THE RELATIONSHIP BETWEEN MIRROR MOVEMENTS AND CORTICOSPINAL TRACT CONNECTIVITY IN CHILDREN WITH UNILATERAL SPASTIC CEREBRAL PALSY

Hsing-Ching Kuo

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ABSTRACT

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Unilateral Spastic Cerebral Palsy (USCP) is caused by an early brain lesion in which the Corticospinal Tract (CST), the primary pathway controlling upper extremity (UE) movements, is affected. The CST connectivity after early brain injury (i.e., an ipsilateral, contralateral, or bilateral connectivity) may influence treatment outcomes. Transcranial magnetic stimulation (TMS) is a common method to probe CST connectivity. However, TMS is limited to children without seizures. Mirror movements (MM), an involuntary imitation of movements by one limb during the contralateral limb voluntary movements, are common in USCP. MM may result when both UEs are controlled by the contralesional motor cortex. Here we investigated the relationship between MM and CST connectivity in children with USCP. We hypothesized that stronger MM were associated with an ipsilateral connectivity. Our secondary aim was to investigate whether the amount of MM was reduced after intensive therapy. Thirty-three children with USCP (mean age=9yrs 6mos; MACS: I-III) participated and were randomized to receive 90hrs of unimanual (n=16) or bimanual (n=17) intensive training. Assessments were measured at baseline and immediately after training. We used TMS and diffusion tensor imaging (DTI) to determine the CST connectivity. We used three approaches to quantify MM: 1) behavioral MM assessment during contralateral movements, including hand opening/closing, finger opposition, finger individuation, and finger walking, 2) involuntary grip force oscillations recorded by force

transducer (FT) when the contralateral hand performed repetitive pinching, and 3) involuntary muscle contractions measured by electromyography (EMG) when the contralateral hand performed pinching. Results showed that strong MM (scores ≥ 3) in the more-affected hand while hand opening/closing were associated with an ipsilateral pathway (Fisher's exact test, p= 0.02). This association was not found in the remaining tasks (Fisher's exact test, opposition, p≥ 0.99; individuation, p≥ 0.99; finger walking, p≥ 0.99). Involuntary GF oscillations were measured in a subset of 16 children. Presence of FT-measured MM in the less-affected hand (> 0.3N) was not associated with TMS-probed connectivity (Fisher's exact test, p= 0.59). Nevertheless, presence of FT-measured MM was associated with DTI-assessed connectivity (Fisher's exact test, p= 0.0498). Similarly, presence of EMG-measured MM in the more-affected hand was not associated with TMS-probed connectivity (Fisher's exact test, p= 0.59). Nevertheless, presence of EMG-measured MM was associated with DTI-assessed connectivity (Fisher's exact test, p= 0.03). The amount of MM did not change after training (p> 0.06 among all measures). In conclusion, strong MM in the more-affected hand while hand opening/closing may be indicative of an ipsilateral connectivity identified by TMS. Presence of MM measured by FT may be a predictor of DTI-assessed CST pattern. Findings of this study may help researchers and clinicians understand the relationship between the CST connectivity and its behavioral manifestation in children with USCP. Such relationship may further guide therapeutic strategies in a wider range of children with USCP.

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DEDICATION

All my Ph.D. work at Teachers College is dedicated to my parents.

謹以這份論文感謝爸爸媽媽對我永遠的支持

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I. INTRODUCTION

Cerebral Palsy (CP) is the primary cause of motor deficits in children. It is an umbrella term that encompasses a group of developmental disorders. Clinically, it can present with movement, postural, cognitive, tactile deficits, or seizures (Bax et al., 2005). Unilateral spastic cerebral palsy (USCP), the most common type of CP (Himmelmann, Hagberg, Beckung, Hagberg, & Uvebrant, 2005), has motor deficits lateralized to one side of the body. Prenatal or perinatal brain injury or immaturity in early developmental process may cause CP. Studies using Magnetic Resonance Imaging (MRI) have demonstrated that the etiology may include middle cerebral artery occlusion or hemorrhage, hypoxic-ischemic encephalopathy, brain malformation, and periventricular leukomalacia or injury (Himmelmann, Beckung, Hagberg, & Uvebrant, 2006; Krageloh-Mann & Horber, 2007). Damage areas may include the cerebral cortex, subcortical structures (Kuban & Leviton, 1994), and the descending corticospinal tract (CST) (Bleyenheuft, Grandin, Cosnard, Olivier, & Thonnard, 2007; Duque et al., 2003).

