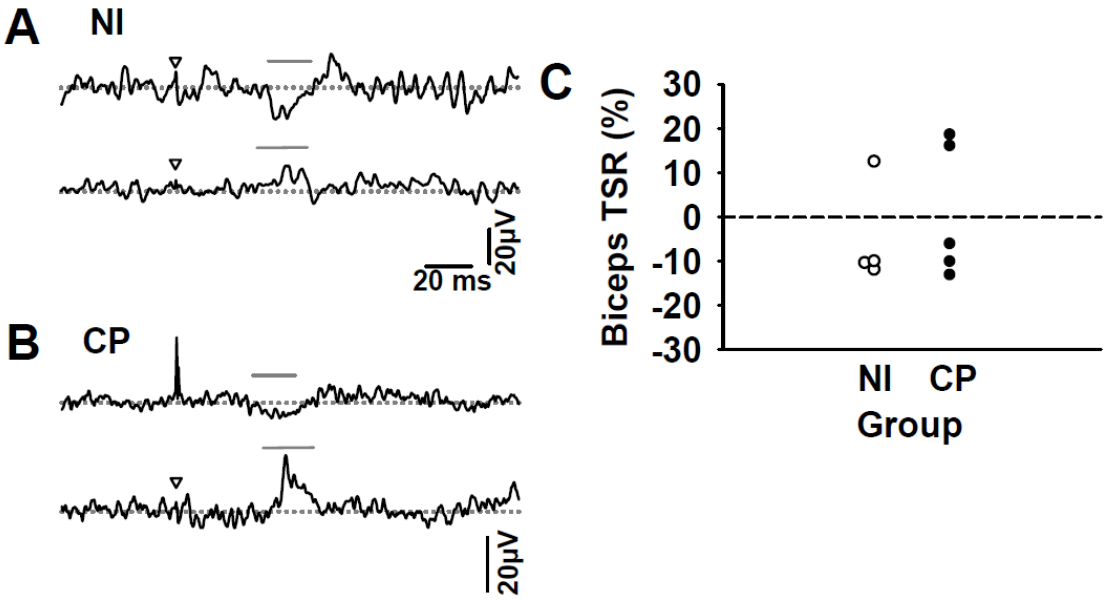

Figure 2-9. Biceps TSR. Example biceps EMG traces from two NI control participants (A) and two participants with CP (B). Rectified and averaged EMG from 200 stimuli of trigeminal nerve at 1.2xMT. White triangles indicate the time TNS. Dotted lines indicate the mean background EMG 100ms before the stimulation. Grey solid lines indicate the window that was used to measure the TSR. Top traces show EMG suppression and bottom traces show EMG facilitation during TSR. C) Amplitude of biceps TSR (white circles NI, black circles CP) calculated as per the SCM TCR.

Discussion

This study demonstrates altered brainstem circuitry in participants with CP as evidenced by the long-latency component of the TCR being more facilitatory compared to age and sex-matched controls. Within CP participants, the activation of brainstem circuitry form trigeminal nerve stimulation produced modulation of SCM MEPs that was most facilitatory at the 50ms ISI, and in some, an abnormally large and long-lasting

response was produced in both neck and upper arm muscles when cortical and trigeminal afferent stimulation were combined. We propose that the excessive responses evoked from trigeminal afferents in CP are produced by heightened excitability of brainstem circuits and may contribute to abnormal postural control of the head and neck. On the other hand, a more excitable brainstem may facilitate the activation of cortico-reticulospinal pathways to activate the spinal cord better in response to damage of more direct corticospinal tract (CST) pathways.

Hyper-excitable TCR in CP

Similar to the TCR evoked from stimulation of the supraorbital branch of the trigeminal nerve (Nakashima et al., 1989), we observed, in control participants, a long-latency suppression of ongoing SCM activity when stimulating the mandibular branch. The TCR may share some of the pathways associated with the startle reflex, given its long latency and coordinated muscle activation (Davis et al., 1982, Siebner et al., 1999, Serrao et al., 2003). A possible polysynaptic pathway could include afferent projections from the Pr5 or spinal trigeminal nucleus (pars oralis), both of which have direct connections to the n.r. PnC to innervate the spinal accessory nucleus via the RST (Yeomans et al., 2002, Schmid et al., 2003). Because the long-latency responses were evoked at low stimulation intensities and were non-noxious (0.8 to 2.0 x MT), they may represent the activation of postural reflexes in response to large diameter cutaneous or muscle afferents that are coordinated by the n.r. PnC (Suzuki et al., 1989, Isa and Naito, 1995). Like with supra- and infraorbital stimulation of the trigeminal nerve, components of the long-latency TCR from stimulation of the mandibular nerve may also involve

pathways from the facial nerve (VII) which has a similar innervation territory in the face (Gupta et al., 2013). Thus, mandibular nerve stimulation may evoke multiple brainstem pathways that seem to produce, at least in control participants, inhibition of ventral neck muscles to help with backward movements of the head in response to non-noxious sensory inputs from the face.

The reduced inhibition observed in the TCR of participants with CP is likely due to a decrease in the activation of inhibitory neurons and/or an increased activation of excitatory neurons in these brainstem circuits. In support of this, increases in serotonin, glutamate decarboxylase and the receptor for Neurokinin 1, all of which can increase neuronal excitability, occur in the spinal trigeminal nucleus of rats subjected to mild traumatic brain injury (Mustafa et al., 2016). In CP, such changes in neuronal excitability can result from direct damage to the brainstem or indirectly from its altered activation by damaged cortical inputs. In cases where there is direct damage to the brainstem, as occurs in amyotrophic lateral and multiple sclerosis, there is a decrease in inhibitory masseter reflexes, augmentation of excitatory blink reflexes (Cengiz et al., 2017) and a delayed latency of various other brainstem reflexes including the TCR (Magnano et al., 2014). Given that the brainstem in CP can show decreases in mean volume (Kulak and Sobaniec, 2007) and fractional anisotropy (Mu et al., 2014), alterations in TCR excitability may arise from direct damage to brainstem structures.

Alterations in cortical drive to the brainstem may also increase excitability in brainstem circuits, similar to that shown for spinal circuits in CP (Condliffe et al., 2016). Activation of descending pathways originating in the motor cortex produces both short- and long-lasting excitation of neurons in the ponto-medullary reticular formation (Fisher

et al., 2012). Thus, excessive cortical drive onto excitatory networks, potentially resulting from reduced tonic activity in GABA-ergic interneurons of the motor cortex in CP (Berweck et al., 2008), may produce a tonic excitation of the ponto-medullary reticular formation to mediate hyper-excitable TCRs. On the other hand, cortical inputs have also been shown to inhibit ongoing neural activity in the reticular formation (Fisher et al., 2012). Thus, it is equally plausible that a reduced drive onto inhibitory circuits in the ponto-medullary reticular formation from a fewer number of cortical inputs may bias the TCR to becoming more facilitatory. Individuals with lesions localized in one cerebral hemisphere due to stroke have shown enhanced acoustic startle reflex responses in the SCM, biceps, and tibialis anterior (Voordecker et al., 1997, Jankelowitz and Colebatch, 2004) which was attributed to a loss of inhibitory cortical drive to the reticular formation (Voordecker et al., 1997). It is interesting to note that participants in the present study with more severe damage to cortical and white matter structures had more facilitatory TCRs (Fig. 2-4). This suggests that larger impairments in cortical drive may produce larger increases in brainstem excitability, although the numbers of participants examined here is too small to make firm conclusions.

Facilitation of SCM MEPs by TNS: possible sites of action

The facilitation of the early-latency SCM MEPs by a prior trigeminal afferent stimulation only occurred at the 50 ms ISI, especially for the participants with CP. Given the long-latency of the facilitation, it is difficult to determine where the interaction between the cortical and trigeminal afferent stimulation is occurring, be it cortical, brainstem or spinal. A cortical site of facilitation is unlikely, given that somatosensory evoked potentials elicited from trigeminal afferent stimulation have a latency of 5 to 22

ms (Richardson and Cody, 1977, Van Loven et al., 2001) and likely occur too early to facilitate the SCM MEP when TMS is applied to the cortex 50 ms after nerve stimulation. Likewise, there was no significant modulation of the SCM MEP at the 20 ms ISI when activation of the cortex by trigeminal afferents was likely to occur.

A more likely possibility is that trigeminal inputs excite brainstem nuclei (like the n.r. PnC) to facilitate cortico-reticulospinal pathways mediating the MEP (Fisher et al., 2012). This is suggested by the data from some of the participants with CP where the sum of the evoked responses, in both the SCM and biceps, when TMS and trigeminal afferent stimulation were given alone, were less than the response produced when the two stimuli were paired together, especially at the 50 ms ISI (Fig. 2-7 & Fig. 2-8). The facilitation of MEPs in multiple muscles could potentially be produced from poly-synaptic, slowly conducting RST activating multiple cervical spinal cord segments (C1 to C6), i.e., the segments innervating both the SCM and biceps muscles. For example, the spino-reticulo-spinal pathway in cats is relayed through the pontine reticular formation producing long-latency responses along multiple spinal cord segments at 15 ms to 120 ms (Shimamura and Livingston, 1963, Gokin et al., 1977, Shimamura and Kogure, 1979). This contrasts with the short-latency TCR that routes only through the spinal trigeminal nucleus where reflexes are confined to more proximal muscles such as the trapezius, splenius and SCM (Di Lazzaro et al., 1995). The facilitation of the biceps MEP in the three participants with CP was likely not mediated solely by a hyper-excitable TSR to the biceps (see Fig. 2-9) but required the dual activation of trigeminal afferents and cortical inputs. Stimulation of trigeminal afferents can also interfere with motor output to hand muscles. In uninjured controls, stimulation of the supraorbital branch of the trigeminal nerve 30 –

60 ms before cortical stimulation induces bilateral inhibition of MEPs of the first dorsal interosseous muscle. Effects from trigeminal stimulation are thought to take place at a subcortical level, as inhibition was produced in MEPs evoked from cortical activation of descending motor pathways with TMS and from subcortical activation of axons with transcranial electrical stimulation (TES; Siebner et al., 1999).

Another piece of evidence supporting facilitation of cortico-reticulospinal pathways by trigeminal afferents comes from the trigeminal afferent conditioning data in CP-10 (Fig. 2-7). Here, TMS to the ipsilateral motor cortex did not produce an evoked response and thus no cortico-motoneuronal or cortico-reticulospinal pathways were activated strongly enough to recruit SCM motoneurons. However, when the TMS was conditioned by a prior trigeminal afferent stimulation, a long-latency (≈ 35 ms), long-lasting response in the ipsilateral SCM was produced without a short-latency MEP. This suggests that the trigeminal afferents facilitated a longer-latency cortico-reticulospinal pathway to the SCM and not the more direct cortico-motoneuronal pathway, of which the former may become more excitable in CP.

Functional considerations

Impairments in the control of head and neck functions such as chewing, swallowing, control of oral secretions and posture may arise from impaired activation of brainstem pathways in CP. Increased excitability of the TCR and the facilitation of MEPs to neck flexor muscles by trigeminal afferent stimulation may be indicative of the alteration in brainstem circuits that are involved in these functions. Although these altered long-latency reflexes may represent an epi-phenomenon to the disorder, it is

interesting that excessive neck flexion occurs in children with spastic CP during squatting (Dan et al., 2000), potentially as a result of abnormal recruitment of brainstem pathways that are shared with the TCR.

The increased excitability of these brainstem reflexes may also be an indicator of increased excitability in cortico-reticulospinal pathways in response to damage of the CST in CP. In animal models of CST injury, gross motor recovery was associated with increases in the activation of the RST, especially to flexor muscles (Zaaimi et al., 2012). Evidence for a reduced CST, but increased RST activation of limb muscles in CP, comes from a reduction in intramuscular coherence in frequency bands attributed to common drive from cortical (monosynaptic) inputs and a broader cumulant density peak, suggestive of polysynaptic (e.g., RST) pathways (Petersen et al., 2013). Although not as accurate or well controlled, increased activation of cortico-reticulospinal pathways in CP may provide some enhancement of motor function, including that for the head and neck.

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Chapter 3. Descending Motor Pathways in Children with Perinatal Stroke

Introduction

Perinatal ischemic stroke occurs following a blockage of blood vessels within the brain, which can cause damage to structures involved in motor control (Govaert, 2009, Kirton, 2013). Perinatal stroke is the most common cause of hemiplegic cerebral palsy (CP; Shevell et al., 2009). Individuals with hemiplegic CP experience problems with motor control mainly contralateral to the injured cortex where most severe consequences are often present in the upper extremity. Individuals also experience lower limb weakness (Elder et al., 2003, Hussain et al., 2014) and co-contraction of antagonist muscles in the legs (Tedroff et al., 2008, Gross et al., 2015). Gait abnormalities including drop foot in the swing phase, tight heel cord in the stance phase, and restricted motion of the knee, hip and ankle can be experienced by individuals with hemiplegic CP (Winters et al., 1987), making mobility an important target for rehabilitation.

The corticospinal tract (CST), a major descending pathway from the sensorimotor cortex to spinal motor neurons, is important for the control of lower extremity muscles during walking – most specifically shown in the modulation of ankle dorsiflexor muscle activity during every step (Petersen et al., 2012). The CST undergoes significant development in the prenatal and postnatal periods. As studied in the upper limb, CST axons that initially project bilaterally are refined whereby uncrossed connections (ipsilateral) are pruned and crossed connections (contralateral) are reinforced by 2 years of age (Eyre et al., 2001, Staudt, 2010). In adults, 8-15% of uncrossed ipsilateral CST axons remain, leaving the spinal cord innervated mainly by the crossed, contralateral CST

(Eyre et al., 2001, Clowry, 2007, Staudt, 2010). The CST and sensorimotor cortex are common sites of damage in the event of prenatal and perinatal brain injury (Khwaja and Volpe, 2007). Interference of typical brain development can result in atypical development of the CST. For example, in unilateral motor cortex damage, contralateral CST connections from the damaged cortex develop weakly, while ipsilateral connections from the undamaged motor cortex to the paretic hand persist. (Farmer et al., 1991, Carr et al., 1993, Eyre, 2007). Persistent ipsilateral projections from the undamaged hemisphere and reduced contralateral projections from the damaged hemisphere to the hand are correlated with poor hand function, especially in individuals with hemiplegic CP (Carr et al., 1993, Holmstrom et al., 2010). Most of the knowledge regarding CST and aberrant development after stroke is only known for the upper limb. Similar development of the CST from the motor cortex to leg motor neurons likely exists; however, the organization and response to brain damage is unknown.

Activity-based therapies that promote self-initiated movements have been beneficial in the functional ability of children with CP. Constraint-induced movement therapy (CIMT) is most widely applied to the upper limb where restraint of the uninjured limb causes forced use of the most impaired limb (Taub et al., 1999) . CIMT has shown improvement in the use of the more affected hand (Taub et al., 2004, Eliasson et al., 2005) and development of new motor behaviours after as little as 3 weeks of therapy (Taub et al., 2004). An alternative approach of bimanual therapy has demonstrated improved dexterity and coordination of both hands (Gordon et al., 2007). Fewer activity-based interventions have been applied in the lower limb. Muscle strengthening in various leg muscle groups has produced increased muscle strength, step length, improved gross

motor function (Damiano et al., 1995, Damiano and Abel, 1998), and increased muscle volume (McNee et al., 2009). Intensive treadmill walking in children with CP resulted in improved ankle dorsiflexion (Phillips et al., 2007) and gross motor function (Mattern-Baxter et al., 2013). Similarly, a body weight-supported system to allow for unconstrained movements improved gross motor function and increased rates of motor development (Prosser et al., 2012).

In addition to influencing functional outcome, activity-based therapies also impact underlying motor pathway development. In cats who received inactivation of the primary motor region, CIMT combined with active training restored previously impaired CST connections to ventral regions of the spinal cord, restored the motor cortical map and increased the number of spinal cholinergic interneurons (Friel et al., 2012). Children and young adults trained with CIMT in the hand showed an increase in motor cortex excitability of contralateral responses from the injured cortex (similar to typical motor pathway organization) and a decrease in excitability of ipsilateral responses from the uninjured cortex (motor pathway organization associated with poor motor function) as shown with TMS and fMRI (Juenger et al., 2013). In children who received bimanual training, an increase in cortical map size (Bleyenheuft et al., 2015, Friel et al., 2016), and amplitude of MEPs evoked from the affected cortex (Friel et al., 2016) was seen. This evidence suggests that underlying motor systems benefit positively from activity-based therapies and help to improve movement control.

The influence of rehabilitation on establishing a functional motor system in CP can largely depend on the age at which it is applied. During specific periods of infancy, development of the CST is largely shaped by motor activity (Basu, 2014, Herskind et al.,

2015). Thus, the greatest potential effects of an intervention occur when it is applied at an optimal age. In a cat model of CP, the most functionally effective motor recovery occurred following rehabilitation administered during an ideal critical period. Inactivation of the primary motor region during postnatal weeks 3-7 resulted in drastic motor system damage, leaving kittens unable to reach or grasp food (Martin et al., 2011, Friel et al., 2012). Motor training administered between postnatal weeks 8 to 10 restored limb control and skilled movement, while training administered after postnatal week 10 resulted in less effective functional recovery. Early rehabilitation repaired the distribution of CST terminations in the spinal cord, restored the motor cortical map and increased the number of spinal cholinergic interneurons (Friel et al., 2012) – a combination of underlying systems that would make large functional improvements possible. Thus, rehabilitation can contribute to establishing a functional motor system, especially when applied during a critical period.

As studies of early rehabilitation applied in kittens with inactivation of the primary motor region have shown benefits to the developing functional motor system (Martin et al., 2011, Friel et al., 2012), it is likely that infants with CP also have the potential to benefit greatly from early rehabilitation. Children in the present study (ongoing) have had unilateral perinatal ischemic stroke, confirmed by MRI. Children receive intensive physiotherapy, which encourages self-initiated movements with emphasis on activity of the most functionally impaired leg as described in Hurd et al., 2017. It is possible that a similar critical period where interventions would be most functionally advantageous, as outlined in cats (Friel et al., 2012), also exists in humans; however, the time window of this period and the degree to which underlying motor

systems is influenced is not yet understood. Children in the present study received physiotherapy administered at different ages (ranging from starting at 9 to 38 months old). Here, we investigate the descending motor pathways from the motor cortex to ipsilateral and contralateral leg muscles. We hypothesize that descending motor pathways will become more developed with an increase in age – thus, we predict a greater prevalence of muscle responses to activation of descending pathways that establish shorter onset latencies as age increases. Given the restoration of typical CST organization (i.e. crossed contralateral projections from both cortices) in cats with inactivation of the primary motor region who received intensive physiotherapy (Martin et al., 2011, Friel et al., 2012), we hypothesize that intensive physiotherapy will promote increased prevalence of contralateral MEPs evoked from the affected cortex following the training period. Moreover, given the reduction in aberrant ipsilateral motor pathways to hand muscles in children with hemiplegic CP who received CIMT (Juenger et al., 2013), we also hypothesize a decrease in the heightened prevalence of ipsilateral pathways primarily from the unaffected leg motor cortex in response to training.

Methods

Participants

This study was approved by the Heath Research Ethics Board at the University of Alberta (Pro00072587). Written informed consent was obtained from a parent/guardian of all participants. Thirty children have enrolled in the study as of December 2016; three children have withdrawn from the study (E004 was excluded because of behavioral impairments, no data was collected; C001 moved out of the province after the post delay

time period, the delay data was not used because of inconsistent documentation; C006 withdrew due to family issues after the post delay time period, the pre and post delay data is included). Thus, our sample includes 16 children from Edmonton and 12 children from Calgary (see Table 3-1 for participant details). In addition, 5 pilot children were enrolled at the beginning of the study to test parameters for TMS and outcome measurements; their data is not included here.

Participant ID	Type of stroke	Affected cortex	Age (months)	Study group
E001	MCA	L	18	Delay
E002	MCA	L	35	Delay
E003	MCA	R	9	Immediate
E005	MCA	L	23	Delay
E006	MCA	L	10	Delay
E007	PVI	L	10	Immediate
E008	MCA	L	13	Immediate
E009	MCA	L	14	Immediate
E010	MCA	R	13	Delay
E011	MCA	L	10	Immediate
DE002	PVI	L	26	Immediate (parent train)
DE003	PVI, arterial damage	R	28	Immediate (parent train)
DE004	MCA	R	11	Immediate (parent train)
DE005	MCA, APPIS	R	11	Immediate (parent train)
DE006	PVI	R	27	Immediate (parent train)
DE007	MCA	L	14	Immediate (parent train)
C002	MCA	L	13	Immediate
C003	MCA	R	17	Delay
C004	PVI	L	17	Immediate
C005	PVI	L	12	Immediate
C006	PVI	L	31	Delay
C007	Arterial ischemic, non specific distribution	R	29	Delay
C008	PVI	L	15	Delay
C009	MCA	R	26	Immediate
C010	PVI	R	11	Immediate
C011	PVI	R	17	Delay
C012	PVI	L	17	Immediate
DC001	PVI	R	22	Immediate (parent train)

Table 3-1. Characteristics of participants. Columns list the participant's study ID, type of stroke, most affected cortex, age (at the time of enrollment in the study, in months), and the study group to which children were assigned. Participants that received training at home from their parents are identified as "parent train". DE001 was reclassified as E001 after moving to Edmonton. E, Edmonton; DE, distance Edmonton; C, Calgary; DC, distance Calgary; L, left; R, right; MCA, middle cerebral artery; PVI, periventricular venous Infarction; APPIS, arterial presumed perinatal ischemic stroke.

Inclusion criteria for participants included MRI-confirmed diagnosis of unilateral perinatal injury (categorized as neonatal arterial ischemic stroke [Fig. 3-1A&B], arterial presumed perinatal ischemic stroke or periventricular venous infarction [Fig. 3-1C&D]), born ≥32 weeks gestational age, and are within 8 months and 3 years old at time of enrollment. Exclusion criteria included botulinum toxin injections in the lower legs in the 6 months previous to participation, contraindications to TMS (i.e. regular seizures), or other extensive brain, musculoskeletal, cognitive, or behavioral impairments.

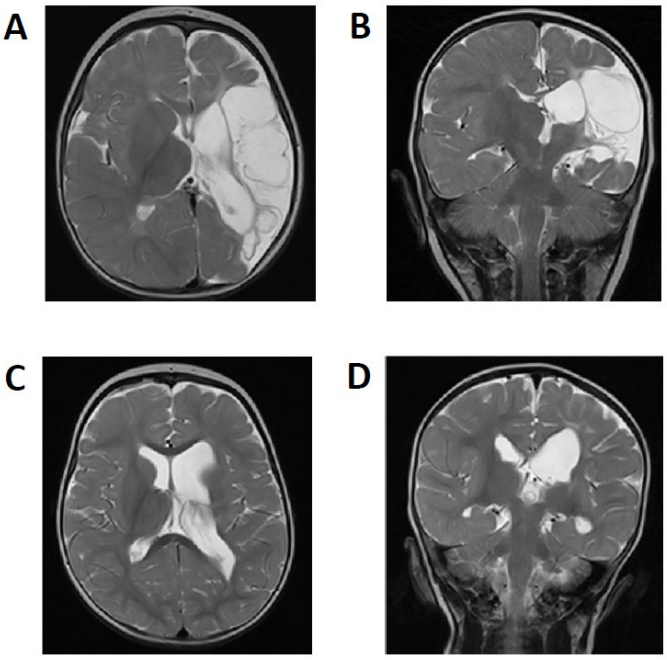

Figure 3-1. MRIs of perinatal stroke. Axial (A) and coronal (B) slices of a T2 weighted MRI of a child with arterial ischemic stroke. Axial (C) and coronal (D) slices of a T2 weighted MRI of a child with periventricular venous infarction.

Study Design and Intervention

The present study is being carried out in Edmonton and Calgary, AB. Children in the single-blind randomized controlled trial (Fig. 3-2) are randomly allocated to start training either immediately or after a delay period. The delay group serves as controls who do not receive training. For children with families who do not live within commuting distance, participation happens at home with training provided by the

parent/guardian while receiving guidance from physiotherapists. Following training, all children have a follow-up period in which there is no training. Each period (delay, train and follow up) is 3 months in duration. Neurophysiological measures are taken at the beginning and end of each study period and again at 4 years of age (arrows in Fig. 3-2). Data from some children at different study time points are not included: 2 children did not have post-delay/ pre-train data because of inconsistent documentation or technical problems during data collection; 10 children did not have follow-up data because experimental sessions were initially not scheduled, or the child was uncooperative during data collection; 17 children do not have 4 year follow-up data because the child has not reached 4 years of age as of July 2017, or the child was uncooperative during data collection, or the child withdrew from the study.

Therapy was administered by physiotherapists (or by parents at home while receiving guidance from physiotherapists) for 1 hour/day, 4 days/week, for 3 months. Intensive therapy encouraged self-initiated movements with emphasis on activity of the affected leg. Activities included walking (on even and uneven ground), stepping over obstacles, ascending and descending stairs and ramps, standing and balancing, and squatting. To increase intensity for children with greater endurance, weights were placed on top of the foot (~20g) and around the ankle (≥110g) of the affected limb.

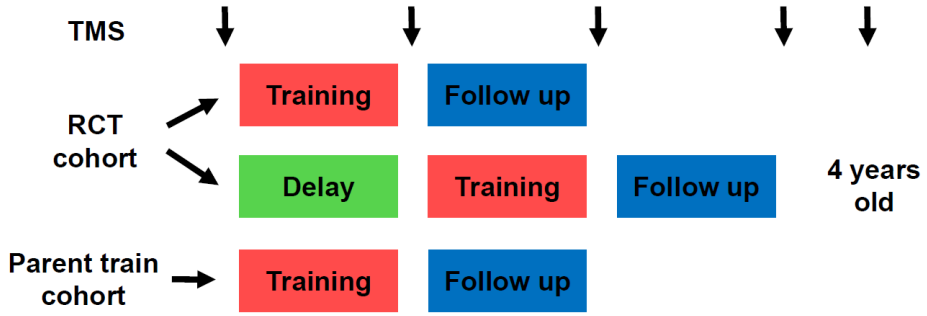

Figure 3-2. Experimental design. Children in the randomized controlled trial (RCT) are randomly assigned to start training either immediately (pink boxes) or after a delay period (green box). The delay group participants serve as controls who do not receive training. The RCT cohort receives training from a physiotherapist at the UofA, while the out of town cohort receive training from parents at home. Following training, all children have a follow-up period (blue box) during which time there is no training. Each period is 3 months in duration. Neurophysiological measurements using transcranial magnetic stimulation are taken at the beginning and end of each study period and at 4 years of age.

Transcranial Magnetic Stimulation (TMS)

Motor pathway excitability was measured at the beginning and end of each study period and again at 4 years of age. TMS was applied over the leg representation of each motor cortex while electromyography (EMG) was recorded from 4 major leg muscle groups which included proximal, distal, anterior and posterior locations of the leg. As motor-evoked potentials (MEPs) are more easily evoked with a background contraction, children were placed in a standing position and tilted backwards by a therapist to evoke muscle activity.

Pairs of conductive adhesive hydrogel electrodes (Covidien Ltd., Dublin, Ireland, 3.81 x 2.24 cm) were placed over the muscle belly of tibialis anterior (TA), gastrocnemius-soleus (Sol), quadriceps (Quad), and hamstrings (Ham) on both legs, with a spacing of 1cm. Surface EMG signals were amplified (1k gain), digitized and sampled

at 5kHz using Axon Instruments hardware (Digidata 1440A) and software (Axoscope 10.3, Molecular Devices LLC, Sunnyvale, USA). TMS was applied to each leg motor cortex, separately, using two MagStim 200 stimulators (The Magstim Company Ltd, Carmarthenshire, UK) connected by a Bistim module and a 110 mm double-cone coil. For one child (E011) with small head dimensions (26 cm ear to ear; 27cm nose to inion), a less powerful custom batwing coil (P/N 15857: 90 mm wing diameter) was used to minimize discomfort. Stimulation was typically applied 1 to 2 cm lateral from vertex. Double-pulse stimulation was applied with an interstimulus interval (ISI) of 10ms, and each stimulus at an intensity of 80% maximum stimulator output (MSO). Single and double pulse (with ISIs from 5ms to 20ms) protocols were performed on 5 pilot participants to determine the reliability of resulting MEPs. Since double-pulse stimulation at an ISI of 10ms produced the most robust MEP, this parameter was used in the rest of the TMS sessions. Stimulation was applied for up to 5 stimuli per cortex; however, testing stopped if the child objected to receiving the TMS. TMS was well tolerated in the majority of participants.