Dysgenesis of the corticospinal tract (CST), the primary descending motor pathway, may contribute to impairment in skilled upper extremity (UE) movements (Lemon, 2008; Martin, Friel, Salimi, & Chakrabarty, 2009). In a feline model of USCP, it has been established that CST development is driven by activity-dependent competition (Friel & Martin, 2007; Martin & Lee, 1999) and motor experience (Martin, Choy, Pullman, & Meng, 2004). The mechanism of injury and subsequent recovery in the corticospinal system in children with USCP resembles that in the animal model of CP (Eyre, Taylor, Villagra, Smith, & Miller, 2001; Friel, Williams, Serradj, Chakrabarty, & Martin, 2014). This mechanism has been tested in typically developing (TD) children

and those with USCP by means of transcranial magnetic stimulation (TMS). Typically developing (TD) children have bilateral motor evoked potential (MEP) responses when stimulating either motor cortex (M1) with TMS at term age. At 3-6 months of age, evidence of withdrawal of the ipsilateral CST occurs, that is, the ipsilateral MEP begins to have longer latency and smaller amplitude as compared to the contralateral MEP (Eyre et al., 2007; Eyre et al., 2001). By 2 years of age, there is primarily contralateral MEP response with sparse ipsilateral response in typical development. In contrast, children with USCP exhibit a distinctive pattern when probing with TMS. The diminished activity in the more-affected M1 strengthens the ipsilateral connections originating from the less-affected M1 in children with USCP. Starting at ~6 months of age, stimulating the less-affected M1 with TMS often elicits muscle responses on both UEs, whereas stimulating the more-affected M1 with TMS elicits decreased or no contralateral MEP responses. By 2 years of age, stimulating the less-affected M1 elicits MEPs in both UEs with similar onset latencies. Approximately 50% of children with USCP have their more-affected UE controlled by the ipsilateral CST projecting from the less-affected M1 (Lotze, Sauseng, & Staudt, 2009).

The extent of CST dysgenesis could impact hand function in children with USCP. Studies showed that a greater asymmetry index (calculated by the cross-section areas of bilateral cerebral peduncle) predicts a more severe level of hand impairment by using structural Magnetic Resonance Imaging (MRI) (Duque et al., 2003; Friel, Kuo, Carmel, Rowny, & Gordon, 2014; Staudt et al., 2002) or more precisely by using Diffusion Tensor Imaging (DTI) (Bleyenheuft et al., 2007). Brain lesion extent may impact hand function (Staudt, Niemann, Grodd, & Krageloh-Mann, 2000) and the CST reorganization

in patients with PVL (Staudt et al., 2002). In the latter, they showed that patients with smaller lesions had a preserved contralateral CST, whereas patients with larger lesions developed an ipsilateral connectivity. Importantly, Staudt et al. (2004) demonstrated that the timing of brain lesion may affect hand function. Children with brain malformation (1st and 2nd trimester lesion) had better hand function than children with PVL (early 3rd trimester lesion), who had better hand function than children with middle cerebral artery hemorrhage or occlusion (late 3rd trimester lesion). Holmstrom et al. (2010) demonstrated that the most impaired hand function (measured by Box and Blocks test) was found in children with an ipsilateral CST connectivity, whereas the least impaired hand function was found in children with a contralateral CST connectivity. Hand function outcome cannot be explained by a single factor and may dependent on a combination of the lesion extent, type, and location (Holmstrom et al., 2010; Staudt et al., 2004). Two reviews proposed to use the reorganization or "rewiring" of CST as a biomarker to guide therapeutic applications and inform hand function outcome (Gordon, Bleyenheuft, & Steenbergen, 2013; Jaspers, Byblow, Feys, & Wenderoth, 2015).

Intensive hand therapies have been shown to improve hand function in children with USCP in several randomized controlled trials (RCTs) and are strongly recommended to be better approaches in improving the level of activities in a systematic review (Gordon et al., 2011; Novak et al., 2013; Sakzewski et al., 2011). Two forms of evidence-based intensive therapy have been developed based on motor learning principles. Unimanual intensive therapy focuses on mass practice of children's more-affected hand while the less-affected hand is constrained; bimanual intensive therapy focuses on ameliorating bimanual coordination. Both forms of intensive hand therapy

can produce long-term improvements in hand function. However, it is costly (thousands of dollars per child) and time consuming (60-90 hours) (Wallen, Ziviani, Herbert, Evans, & Novak, 2008). Thus, it is imperative to target specific types of therapy to children who are most likely to benefit. As discussed previously, the CST connectivity is important because it may impact how children respond to different forms of intensive hand therapies. Kuhnke et al. (2008) demonstrated that children with ipsilateral CST connectivity responded poorer than children with contralateral CST connectivity in the speed component of the Wolf Motor Function Test after Constraint-Induced Movement Therapy (CIMT). In addition, our recent findings (Friel et al., 2016, see Appendix D) showed that children with an ipsilateral CST responded equally well as those with a contralateral CST to intensive bimanual therapy (Hand-arm Bimanual Intensive Therapy). These findings suggested that there might be an association between the CST connectivity and improvements after a certain therapy.