Functional Measures

Multiple functional outcome measures were collected throughout the study period (Hurd et al., 2017); only the Gross Motor Function Measure (GMFM-66) and toe clearance during treadmill walking, before and after the delay and training periods, are reported here. GMFM-66 is an observational measure which scores 66 gross motor tasks to measure gross motor development (Russell et al., 2010). GMFM-66 was measured by pediatric physiotherapists who were blinded to the child's group assignment. Two baseline GMFM-66 assessments were averaged together to give a single baseline

measure. Children were videotaped as they walked (with support if unable to walk independently) on a treadmill. Toe clearance was measured as the maximum vertical distance between the toe and the ground when the foot travels forward during the swing phase of walking. Further explanation of the quantification of these measures is included below.

Data Processing

MEPs: To determine the presence of an MEP, EMG trials in which a possible MEP was present were first identified. MEPs typically occurred in multiple muscle groups at similar onset latencies. In these trials, a time window of differing size was selected to contain the MEP. An MEP was considered present if the mean of the rectified EMG within the selected window was at least 30% larger than the mean of the rectified background EMG, the latter calculated for a 50 ms window just prior to the TMS stimulus. In cases where the mean MEP EMG was not 30% larger than the background EMG, an MEP was still considered to occur if a repeatable waveform was visible within the MEP window for 2 trials or more. The onset latency of the MEP was visually measured from the raw, unrectified EMG traces.

MEP Prevalence per Experiment: To determine how readily MEP responses could be evoked in our sample of children, we first determined, in each leg, how many muscles displayed an MEP response out of the total number of muscles that were recorded in a given experimental session: MEP Prevalence/Experiment = number of muscles with an MEP/number of muscles that were recorded from (x 100%). Four separate recording configurations were used where TMS was applied: 1) ipsilateral to the

unaffected cortex; 2) contralateral to the unaffected cortex; 3) ipsilateral to the affected cortex; and 4) contralateral to the affected cortex. Typically recordings were taken from 4 muscles (TA, Sol, Quad, Ham); thus, values would normally range from 0, 25, 50, 75, or 100% to indicate that 0, 1, 2, 3 or 4 muscles, respectively, had an MEP response.

Change score of MEP Prevalence/Experiment over study period: To determine if the prevalence of MEPs changed after each study period (i.e., delay, train or follow up), the MEP Prevalence/Experiment before and after each study period was compared in each child (change score = MEP Prevalence/Experiment after study period – MEP Prevalence/ Experiment before study period). Data from the immediate- and delay-trained groups are displayed separately along with data from the 4 different recording configurations. Data from physiotherapist- and parent-trained children showed no difference in change of MEP prevalence over any study period; thus, data are grouped together in the immediate-train and immediate-follow up groups.

MEP Prevalence across muscles: To determine if the prevalence of MEPs was distributed differently across muscles, in each child, the number of experiments in which each muscle showed an MEP was divided by the number of experiments from which that muscle was recorded (x 100%). For example, in a given recording condition, if we recorded from the TA muscle in 3 experiments (e.g., before training, after training and at 3 months follow up) but only measured a TA MEP in 2 experiments (e.g., after training and at the 3 month follow up), a MEP Prevalence/muscle score of 66.7% was given (2/3 x 100%). Data from the 4 different recording configurations were plotted separately.

MEP Prevalence across age: To determine if the prevalence of MEPs changed with age, the MEP Prevalence/ Experiment (see above) was calculated for each

experimental session and plotted against the child's age. The data were then grouped into five 8-month bins: 9-17, 18-26, 27-35, 36-44 and 45-53 months (Fig. 3-9A&B). As such, the same child was plotted in multiple age bins – once for each experimental session. Data from the 4 different recording configurations were plotted separately.

Functional Measures: A Matlab script was used to quantify toe clearance as the maximum vertical distance between a toe marker and the ground during the swing phase of walking. The degree of symmetry of toe clearance between both feet was quantified as [(affected leg score – unaffected leg score) / (affected leg score + unaffected leg score)]. The change of toe clearance symmetry and GMFM-66 scores after the delay and training periods was calculated as (after score – before score).

Association between changes in MEP prevalence and functional scores: The change in MEP Prevalence/Experiment after the delay and training study periods was plotted against the corresponding change in GMFM-66 total score (Fig. 3-7) and toe clearance scores (data not shown) in each child. Because there were no significant differences between the MEP prevalence of ipsilateral and contralateral MEPs evoked from either the unaffected or affected motor cortex after the delay or training study periods (using Mann Whitney Rank Sum tests, all $p > 0.2$), ipsilateral and contralateral MEP data were averaged together. Correlations between the change in MEP Prevalence and the change in functional scores (GMFM-66 and toe clearance) were calculated with Pearson's correlation coefficients (r) for the delay and training periods. Data was plotted separately for the delay- and immediate-trained children.

Statistics

All statistical analysis was performed using SigmaPlot (version 11.0). To determine if data from the delay- and immediate-trained children needed to be considered separately, Mann-Whitney Rank Sum tests were used to compare the ages between the delay- and immediate-trained children at the pre train, post train, follow up, and 4 year study time points. To determine if there was a delay or training effect on MEP Prevalence/Experiment, the change scores for the delay, train and follow-up periods were plotted for each recording configuration in both the delay-trained and immediate-trained children. Given that the median change scores were 0% across all but one measurement (Fig. 3-6), in subsequent data analysis (ie., MEP Prevalence/ muscle, MEP Prevalence/ age and MEP latency), values from the different experiment periods were combined together.

MEP Prevalence across muscles and age: Because there were no significant differences in MEP Prevalence between ipsilateral and contralateral MEPs evoked from either the unaffected or affected motor cortex for a given muscle or age bin (Mann Whitney Rank Sum tests, all $p > 0.3$), ipsilateral and contralateral MEP data were averaged together. To compare whether MEPs from a given muscle (TA, Sol, Quad, Ham) were more readily activated from the affected versus unaffected cortex, separate Mann Whitney Rank Sum tests were used to compare MEP Prevalence between the unaffected and affected cortex (Fig. 3-8C&D) with Bonferroni correction to account for multiple comparisons [significance set to $p = 0.0125$ ($p = 0.05/4$)]. To determine whether there were differences in MEP Prevalence between the 5 age bins, a Kruskal-Wallis One Way ANOVA on Ranks were used. The difference in MEP Prevalence was also assessed

before and after approximately 2 years of age, at which time motor pathways typically undergo refinement and strengthening (Eyre et al., 2001, Staudt, 2010, Friel et al., 2013, Kirton, 2013). To do this, data for each cortex in children younger than 26 months (combining bins 9 - 26 months) was compared to data from children older than 26 months (combining bins 27- 53 months) with Mann Whitney Rank Sum tests (Fig. 3-9C&D)

Onset latency of MEPs across age: The onset latencies of MEPs were plotted against age and displayed separately for each recording configuration. Data were fit with a linear or exponential decay [f=a*exp(-b*x)] depending on the goodness of fit as determined from the coefficients of determination (r^2). To determine if onset latency of the MEP was correlated with age, separate Pearson's correlation coefficients (r) were calculated for each recording configuration.

Results

EMG analysis

MEPs were generally evoked more often following TMS applied to the unaffected cortex compared to the affected cortex. This can be observed in Fig. 3-3, where stimulation of the unaffected cortex in a 39-month-old child produced MEPs in all ipsilateral and contralateral leg muscles (Fig. 3-3A, see insets for recording configurations). The onset latency of both ipsilateral (Fig. 3-3A, black traces) and contralateral (Fig. 3-3A, blue traces) MEPs were shorter in more proximal muscles (Quad and Ham) compared to more distal muscles (TA and Sol), as indicated by the red arrows. A characteristic silent period can be seen following the MEPs, indicating a refractory

period in motor neurons and the activation of inhibitory circuitry in the motor cortex (Triggs et al., 1993). In contrast, stimulation of the affected cortex produced MEPs in a fewer number of muscles. As demonstrated for the same participant in Fig. 3-3A, MEPs are present in 1 out of 4 ipsilateral muscles (Fig. 3-3B, black traces) and 2 out of 4 contralateral muscles (Fig. 3-3B, blue traces).

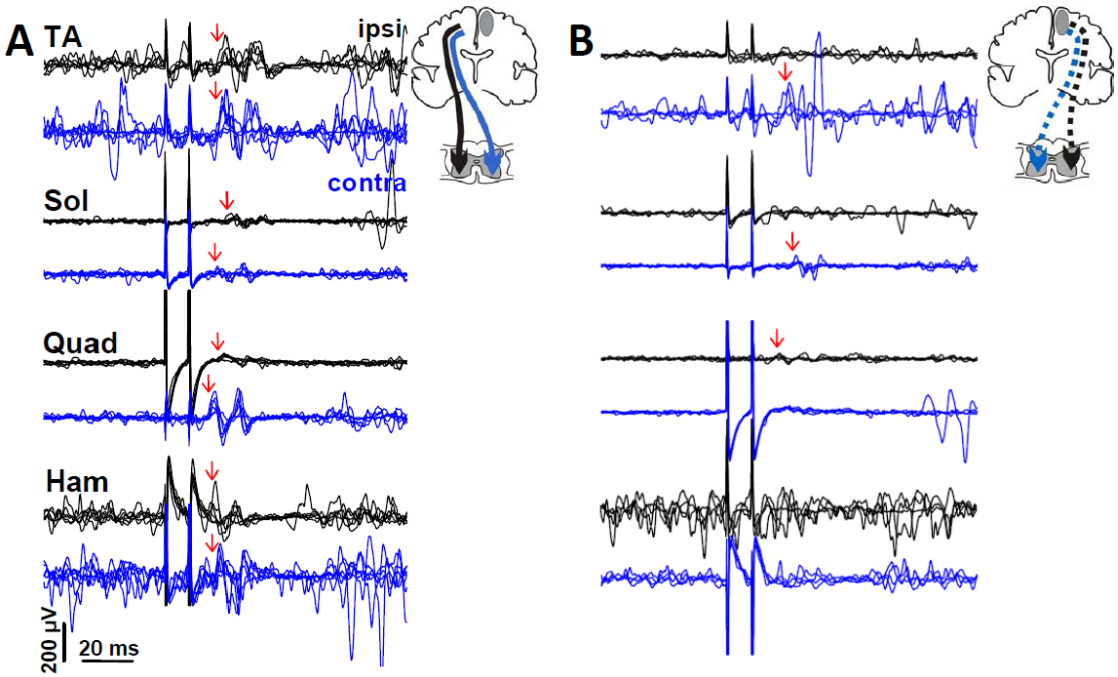

Figure 3-3. Overlaid EMG traces in a 39 month old child. EMG from 4 leg muscles (TA = tibialis anterior; Sol = soleus/ gastrocs; Quad = quadriceps; Ham = hamstrings) following TMS applied to the unaffected (A) or affected (B) motor cortex. The onsets of motor-evoked potentials (MEPs) are indicated with red arrows. The recording configuration is shown by the schematic inset.

Some children showed ipsilateral MEPs occurring independent of contralateral MEPs, representing the possibility to isolate activation of ipsilateral descending pathways from each cortex. Sixteen of the 28 children displayed ipsilateral MEPs only and no contralateral MEPs to a given muscle when the unaffected cortex was stimulated. Similarly, 14 children displayed only ipsilateral MEPs and no contralateral MEPs to the

same muscle when the affected cortex was stimulated. The pre-stimulus EMG was similar between ipsilateral and contralateral muscles while children were standing with weight support on both feet; thus, the level of muscle contraction was likely not solely responsible for this difference.

Age and Functional Progression of Participants throughout the Study

Since development is dependent on age, and because children are aging throughout the study, the age progression of children was plotted (Fig. 3-4). Children in the delay group had a higher median age than the children in the immediate group at the pre-train [(delay 20.5 (13 – 38) vs. immediate 13.5 (9 - 28 months)] and post-train [(delay 23 (16 – 41) vs. immediate 17 (12 - 31 months)] time points (both $p < 0.04$).

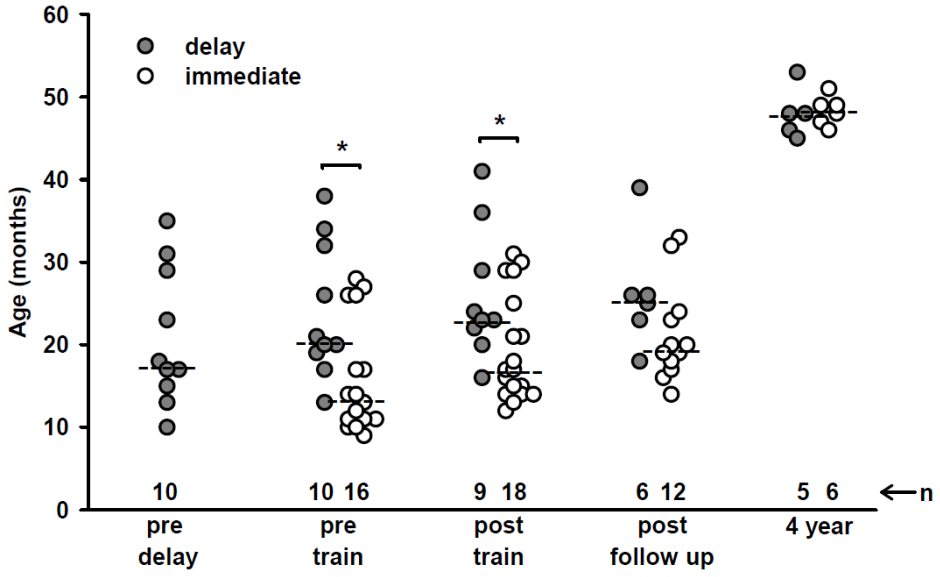

Figure 3-4. Age progression of participants. The age of children in the delay- (dark circles) and immediate- (white circles) trained groups at each study time point. Median age in each group at each time point is plotted as a horizontal line.

Progression in motor function was also apparent throughout the study. GMFM-66 scores plotted before and after each study period revealed an overall increase in gross motor function for the delay-trained (Fig. 3-5A) and immediate-trained (Fig. 3-5B) children. The greatest change in GMFM-66 scores in most delay- and immediate-trained children was present following the training period (steeper slopes as seen between pre-train and post-train time points).

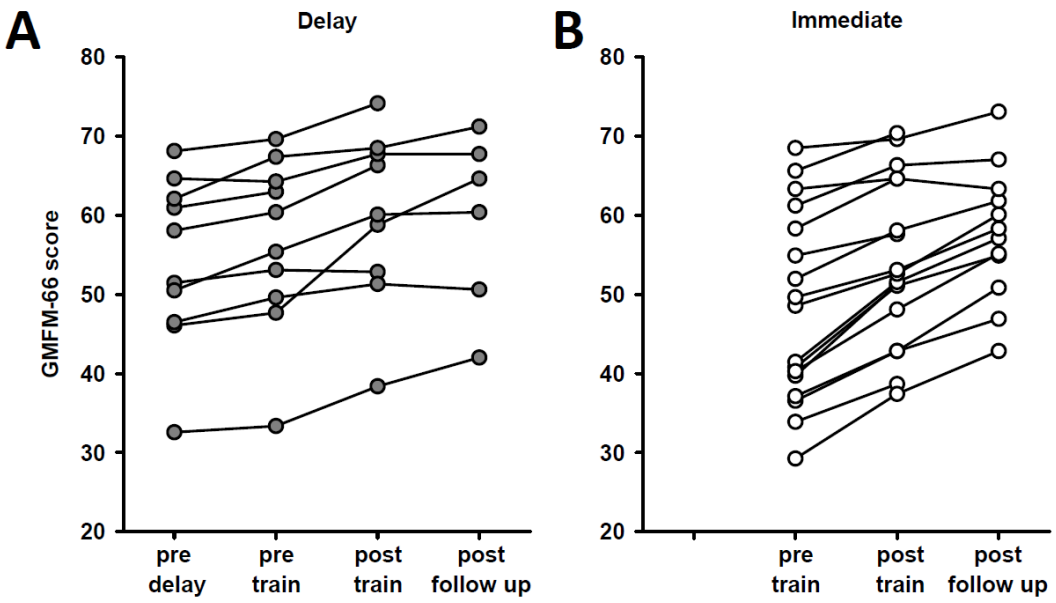

Figure 3-5. Gross motor function progression of participants. The GMFM-66 scores of delay- (A, dark circles) and immediate- (B, white circles) trained groups at each study time point. Individual children's scores are connected (solid lines).

Changes in MEP prevalence and the association with functional changes

To determine if the study periods influenced the prevalence of MEPs, the change in MEP prevalence (see Methods for calculation) was investigated before and after each period (delay, train and follow-up). Data from delay trained and immediate trained children are plotted separately given their differences in age. Overall, no trends in MEP

prevalence were apparent given that the median change values were 0% in most groups. For example, changes after the delay (Fig. 3-6A) and training (Fig. 3-6B) periods had a large variability with a median 0% change in ipsilateral and contralateral muscles from both unaffected and affected cortices (range from -50 – 75% after the delay period; range from -100 – 100% after the train period in immediate-trained children; range from -50 – 50% after the train period in delay-trained children).

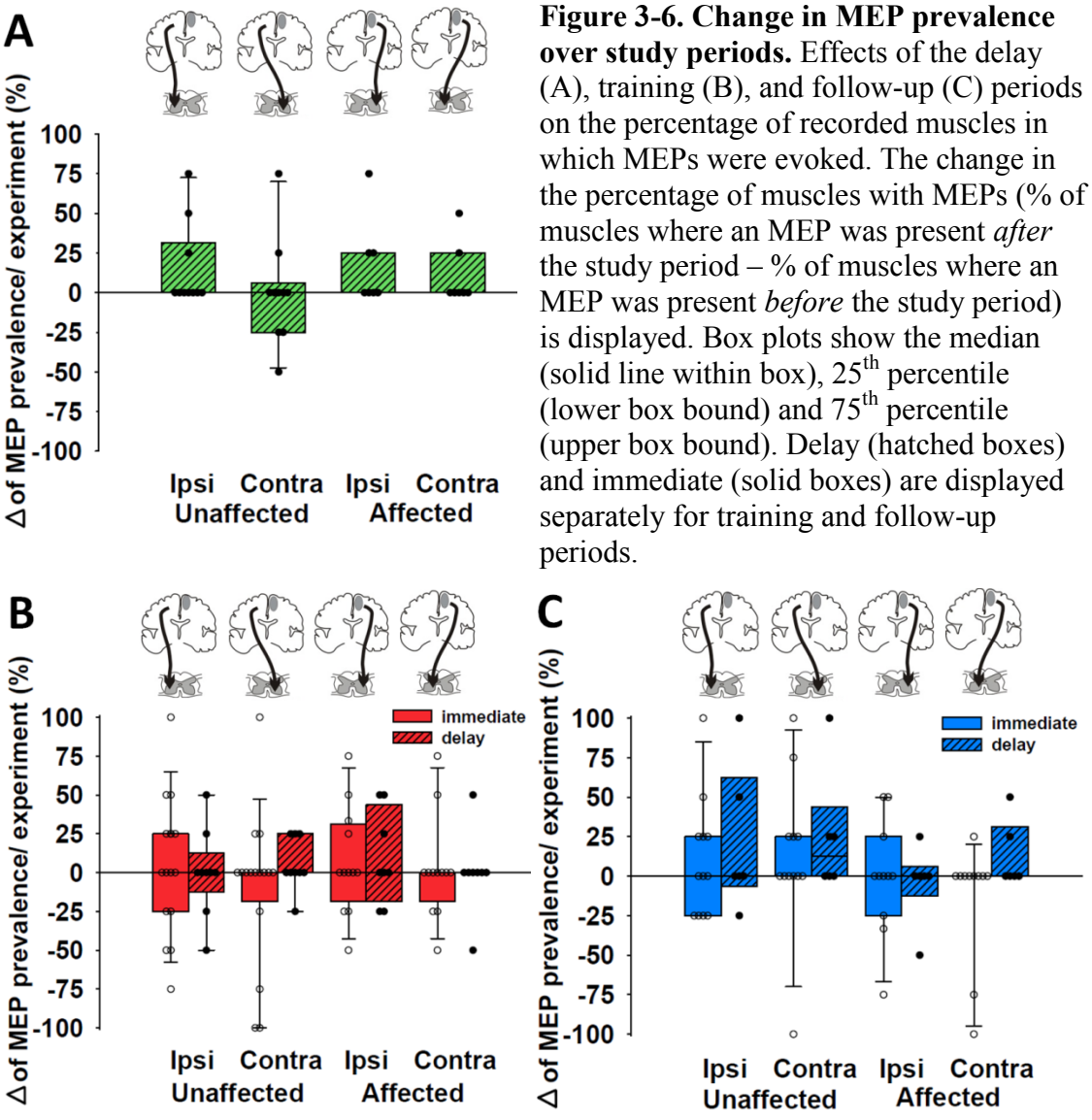

Figure 3-6. Change in MEP prevalence over study periods. Effects of the delay (A), training (B), and follow-up (C) periods on the percentage of recorded muscles in which MEPs were evoked. The change in the percentage of muscles with MEPs (% of muscles where an MEP was present *after* the study period – % of muscles where an MEP was present *before* the study period) is displayed. Box plots show the median (solid line within box), 25th percentile (lower box bound) and 75th percentile (upper box bound). Delay (hatched boxes) and immediate (solid boxes) are displayed separately for training and follow-up periods.

MEP prevalence changes after the follow-up period was typically 0% (Fig. 3-6C) and also showed large variability across children in both the delay- (range from -50 – 100%) and immediate-trained groups (range from -100 – 100%). Thus, no consistent trends were apparent throughout the study periods

To determine if some of the observed increases in MEP prevalence mainly occurred in children with larger improvements in motor function, we plotted MEP prevalence change scores against change scores in motor function. The change in MEP prevalence after the delay and training study periods were then correlated with the corresponding change in GMFM-66 scores (Fig. 3-7) and toe clearance symmetry scores (data not shown). Changes in MEP prevalence were not significantly correlated with changes in GMFM-66 after the delay or training periods in both the delay-trained children (Fig. 3-7, solid circles) and immediate-trained children (Fig. 3-7, open circles) (all $p > 0.07$ ranging from 0.07 – 0.8). Likewise, changes in MEP prevalence were not significantly correlated with changes in toe clearance after the delay or training periods (data not shown, all $p > 0.1$, ranging from 0.1 – 1.0).

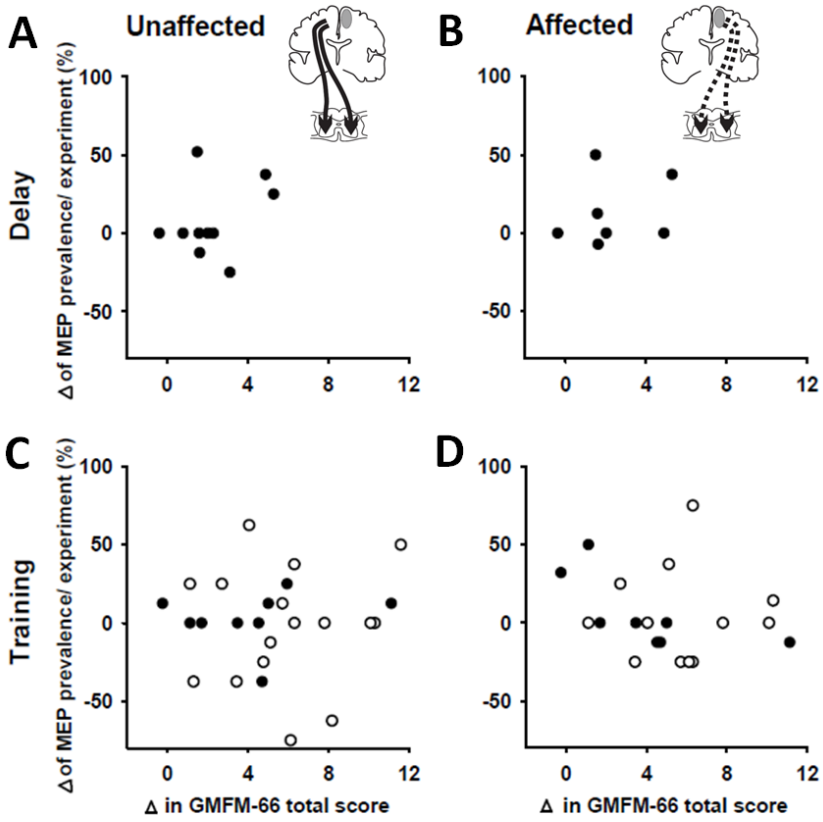

Figure 3-7. Correlation of MEP prevalence and gross motor function scores. Change in MEP prevalence for each child plotted against change in GMFM-66 score after the delay (A and B) and training (C and D) periods in the unaffected (A and C) and affected (B and D) cortices. Delay- (black symbols) and immediate- (white symbols) trained groups are displayed separately in the training plots.

Prevalence of MEPs across Muscles

Because changes in MEP prevalence across study periods did not show consistent trends in either the delay- or immediate-trained children (Fig. 3-6) or its relation to functional scores (Fig. 3-7), further characterization of MEPs was conducted by grouping data from across all study periods and from delay- and immediate-trained children. MEP prevalence across muscles and ages, and onset latency across ages was investigated.

MEP prevalence across muscles is presented in figure 3-8 where ipsilateral and contralateral data are presented separately (Fig. 3-8A&B). Ipsilateral and contralateral MEP data were averaged together for each muscle when comparing between muscles and between cortices (Fig. 3-8C&D). When comparing MEP prevalence between muscles, the median MEP Prevalence for muscles activated from the unaffected cortex were all greater than 0% (TA: 20.8%, Sol: 25%, Quad: 30%, Ham: 32.9%) in contrast to the affected cortex (TA: 0%, Sol: 0%, Quad: 0%, Ham: 14.6%). When comparing muscle groups between unaffected and affected cortices, the MEP prevalence of the quadriceps muscles from the unaffected cortex was higher than the affected cortex ($p = < 0.001$). In contrast, no differences in prevalence between cortices were seen in the tibialis anterior ($p = 0.21$), soleus ($p = 0.03$) and hamstring ($p = 0.07$) muscles.

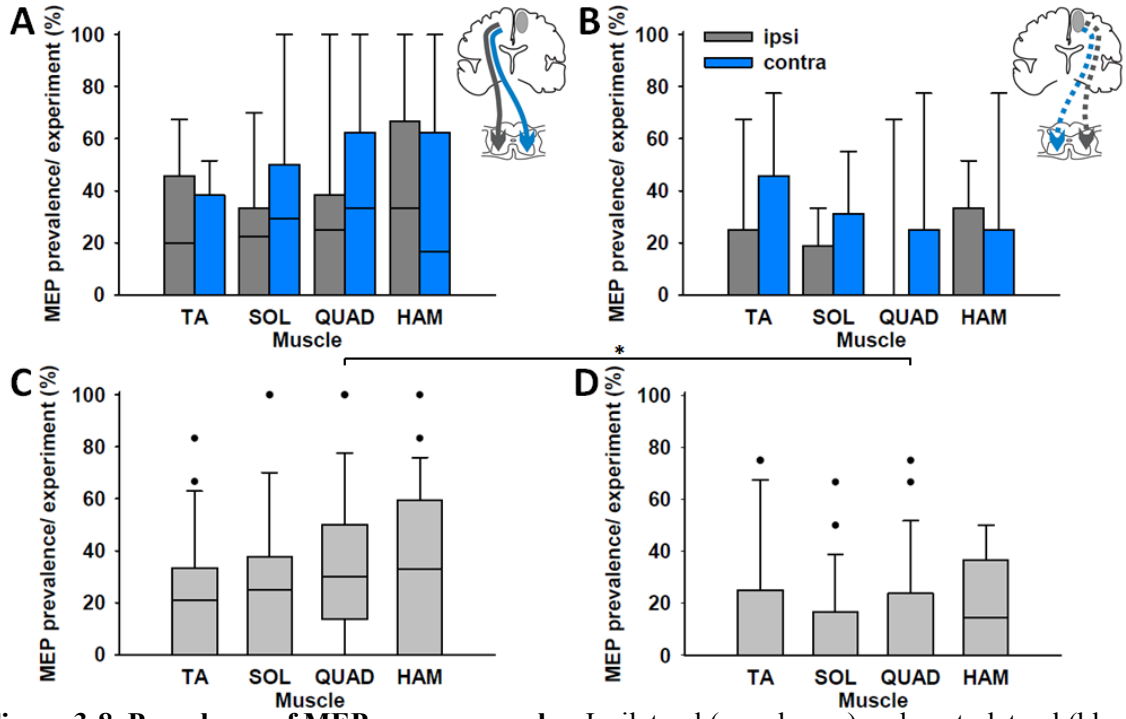

Figure 3-8. Prevalence of MEPs across muscles. Ipsilateral (grey boxes) and contralateral (blue boxes) MEPs in leg muscles following stimulation of the unaffected (A) and affected (B) cortex. The % that each child displayed an MEP in each separate muscle across experiments (number of experiments in which muscle showed MEP / total number of experiments from which muscle was recorded x 100) is displayed. Ipsilateral and contralateral MEP data were averaged together for each muscle for the unaffected (C) and affected (D) cortex. The box plot shows the median (solid line within the box), 25th percentile (lower box bound), 75th percentile (upper box bound), and 95th percentile (whiskers). n= 28 (A and B).

Prevalence of MEPs across Age

When comparing MEP prevalence between age bins (ipsilateral and contralateral MEP data averaged together for each age bin), there was no significant difference between bins for MEPs from the unaffected (Kruskal-Wallis One Way ANOVA: p = 0.14) or affected cortex (Kruskal-Wallis One Way ANOVA: p = 0.07, Fig. 3-9). However, when comparing MEP prevalence before and after roughly 2 years of age (combining MEP data within bins 9-26 months and bins 27-53 months) from the unaffected cortex (Fig. 3-9C), there was a higher prevalence of MEPs in the 27-53 month group (median 28.6%) compared to the 9-26 month group (median 12.5%) from the unaffected cortex (p = 0.02). In the affected cortex (Fig. 3-9D), there was no significant difference in MEP prevalence between the 27-53 month group (median 12.5%) than the 9-26 month group (median 0%, p = 0.21).

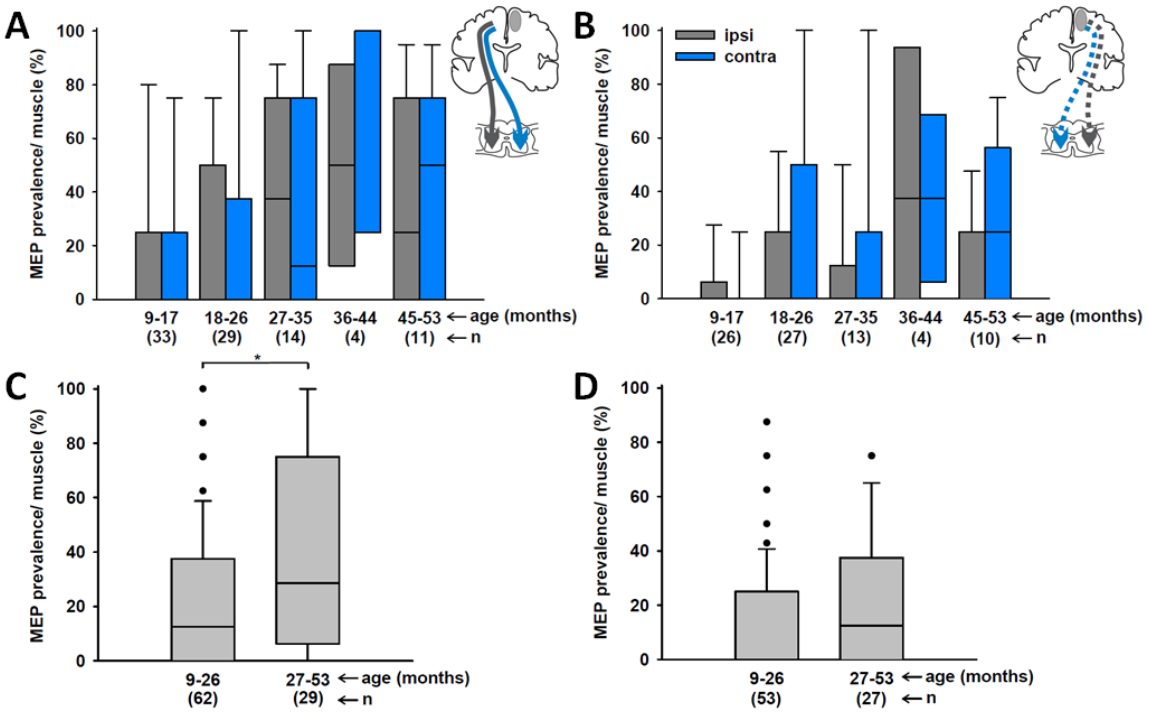

Figure 3-9. Prevalence of MEPs across age. Ipsilateral (grey boxes) and contralateral (blue boxes) MEPs in leg muscles following stimulation of the unaffected (A) and affected (B) cortex. The % of muscles in which MEPs were evoked in each experiment, grouped according to age into 8 month age bins is displayed. Data in children younger than 26 months (combining bins 9 - 26 months) was compared to data from children older than 26 months (combining bins 27- 53 months) for the unaffected (C) and affected (D) cortex. The box plot shows the median (solid line within the box), 25th percentile (lower box bound), 75th percentile (upper box bound), and 95th percentile (whiskers).

Onset latency of MEPs across age

When plotting MEP latency as a function of age, an exponential decay best modeled the data from MEPs ipsilateral and contralateral to the unaffected cortex and ipsilateral to the affected cortex (Fig. 3-10A&B&D) compared to a linear fit (see Table 3-2 for comparisons). In contrast, MEP onset latencies contralateral to the affected cortex could not be fit with either an exponential or linear equation (Fig. 3-10C). Likewise, a decrease in MEP onset latency was significantly correlated with an increase in age in

MEPs ipsilateral and contralateral to the unaffected cortex and ipsilateral to the affected cortex; however, MEP onset latency was not significantly correlated with age in MEPs contralateral to the affected cortex (see Table 3-2 for values).

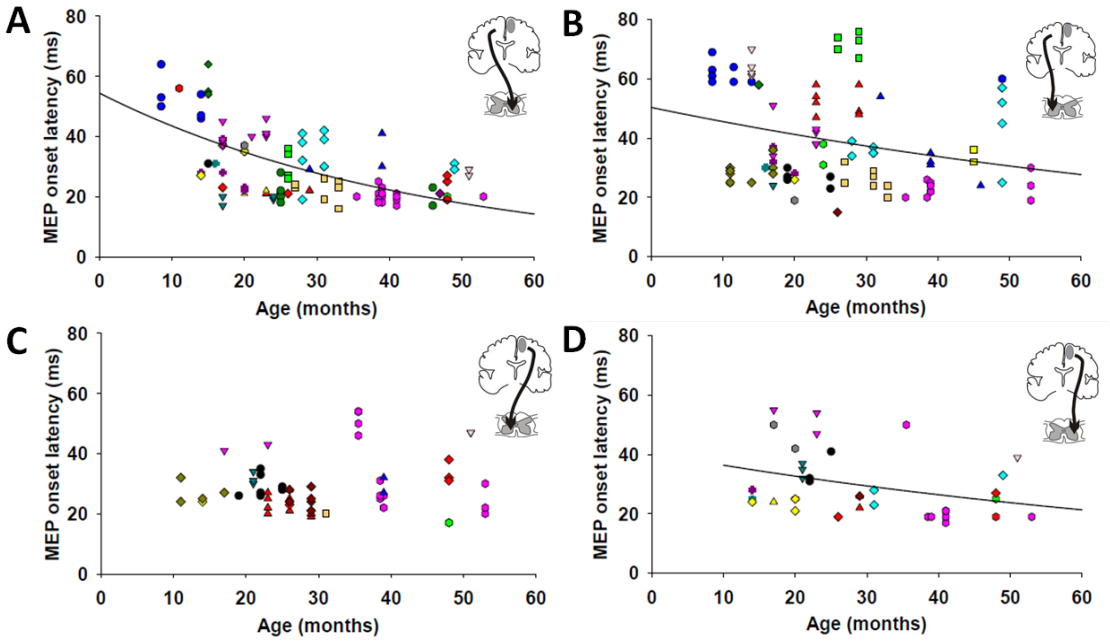

Figure 3-10. MEP onset latency across age. Onset latency of contralateral (A and C) and ipsilateral (B and D) MEPs evoked from the unaffected (A and B) and affected (C and D) cortex. Each child is represented with a different symbol. Latency values from all 4 leg muscles are plotted when present. n= 21 (A and B), 14 (C), 16 (D). Regression lines are exponential decay.

Exponential regression	$r^2 = 0.361$, $\mathbf{p = <0.001}$	$r^2 = 0.073$, $\mathbf{p = 0.010}$	$r^2 = 0$, $p = 1$	$r^2 = 0.115$, $\mathbf{p = 0.041}$
Linear regression	$r^2 = 0.313$, $\mathbf{p = <0.001}$	$r^2 = 0.069$, $\mathbf{p = 0.012}$	$r^2 = 0.018$, $p = 0.322$	$r^2 = 0.113$, $\mathbf{p = 0.042}$
Pearson's	$r = -0.559$, $\mathbf{p = <0.001}$	$r = -0.262$, $\mathbf{p = 0.012}$	$r = 0.133$, $p = 0.322$	$r = -0.337$, $\mathbf{p = 0.042}$

Table 3-2. Statistical relationships between MEP onset latency and age. Statistical values from exponential regression, linear regression and Pearson's correlations between MEP onset latency and age as plotted in Fig 3-10. Columns list the recording configurations as shown by the schematic. Bolded *p* values indicate significance of $p = <0.05$. r^2, coefficient of determination; *r*, correlation coefficient.

Discussion

General Summary

Although children in the present study showed functional improvements with GMFM scores and toe clearance symmetry, it remains unclear *if* and *how* intensive physiotherapy during infancy influences descending motor system development. There were no consistent changes in MEP prevalence or correlation between MEP prevalence and functional improvements following any study period in either the delay- or immediate-trained groups. Despite some limitations in TMS testing (described below), there was enough consistency in the data to show some trends, especially for data from the unaffected motor cortex: 1) more MEPs were evoked from the unaffected cortex compared to the affected cortex; 2) MEPs from the unaffected cortex were more prevalent in children older than 2 years compared to children younger than 2 years; and 3) the onset latency of ipsilateral and contralateral MEPs evoked from the unaffected cortex and ipsilateral MEPs evoked from the affected cortex exponentially decreased with an increase in age.

Prevalence of MEPs Did Not Change after Intensive Physiotherapy

There were no consistent trends in MEP prevalence following the training period in either the delay or immediate trained groups. There was also no correlation between MEP prevalence and functional improvements following the delay or training study periods. Lack of consistent changes following training could be because intensive physiotherapy did not strengthen similar descending motor pathways across children or, more likely, because of confounds with the experimental testing. Applying TMS to

children has a number of limitations and barriers. Firstly, the presence and amplitude of MEPs are dependent on the level of muscle contraction, as shown in uninjured adults where increasing strengths of voluntary muscle contraction in triceps surae muscles are correlated with increasing MEP amplitudes (Oya et al., 2008). Thus, because levels of muscle activation across participants were uncontrolled, comparing the prevalence and size of MEPs across our sample is problematic. Although children stood (with support if needed) evenly on both feet to activate relatively equal voluntary contraction across muscles in both legs, contraction levels may not have remained consistent within children, across experiments and across muscles. Future analysis of MEP amplitudes and background activation will give insight into this relationship. As prevalence of MEPs were most prominent in the quadriceps muscles following stimulation of the unaffected cortex, it may be beneficial to focus the testing and control of contraction levels in this single muscle, rather than across 4 groups of muscles.

Normally, MEP responses are identified by characteristics such as amplitude, latency, duration of response and silent periods following the response (Uozumi et al., 1991); however, muscle activation in the present study was uncontrolled, which is known to cause variability in the EMG noise and changes onset latency and amplitude of the response (Valls-Sole et al., 1994, Oya et al., 2008). As a result, objective criteria of MEP characteristics to select EMG trials with MEPs could not be used alone. Instead, we used subjective criteria (i.e. appearance of MEPs in multiple muscle groups with similar onset latencies) to identify trials in which a possible MEP was present and further objective criteria of MEP size and repeatable waveforms across multiple trials to confirm the presence of MEPs. This analysis process might introduce a bias, given that the researcher

was not blind to the participant or study period during analysis. Alternatively, analysis from multiple researchers being blinded to the participant and study period to which the EMG traces belong, may improve the validity of the identification of EMG trials with MEPs.

Another limitation with the TMS protocol was that precise motor cortex mapping to locate the leg motor cortex "hot spot" was not possible because of limited attention and stillness of children. Stimulation was applied in the same general location for all participants (typically 1-2 cm lateral from vertex, see Maegaki et al., 1999, Garvey and Mall, 2008) and at the same stimulation intensity (80 % MSO). A lateral shift away from the midline of the optimal TMS "hot spot" for upper and lower extremity muscles has been shown in young adults with unilateral and bilateral CP (Maegaki et al., 1999, Kesar et al., 2012). While the children in the present study have varying degrees of damage to the motor cortex, it is likely that the development of the spatial representation of lower extremity muscles is disrupted. Thus, the inability to locate the precise leg motor cortex "hot spot" – which may not be in the same location for all children – could account for the lack of MEPs evoked in some children; however, this could not account for the lack of training effect as the same stimulation location was used in all experiments. With regard to stimulation intensity, although TMS was not applied at an intensity based on the cortical excitability for each child (i.e. relative to motor threshold), applying supra-threshold stimulation overcame the differences in cortical excitability across participants, which likely activated any present descending motor pathways within the leg motor cortex region. Using double pulse stimulation with each pulse at a very high stimulation intensity of 80% MSO with a double cone coil allowed maximum activation of the leg

motor cortex; however, in events where no muscle responses were evoked, higher intensities were not explored in order to avoid producing uncomfortable facial activation. Overall, more experiments should be designed to address the challenges in motor cortex mapping and stimulation in children with CP.

Lastly, inferring the organization and development of underlying motor pathways based on TMS data alone is difficult, as the severity and location of injury are different for each participant. Future categorization of all MRIs, based on lesion severity and location, will give further insight into how underlying brain injury is related to functional abilities.

In summary, TMS applied to children with unilateral CP with the parameters outlined in the present study did not reveal changes in motor pathway development in response to training, either because motor therapy did not change descending pathway strength or that the TMS protocol was not sensitive enough to measure any changes that did occur. Thus, we are unable to conclude *if* and *how* motor pathways are influenced by intensive physiotherapy to facilitate functional improvements.

Similar prevalence of ipsilateral and contralateral MEPs across muscles

Despite limitations in TMS testing and the lack of consistent changes of MEP prevalence following any study period, there was enough consistency in the data to show some trends when data from all experiments were combined. More MEPs were evoked from the unaffected cortex compared to the affected cortex, which is likely due to less damage contralateral to the lesioned cortex. When comparing muscle groups between cortices, there were more MEPs in the quadriceps muscle group from the unaffected

cortex compared to the affected cortex. The standing position of the children during the experiment may require a higher background contraction in the quadriceps compared to the other muscle groups which showed no difference in prevalence between cortices. Interestingly, there was no difference between the prevalence of ipsilateral and contralateral MEPs across muscles within each cortex. Similarly, ipsilateral and contralateral MEPs to the same muscle have been reported in children with hemiplegic CP (Carr et al., 1993) and typically developing children (Muller et al., 1997); however, these results raise the question as to whether motor cortices were being activated in isolation to activate separate descending motor pathways. It is possible that current spread from the TMS could activate both leg motor cortices, as they are located on adjacent sides of the longitudinal fissure (Penfield and Boldrey, 1937). While some children showed ipsilateral MEPs occurring independent of contralateral MEPs, suggesting the possibility to isolate activation of ipsilateral pathways, further experiments should be designed to address the likelihood of current spread of TMS in the leg motor cortices of children.

The Influence of Age on MEP prevalence

Children older than 2 years of age showed an increased prevalence of MEPs evoked from the unaffected cortex compared to children younger than 2 years of age. A combination of developmental processes likely contributes to the results in support of our hypothesis of greater prevalence of MEPs as children age. Firstly, the CST undergoes vast myelination changes throughout development – a process that largely occurs between the 3rd trimester to 2 or 3 years of age (Brody et al., 1987, Kinney et al., 1988, ten Donkelaar et al., 2004). Myelin forms in a proximal to distal manner, descending

from the brain to the lumbar spinal cord (Stanfield, 1992, ten Donkelaar et al., 2004, Purger et al., 2016), where myelination of large diameter axons of the CST occurs first (Stanfield, 1992, Purger et al., 2016). Coinciding with the time period of myelination, there is elimination of less effective axons and growth of terminal branches of the most effective axons within the spinal grey matter (Eyre et al., 2001, Staudt, 2010). In kittens, clusters of presynaptic boutons containing synaptic vesicles, in which neurotransmitters are stored, are formed on CST terminals to create functional connections with spinal neurons (Li and Martin, 2002, Meng et al., 2004). When kittens reach 2 months of age (approximately 2 years in children), further development of the cortical motor map occurs where limb muscles begin to be represented in a topographic arrangement on the motor cortex (Bruce and Tatton, 1980). Physiological changes to the corticospinal system during development, including myelination and refinement of CST terminals by 2 years of age, followed by the development of cortical motor maps, allow for effective activation of spinal motor circuits from descending cortical pathways (Martin, 2005). Thus, the time course of development of a functionally effective CST coincides with the increased prevalence of MEPs seen from the undamaged cortex in children with CP who are ~ 2 years of age and older.

Within all age bins, there was no difference in prevalence between contralateral and ipsilateral MEPs where ipsilateral MEPs from both the unaffected and affected cortex continued to persist up to 4 years of age. Early in development, from 18 months to roughly 10 years, typically developing children have ipsilateral responses in the upper and lower limbs with longer onset latencies, higher thresholds, and smaller amplitudes than contralateral responses (Maegaki et al., 1999, Eyre et al., 2001). After 10 years of

age, ipsilateral MEPs are not detected in typically developed adults without voluntary muscle contraction (Muller et al., 1997, Ziemann et al., 1999). In contrast, children with hemiplegic CP have ipsilateral responses in the upper limb with onset latencies, thresholds and amplitudes similar to contralateral responses from 18 months into adulthood (Carr et al., 1993, Eyre et al., 2001). In individuals with bilateral CP aged 10 to 49 years, ipsilateral responses in lower limb muscles can be evoked easily following stimulation of the less damaged hemisphere where voluntary contraction is often not required (Maegaki et al., 1999). Persistence of ipsilateral pathways in individuals with interference to brain development is attributed to the resultant reorganization of the corticospinal system, whereby ipsilateral CST projections persist from the undamaged or the least damaged hemisphere to innervate areas that are no longer innervated by contralateral projections from the injured cortex (Farmer et al., 1991, Carr et al., 1993, Eyre, 2007). It is possible that the ipsilateral MEPs observed in leg muscles in our participants may continue to persist throughout their lifetimes.

The Influence of Age on MEP onset latency

Myelination of the CST and greater synaptic efficacy between CST axons and spinal motor neurons also facilitates fast conduction of action potentials down axons. Our sample showed progressively shorter MEP onset latencies with increases in age. Contralateral MEPs from the unaffected cortex showed the most consistent trend of exponential decay in MEP onset latency, typically with latencies of 60ms at 10 months of age to 20ms at 2 years of age and older. Changes in onset latency during infancy reflect developmental changes in the *central* and *peripheral* nervous systems. During the first two years of life, a rapid decline of central motor conduction times occurs where adult

values are reached by approximately 2 years (Eyre et al., 1991, ten Donkelaar et al., 2004) – after which time CST myelination is largely complete (Brody et al., 1987, Kinney et al., 1988, ten Donkelaar et al., 2004). Peripheral conduction times also decrease initially but after 5 years, progressively increase in proportion to limb length (Eyre et al., 1991). Thus, the average MEP latency of 23.6 ± 4.6 ms (across all muscles contralateral to the unaffected cortex) in our sample of 4 year olds will increase in proportion to leg length until adult latencies of approximately 30ms (Remaud et al., 2014) are reached. The physiological processes of central and peripheral nervous system development likely explain why a regression of exponential decay best modeled the MEP latency changes with increases in age; however, a model of exponential decay with a subsequent increase in MEP latency after 5 years to reach adult values would likely be more reflective of latency changes throughout a longer time period of development. Direct activation of the CST with transcranial electrical stimulation (TES) has evoked MEPs in the leg of children of 6 months with latencies < 40ms (F. Roy, personal communication). Thus, our longer latency MEPs (>40ms) are likely mediated by a polysynaptic pathway.

Ipsilateral MEPs from the unaffected and affected cortices showed similar exponential decay of MEP onset latencies, but with greater variability. Variable onset latencies suggest ipsilateral MEPs are mediated by multiple descending pathways. CS axons that directly innervate the spinal cord – either from pathways that descend ipsilaterally or pathways that initially descend contralaterally and re-cross the spinal cord (Muller et al., 1997, Rosenzweig et al., 2009, Yoshino-Saito et al., 2010) – may be organizations possible for fast-conducting MEPs. Oligosynaptic cortico–brainstem-

spinal pathways may mediate slow-conducting MEPs with projections from the cortex to brainstem nuclei which in turn innervate both sides of the spinal cord. In animals models of CST injury, gross motor recovery of the upper limb was associated with increased involvement of reticulospinal pathways from the brainstem to bilateral flexor muscles (Zaaimi et al., 2012). Changes in MEP onset latency in the children throughout their lifetime may give more insight into the organization of ipsilateral pathways.

Brainstem neurons can be activated at long latencies in response to the discharge sound of a TMS coil (Fisher et al., 2012). To determine if the sound of the TMS coil was evoking a startle response – a reflex pathway known to route through the brainstem to activate muscles throughout the body (Yeomans et al., 2002) – which could be responsible for long latency muscle responses, additional acoustic startle experiments were conducted as per Fisher et al., 2012. In 2 children in the present study, a styrofoam spacer (1cm width) was placed between the coil and the scalp to lift the TMS coil off the head (with a reduced ability to excite neural elements) but to allow the click sound of the TMS coil to be heard by participants. Long-latency MEPs were not present in both children tested suggesting that the long latency MEP were not evoked from a startle response but from activation of descending CS pathways.

Conclusion

In summary, axon myelination and increased synaptic efficacy of the CST during infancy likely contribute to increased prevalence of contralateral MEPs from the unaffected cortex, with deceased onset latencies as children with hemiplegic CP increased in age. Persistent aberrant ipsilateral MEPs across all leg muscles with variable onset latencies are similar to studies showing ipsilateral pathways innervating hand

muscles in children with hemiplegic CP (Farmer et al., 1991, Carr et al., 1993). Variable latencies of ipsilateral MEPs suggests ipsilateral pathways from both the affected and unaffected motor cortices are mediated by multiple descending pathways. Overall, more experimental methods/ technology should be designed to address the challenges in motor cortex mapping and stimulation in children with CP to investigate *if* and *how* intensive physiotherapy during infancy influences descending motor system development.

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Chapter 4. Discussion

Thesis summary

In this thesis, we have explored the organization of sensorimotor pathways in adults and children with cerebral palsy (CP). In Chapter 2, the function of brainstem circuitry and the interaction between brainstem and cortical motor pathways in adults with bilateral CP were examined. Increased excitability of the long-latency component of the trigeminal cervical reflex (TCR) in CP compared with controls, and facilitation of motor-evoked potentials (MEPs) following trigeminal nerve stimulation in CP, may indicate altered brainstem circuits that are involved in the control and head and neck functions. Additionally, some participants with CP had abnormally large and long-lasting responses produced in both neck and arm muscles when cortical and trigeminal stimuli were combined. This may indicate that cortico-reticulo-spinal pathways are more excitable in CP, which could provide some enhancement of motor function.

In Chapter 3, the development of the corticospinal tract (CST) in children with hemiplegic CP was examined. It remains unclear *if* and *how* intensive physiotherapy during infancy influences descending motor system development, as no consistent changes in MEP prevalence or correlation between MEP prevalence and functional improvements were seen following any study period. MEPs in leg muscles were more prevalent from the unaffected cortex in children older than 2 years of age, likely as a result of underlying refinement of the CST, including myelination and activity-dependent pruning processes (ten Donkelaar et al., 2004, Martin, 2005). Ipsilateral pathways from the undamaged hemisphere to leg muscles persisted throughout infancy in children with CP. In the upper limb, persistence of ipsilateral pathways is more pronounced in children

and adults with CP compared to controls (Muller et al., 1997). This is attributed to the resultant reorganization of the corticospinal system, whereby the ipsilateral CST persist from the least damaged hemisphere to innervate areas that are no longer innervated by contralateral projections from the injured cortex (Eyre, 2007). Lastly, our sample showed progressively shorter MEP onset latencies with increases in age, suggesting greater myelination of the CST and synaptic efficacy between the CST and spinal motor neurons at 3 years and older (Eyre et al., 1991, ten Donkelaar et al., 2004).

In the following sections, I discuss potential pathways that mediate trigeminal reflexes (1a), potential sites of interaction between cortical and trigeminal afferent stimulation (1b) and the clinical significance of motor pathway development in children with CP (2). Future directions for both projects will also be suggested.

1a. Potential pathways that mediate trigeminal reflexes

The anatomical pathways mediating the long-latency component of the TCR and trigemino-spinal reflex (TSR) are not well known; however, these reflexes share some commonalities with the startle response, whose anatomical pathway traveling through the brainstem is well-known. A startle reflex can be initiated with a loud noise, which activates auditory receptors in the cochlea, from which the vestibulocochlear nerve (8^{th} cranial nerve) projects to cochlear root neurons (CRNs). As seen in Figure 4-1, CRNs project to the ipsilateral and contralateral nucleus reticularis pontis caudalis (n.r. PnC) within the pontomedullary reticular formation (PMRF; Davis et al., 1982, Lee et al., 1996, Gomez-Nieto et al., 2014). As discussed in Chapter 1, the n.r. PnC integrates information from multiple systems, including the auditory, trigeminal and vestibular

systems (see Fig 4-2 in Introduction; Yeomans et al., 2002) to project to facial, cranial and spinal motor neurons in order to activate face and limb muscles on both sides of the body. Thus, it is possible that reflexes evoked from trigeminal or auditory stimuli may share a common anatomical pathway.

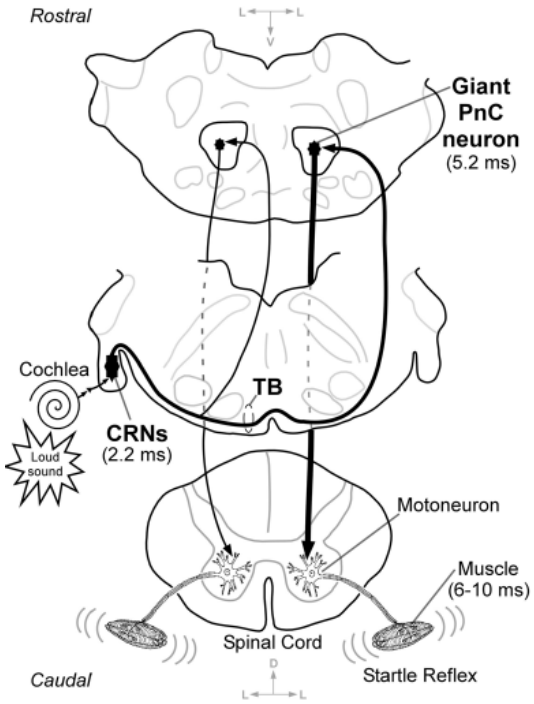

Figure 4-1. Acoustic startle reflex pathway. A loud noise activates the auditory receptors in the cochlea which project to cochlear root neurons (CRNs). CRNs synapse with the ipsilateral and contralateral nucleus reticularis pontis caudalis (PnC) where projections are sent to motoneurons in the spinal cord via the reticulospinal tract. (Gomez-Nieto et al., 2014)

Commonalities between long latency TCR and acoustic startle

To determine if the long-latency component of the TCR shares a similar anatomical pathway as the acoustic startle reflex, in preliminary experiments we compared the profile of responses evoked by trigeminal nerve and acoustic stimuli in the tonically active sternocleidomastoid (SCM) muscle. In control participants, both the TCR and acoustic startle evoked bilateral responses (not shown, see also Milanov et al., 2001), attributed to the bilateral innervation of the n.r. PnC, from which descends the reticulospinal tracts (RSTs) to innervate motorneurons on both sides the spinal cord

(Gomez-Nieto et al., 2014). Moreover, a similar profile of suppression in the active SCM was produced from trigeminal and acoustic stimulation. For instance, the duration of suppression from acoustic stimulation was 52 ms (Fig. 4-2, grey trace) and from trigeminal stimulation was 49 ms (Fig. 4-2, black trace) and both had a peak suppression at approximately 60 ms.

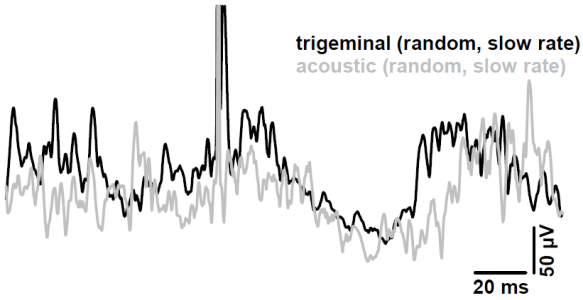

Figure 4-2. Reflex responses in the sternocleidomastoid (SCM) in response to trigeminal and acoustic stimuli. Rectified and averaged EMG traces in a control participant. Trigemino-cervical reflex (TCR) evoked from 20 trigeminal stimuli applied randomly at a slow rate every 5 to 10 seconds (black trace) and the acoustic startle reflex evoked from 10 acoustic stimuli applied randomly every 20 seconds (grey trace).

Another well-characterized property of the acoustic startle reflex is its habituation over time as the magnitude of responses decreases in response to repetitive loud sounds. The degree of habituation depends on the length and variability of the interval between stimuli (ISI). Tones presented with shorter ISIs show greater habituation than tones presented at longer ISIs. For example, rats showed a lower mean percentage startle response (greater habituation) when tones were presented at fast rates of a tone every 2 seconds, compared to slow rates of every 16 seconds (Davis, 1970). The variability of the ISI also influences habituation where tones presented at fixed, predictable intervals evoke greater habituation than tones presented at random, unpredictable intervals. For example, rats showed lower startle response frequency (greater habituation) when tones were presented at a fixed ISI of 8 seconds compared to random ISIs between 2 and 18 seconds (Davis, 1970). If the TCR shares a similar pathway of activation through the brainstem to

that of the acoustic startle, TCR responses should habituate to repetitive stimuli, particularly to those with fixed ISIs presented at a fast rate.

We examined the properties of habituation in the long latency component of the TCR with two stimulation conditions. Responses were recorded from the SCM following 20 stimuli of the mandibular branch of the trigeminal nerve at an intensity of 2.0 x motor threshold of the masseter muscle. Trigeminal stimulation applied randomly at a slow rate (every 5-10 seconds), similar to Davis, 1970, suppressed the ongoing EMG (Fig. 4-3, black trace) for a duration of 49 ms, where the mean SCM EMG was -38.6% of the background EMG (see Methods in Chapter 2 for explanation of reflex calculation). In contrast, trigeminal stimulation applied at fixed intervals at a fast rate (every 0.5 seconds, Fig. 4-3, red trace), reduced the duration of EMG suppression to 20 ms, where the mean EMG was only -19.8% of background EMG – similar to results seen in controls in Chapter 2. Reduced EMG suppression in response to stimuli that are presented at fixed ISIs at a fast rate reveals the TCR shows similar characteristics of habituation as the acoustic startle.

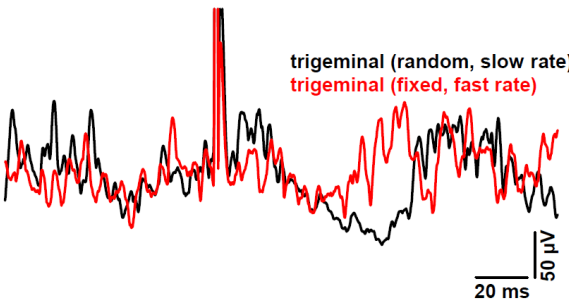

Figure 4-3. Trigemino-cervial reflex (TCR) in the sternocleidomastoid (SCM). Rectified and averaged EMG traces in a control participant. TCR evoked from 20 trigeminal stimuli applied randomly at a slow rate every 5 to 10 seconds (black trace) and at regular intervals at a fast rate of every 0.5 seconds (red trace).

Given the similarities in characteristics between the TCR and acoustic startle reflex, it is possible that these reflexes share similar neuronal pathways. It has been proposed that acoustic and tactile information is integrated in the n.r. PnC within the

brainstem (Yeomans et al., 2002). This point of convergence has been examined in rats and humans where the combination of acoustic and trigeminal stimulation produce stronger startle responses than when stimuli are experienced individually (Li and Yeomans, 1999, Yeomans et al., 2002). Thus, the long latency TCRs, as shown to be more facilitatory in CP in Chapter 2, may be mediated by hyperexcitability of pathways routing though the n.r. PnC. Further characterization of trigeminal and acoustic reflexes may give insight into the neural pathways that mediate them.

1b. Potential sites of interaction between cortical and trigeminal afferent stimulation

Aside from evoking a direct reflex response, a trigeminal or auditory stimulus also influences the excitability of structures along the cortical-brainstem-spinal cord motor pathway. An acoustic stimulus applied 30-60ms before a cortical stimulation inhibits MEPs in the deltoid, first dorsal interosseous and soleus muscles (Furubayashi et al., 2000, Fisher et al., 2004, Ilic et al., 2011, Tazoe and Perez, 2017). Effects from auditory stimulation are thought to take place at a cortical level, as only responses evoked from cortical activation of descending motor pathways with TMS are affected and not responses from subcortical activation of axons with transcranial electrical stimulation (TES; Furubayashi et al., 2000, Kuhn et al., 2004, Di Lazzaro et al., 2008). In contrast, an acoustic stimulus produces opposite effects at the spinal level. Acoustic stimuli can facilitate spinal mechanisms as seen with the increase in amplitude of the H-reflex when conditioned with a previous acoustic tone at 100 to 200 ms (Ilic et al., 2011). Thus, an acoustic tone evokes different mechanisms of interaction at the cortical and spinal levels that are specific to the ISI at which it is applied.

In contrast to inhibition of MEPs from acoustic stimulation, we showed that trigeminal stimulation causes facilitation of MEPs in Chapter 2. A trigeminal stimulus applied 50 ms before TMS facilitates MEPs in the SCM, especially in participants with CP. While acoustic stimulation evokes different mechanisms at different levels of the motor system, it is possible that trigeminal stimulation may also work through different sites to facilitate MEPs. Facilitation of MEPs from trigeminal stimulation may occur by facilitating brainstem circuits rather than cortical structures. A cortical site of interaction from trigeminal stimulation is unlikely, given that somatosensory-evoked potentials elicited from trigeminal afferent stimulation have a latency of 5-22 ms (Richardson and Cody, 1977, Van Loven et al., 2001) and likely occur too early to facilitate the SCM MEP when TMS is applied to the cortex 50 ms after nerve stimulation. In Chapter 2, there was no significant modulation of the SCM MEP at the 20 ms ISI when activation of the cortex by trigeminal afferents was likely to occur. Instead, a brainstem site of interaction is likely when considering that in healthy adults, trigeminal nerve stimulation modulates the longer latency component of the blink reflex by acting through polysnaptic brainstem pathways and does not modulate cortical circuits as tested with short-interval (SICI) and long-interval (LICI) intracortical inhibition, short-interval (SICF) and intracortical facilitation (ICF; Mercante et al., 2015). The n.r. PnC within the brainstem receives inputs from trigeminal (Shammah-Lagnado et al., 1987, Yeomans et al., 2002, Schmid et al., 2003, Serrao et al., 2003) and cortical (Fisher et al., 2012) systems, making it a likely site of interaction between trigeminal and cortical stimuli. If this is the case, then trigeminal stimulation has the capacity to facilitate cortico-reticulospinal pathways mediating the MEP. This may explain why trigeminal stimulation has a facilitatory effect

on MEPs, in contrast to the inhibitory effect from auditory stimulation. This would also explain the larger facilitatory effect of MEPs seen in participants with CP whose facilitated TCR reflexes would suggest they have a hyperexcitable brainstem.

Given the increased excitability of the TCR in CP and the facilitation of MEPs in response to trigeminal stimulation, it is possible that the brainstem is hyper-excitable in CP. The brainstem may play an integral role in motor control in CP, where increased activation of the reticular formation facilitates increased activity of cortico-reticulo-spinal pathways. In animal studies, the reticulospinal and rubrospinal systems play a considerable role in motor control following lesions to the CST. In monkeys who received CST lesions, the ability to grasp food was lost. Within 4 to 6 weeks, the grip strength that monkeys recovered to support their body weight is attributed to the RST, which was the only major surviving descending pathway (Lawrence and Kuypers, 1968, Zaaimi et al., 2012). In monkeys with lesions to the primary motor cortex, functional recovery and responses evoked in shoulder and elbows from microstimulation of the PMRF increased after 12 weeks of rehabilitation. (Herbert et al., 2015). Rubrospinal output has also mediated recovery in hand function in monkeys following CS lesions (Lawrence and Kuypers, 1968). While the rubrospinal system is suggested to be almost absent in humans (Nathan and Smith, 1955, Baker, 2011), the RST is most likely to contribute to functional recovery in humans with CST lesions. Thus, in individuals with CP, reticulospinal pathways may assume a considerable role in mediating motor control where there is a reduction of direct connections between the cortex and spinal cord.

Further investigation into the site of interaction between trigeminal and cortical stimulation needs to be done. Similar to studies conducted with acoustic stimuli

(Furubayashi et al., 2000, Kuhn et al., 2004, Di Lazzaro et al., 2008), the effects of trigeminal stimulation on MEPs evoked from TMS vs TES will help to determine if trigeminal stimulation interacts at the cortical level. However, due to the proximity of the SCM muscle to the head, transmastoid electrical or occipital TMS (both used to activate CST axons directly) has the potential to activate peripheral nerves around the head and neck that could initiate a direct reflex response. Thus, further experiments would need to be done in a more distal muscle such as the biceps or deltoid.

In summary, trigeminal stimulation may facilitate brainstem circuits, rather than cortical structures. More specifically, the n.r. PnC within the brainstem receives inputs from trigeminal and cortical systems making it a likely site of interaction between trigeminal and cortical stimuli. While it is possible that the brainstem is hyper-excitabile in CP, the brainstem may play an integral role in motor control where increased activation of the reticular formation facilitates increased activity of cortico-reticulo-spinal pathways. Further investigation into the site of interaction between trigeminal and cortical stimulation needs to be done.

2. Development of motor pathways in children with CP

Children with hemiplegic CP experience significant development of motor pathways to leg muscles before 4 years of age, as examined in Chapter 3. Although no training effects were observed, notable changes of MEPs include a greater prevalence of MEPs after 2 years of age, potential persistence of ipsilateral MEPs from the uninjured hemisphere throughout infancy and progressive decreases in onset latency of MEPs with

increases in age. These findings match the anatomical maturation and myelination profile of motor pathways during infancy.

Future directions

Continued analysis of MEPs may give insights into how motor pathways change throughout development. In future analysis, the amplitude (peak-to-peak) of MEPs will be measured to estimate the strength of descending motor pathways in response to training and development. The excitability of inhibitory motor circuits can be inferred indirectly, through examination of silent periods – a pause in EMG activity following an MEP (Fig. 2-3 in Chapter 2). The early part of a silent period is attributed to reduced excitability of the motor cortex and spinal cord, while the later part is attributed to a continuation of reduced motor cortex excitability (Fuhr et al., 1991, Chen et al., 1999, Hallett, 2007). Long lasting cortical inhibition occurs due to strong activation of inhibitory interneurons, which release large amounts of the GABA neurotransmitter. Activation of post-synaptic $GABA_B$ receptors is favorable, causing increased conductance of K^+ ions across the membrane of the neuron (McCormick, 1992, Werhahn et al., 1999). A subsequent action potential can be impeded for up to 200ms (McCormick, 1992). It is not known how silent periods develop throughout infancy. In our sample, silent periods could be observed in EMG with and without the presence of a preceding MEP. Investigating whether silent periods are dependent on age, and if so, how they evolve throughout development, may give us a greater understanding of the excitability of the motor system at very young ages.

Inferring the organization and development of underlying motor pathways based on TMS data alone is difficult, as the severity and location of injury is different for each participant. Future categorization of all MRIs to classify injury in terms of type of infarct (arterial/venous), presence of deep gray matter injury, cerebral malformations and severity of injury (mild, moderate or severe), will be performed. This will give further insight into how underlying brain injury is related to functional abilities.

Clinical Significance

Clinical assessment of the neonatal nervous system is largely based on muscle tone and neonatal reflexes (Shepherd, 2014). These techniques give limited insight into the development of a nervous system that can produce complex functions. Understanding how the excitability and anatomical organization of the CST and other descending motor pathways may develop differently in children with CP may give insight into the prognosis of motor functions. MEPs could be evoked in many children in the present study at 4 years of age. Thus, it is possible that TMS could be used as a tool to infer the excitability and strength of motor pathways, and with this information, predict what motor functions could be expected to develop.

The application of activity-based therapies is becoming more popular for individuals with CP, particularly as early intervention during infancy (Yang et al., 2013, Shepherd, 2014). While changes in MEP prevalence following training were not found in the present study, children showed functional improvements. Other forms of activity-based therapies to activate motor pathways have been beneficial during childhood. Repetitive TMS (rTMS) and transcranial direct current stimulation (tDCS) have worked

to restore motor function in individuals with hemiplegic stroke by modulating the excitability of the motor cortex. When applied in children with arterial ischemic stroke, rTMS reduced the imbalance of interhemispheric inhibition between motor cortices and increased in the amplitude of MEPs evoked form the stroke hemisphere (Kirton et al., 2010). The combination of rTMS and motor training has also been beneficial in adult stroke patients with improvements in motor skill acquisition in the paretic hand (Kim et al., 2006, Takeuchi et al., 2008, Kwon et al., 2014). It has been proposed to combine tDCS and treadmill training in children with CP to investigate changes in gross motor function, gait, and cortical excitability (Grecco et al., 2013). It is clear that the developing motor system in humans can be modulated with activity-based therapies; however, it is still not understood how the underlying motor system is changing with these interventions. A greater understanding of the developing motor system would enhance our understanding of how therapy could promote development of the most functionally effective motor pathways.

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Appendix: Modulation of muscle responses in the upper arm with trigeminal nerve stimulation

Introduction

In CP, disrupted development of direct projections from the cortex to the spinal cord may promote the formation of indirect pathways to the spinal cord via the brainstem. As a reflection of this, it is possible that evoked responses from TMS to the motor cortex may be partly mediated by indirect cortico-reticulospinal pathways in addition to the direct corticospinal tract, especially in CP.

To examine the possible contribution of the cortico-reticulospinal tract in mediating motor-evoked potentials (MEPs), activation of neck afferents, which converge onto reticular nuclei along with cortical inputs, have been used to modulate TMS responses (Wilson and Peterson, 2011, Ellis et al., 2012, Fisher et al., 2012) For example, head rotation in the transverse plane (as a means to active neck afferents) can modulate descending motor pathways where ipsilateral MEPs in wrist extensors and biceps show an increase in amplitude (Ziemann et al., 1999, Tazoe and Perez, 2014) and a decrease in onset latency (Ziemann et al., 1999). However, head rotation has little effect on contralateral MEPs (Ziemann et al., 1999). While it is possible that ipsilateral descending motor pathways could be mediated by a multisynaptic brainstem pathway, head rotation may provide the brainstem with an input too diffuse to modulate the robust contralateral responses. Because the reticular formation also receives inputs from trigeminal nerve afferents (Shammah-Lagnado et al., 1987, Schmid et al., 2003), we investigated the effects of electrical stimulation of the trigeminal nerve to modulate MEP responses. Electrical stimulation may provide a stronger activation of the brainstem in a

more controlled manner compared to head rotation in addition to more precisely controlling the timing of the afferent and cortical inputs onto the brainstem.

Trigeminal and cortical projections both activate the reticular formation at latencies near 3-5ms (Fujii, 1977, Fisher et al., 2012, Fregosi et al., 2017). Here, we used stimulation of the trigeminal nerve to condition the biceps MEP at short ISIs (-2 to 6 ms) to determine if the cortically-evoked MEP could be modulated at the level of the brainstem. We also examined longer ISI's using multiple pulses of TNS to provide a large barrage of afferent inputs (Zewdie et al., 2014) to examine long-latency effects of trigeminal afferent activation, similar to what we did with the SCM MEP in Chapter 2. We hypothesize that MEPs in the upper arm will be modulated to a greater degree in participants with CP in comparison to controls, suggesting a greater recruitment of cortico-reticulospinal pathways in response to early brain injury.

Methods

MEPs were recorded in the biceps muscle in response to TMS over the contralateral motor cortex. Biceps MEPs were conditioned with trigeminal nerve stimulation (TNS) using either a single pulse or a 5-pulse train at a variety of inter-stimulus intervals (ISIs), similar to the SCM muscle in Chapter 2. An MEP of approximately half maximum was evoked in the contralateral biceps muscle during a 10% maximum contraction. On average, the size of the test MEP was 676.3 ± 487.9 μV in the participants with CP and 917.5 ± 245.7 μV in NI controls. The biceps MEP was conditioned with single-pulse trigeminal nerve stimulation (TNS) at short ISIs (6, 4, 2, 0, and -2 ms) and at an intensity of 1.2 x MT. Positive ISIs indicate TNS preceded the TMS

pulse, negative ISIs indicate TNS followed the TMS pulse, and 0 ms indicates that both TMS and TNS were given at the same time. The short ISIs were used to study possible interaction effects at the level of the brainstem. Similar to the SCM, 5-pulse TNS was applied 20, 50 and 100 ms before the TMS. The same number of trigeminal alone (n = 10), test alone (n = 10) and conditioned TMS trials (8 per interval) were used for both the single and 5-pulse trigeminal stimulation trials. The short ISI conditioning paradigm was tested in 13 NI controls and 12 participants with CP. The long ISI conditioning paradigm was tested in 10 NI controls and 8 participants with CP.

Because the TNS alone did not produce a reflex response in the biceps muscle during the window of the test MEP, the peak-to-peak amplitude of the test or conditioned MEPs were simply averaged together to provide a mean test or mean conditioned MEP, respectively, for all ISI trials. The mean conditioned MEPs were then expressed as a percentage of the mean test MEP response [(conditioned MEP/test MEP) x 100%]. To compare the difference in the conditioned MEPs across the different ISIs within a group (CP or NI, Figs. 1B and 2B), a Kruskal-Wallis one-way ANOVA was used with post-hoc Mann-Whitney U-tests (Bonferroni correction of $p = 0.01$ for Fig. 1B (0.5 / 5) and $p = 0.017$ for Fig. 2B (0.5 / 3).

Results

There was very little modulation of the biceps MEP at all ISIs tested when using a single pulse of TNS at 1.2 xMT. As shown for a single control participant (Fig. 1A), TMS alone (second trace) evoked a test MEP in the contralateral biceps with an average amplitude of 698.5 µV. The test MEP showed little modulation when conditioned by TNS at ISIs of -2, 0, 2, 4 and 6 ms (all median values are within ±8.6% of test MEP amplitude). In the group data, a Kruskal-Wallis one-way ANOVA failed to reveal a statistically significant effect of ISI for either the CP ($p = 0.75$) or NI ($p = 0.31$) group (Fig. 1B).

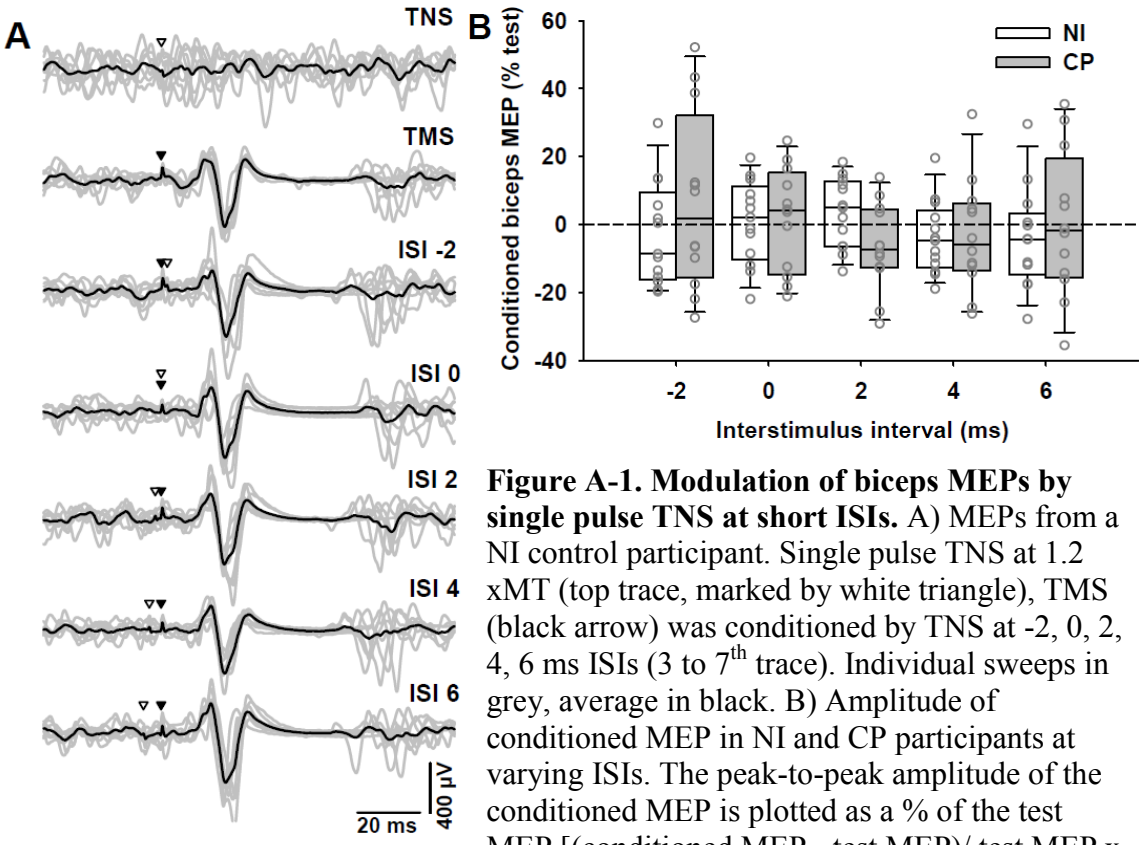

Figure A-1. Modulation of biceps MEPs by single pulse TNS at short ISIs. A) MEPs from a NI control participant. Single pulse TNS at 1.2 xMT (top trace, marked by white triangle), TMS (black arrow) was conditioned by TNS at -2, 0, 2, 4, 6 ms ISIs (3 to 7th trace). Individual sweeps in grey, average in black. B) Amplitude of conditioned MEP in NI and CP participants at varying ISIs. The peak-to-peak amplitude of the conditioned MEP is plotted as a % of the test MEP [(conditioned MEP - test MEP)/ test MEP x 100%]. The box plot shows the median (solid line within the box), 25th and 75th percentiles (box bounds), and 95th and 5th percentiles (whiskers).

Similar to the short ISIs, there was little modulation of the biceps MEP when 5 pulses of TNS were used to condition the biceps MEP at long ISIs as shown for the same NI participant in Figure 2A. Within each group (Fig. 2B), there was variable modulation of the biceps MEP at the longer ISIs with no significant effect for ISI in NI controls ($p = 0.72$) or participants with CP ($p = 0.81$).

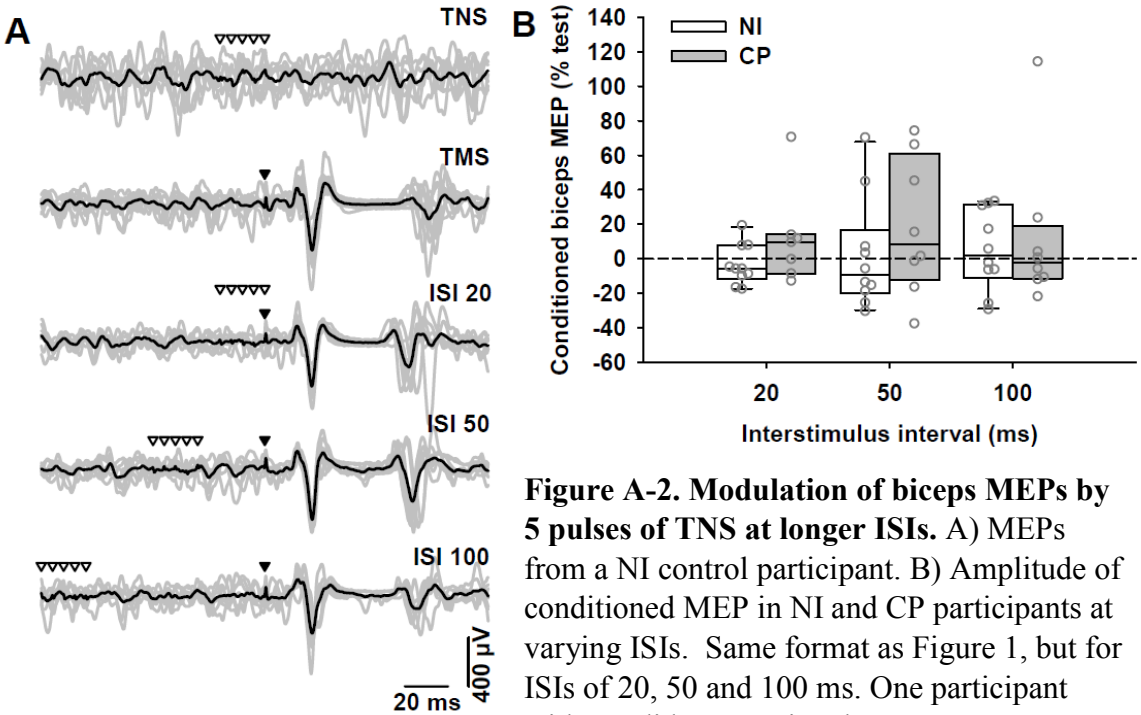

Figure A-2. Modulation of biceps MEPs by 5 pulses of TNS at longer ISIs. A) MEPs from a NI control participant. B) Amplitude of conditioned MEP in NI and CP participants at varying ISIs. Same format as Figure 1, but for ISIs of 20, 50 and 100 ms. One participant with CP did not receive the 20 ms ISI condition.

Discussion

Given that trigeminal and cortical projections have converging inputs onto the reticular formation at similar latencies (Fujii, 1977, Fisher et al., 2012, Fregosi et al., 2017), we conditioned the biceps MEP with TNS at short ISIs to determine if the cortically evoked MEP could be modulated at the level of the reticular formation. Little average modulation of the biceps MEP was present in NI controls and participants with CP at any ISI. A single pulse trigeminal stimulus was ineffective in modulating the MEP,

likely due to the inability to modulate the reticular formation. For this reason, we tried using trains of trigeminal pulses applied at longer latencies of 20, 50 and 100 ms to investigate the effects of a more robust activation of the reticular formation on descending motor pathways (Zewdie et al., 2014). However, this too did not modulate the biceps MEPs in a consistent manner.

Long latencies between the conditioning and test stimuli make it difficult to determine the precise location of the interaction of both stimuli, thus, it would be ideal to apply a more robust activation of the reticular nuclei at very short ISIs to allow for interaction effects precisely at the level of the brainstem. However, the application of trains of pulses is not possible at short ISIs due to overlap of the stimulation with the muscle response. Thus, unlike using head rotation to activate reticulospinal pathways (Ziemann et al., 1999), single pulse stimulation to the mandibular branch of the trigeminal nerve is not useful to demonstrate that contralateral MEPs are mediated by a cortico-reticulospinal pathway. Alternatively, the contralateral biceps MEP, unlike the SCM MEP, may be mainly produced by direct corticospinal tract inputs and thus, show very little modulation by brainstem activation. However, it is important to note that TMS preferentially activates fast-conducting, corticospinal tract neurons which might not have strong cortico-reticulospinal connections compared to slower conducting cortico-reticular pathways.

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