Despite the significant effort and cost associated with the intensive treatment approaches described above, the ability to predict the efficacy of treatment in a given child based on their CST organization would thus be helpful. Conventionally, TMS is a neurophysiological method examining CST connectivity in children with USCP (Eyre et al., 2007; Eyre et al., 2001; Staudt et al., 2004). However, TMS has its limitations. It cannot be applied to children with seizures, a high comorbid in children with CP (35%) (Himmelmann et al., 2005). In addition, the cost of the machine and skills required to perform the experiments poses challenges. We recently demonstrated that Diffusion Tensor Imaging (DTI) can be used as a sensitive (82%) and specific (78%) surrogate for determining CST connectivity in children with USCP (Kuo et al., 2016). However, DTI

is not applicable to children with metal implants and claustrophobia. In addition, families might not have access to MRI facilities that have the capacity and expertise to perform and interpret DTI. This raises a practical need to search for a simple method to identify the CST connectivity. Since the efficacy of hand therapy may be impacted by the pattern of CST reorganization, it is essential to develop a simple screening test for determining CST reorganization that can be applied to a wide range of children with USCP.

Mirror movements (MM) are a common movement pattern in typical development and in children with USCP (Woods & Teuber, 1978). MM depict an involuntary imitation of movements by one limb during the contralateral limb voluntary movements. Different terms have been used interchangeably in the literature to describe this movement pattern, encompassing synkinesia (Marie & Foix, 1916; Westphal, 1874), associated movements (Connolly & Stratton, 1968; Lazarus & Todor, 1987; Todor & Lazarus, 1986), motor irradiation (Cernacek, 1961), motor overflow (Hoy, Fitzgerald, Bradshaw, Armatas, & Georgiou-Karistianis, 2004), or mirror movements (Carr, Harrison, Evans, & Stephens, 1993; Woods & Teuber, 1978). Importantly, several studies have demonstrated some levels of associations between the presence of MM and the CST reorganization in early and acquired brain injury (Carr et al., 1993; Farmer, Harrison, Ingram, & Stephens, 1991; Staudt et al., 2004). A review discussing the mechanisms underlying physiological MM in TD children and pathological MM in USCP can be found in Appendix C.

Two potential mechanisms have been hypothesized to elucidate the presence of MM in children with CP: (a) an ipsilateral CST projecting from the contralesional M1 to both UEs (Carr et al., 1993; Farmer et al., 1991), and (b) co-activation of both M1s

resulting from dysfunctional inhibition between the M1s (Koerte et al., 2011). Most studies in children with USCP supported the first hypothesis that MM are an indicator of an ipsilateral CST controlling bilateral UE. Carr et al. (1993) showed a correlation among strong MM (scores 3-4), a branched ipsilateral CST probed by TMS, and a significant cross correlation during bilateral FDI contractions. This correlation was not demonstrated in those individuals with weak MM (scores 0-2). Although their study sample was a combination of congenital and acquired brain lesions, their finding supported the first hypothesis that the contralesional M1 innervates both hands via an ipsilateral pathway in subjects with strong MM. Similar findings of bilateral biceps activations during unilateral elbow flexion measured by muscle torque and EMG were showed in patients with USCP, but not in control individuals (Sukal-Moulton, Murray, & Dewald, 2013). These findings suggested that MM may be a manifestation of the underlying CST organization in children with USCP.

Evidence supporting the second hypothesis of bilateral M1 co-activation underlying MM was reported in typically developing children (Mayston, Harrison, & Stephens, 1999), congenital MM (Cincotta & Ziemann, 2008; Cohen et al., 1991), unfamiliar task or fatigue-associated overflow in healthy adults (Hoy et al., 2004), elderly (Hoy et al., 2004), and in children with bilateral CP (Koerte et al., 2011). Mayston et al. (1999) reported a lack of cross correlation between bilateral FDI recordings, suggesting no common motor command signaling both hands in TD children. Additionally, the dysfunctional inhibition tested with the interhemispheric inhibition (IHI) protocol using TMS suggested bilateral M1s co-activation in typical development. A study by Koerte et al. (2011) also supported that the second hypothesis may underlie the occurrence of MM

in children with bilateral CP. They found a correlation between the decrease in IHI competence, the structural integrity of corpus callosum (measured by fractional anisotrophy, by using DTI), and the amount of MM in children with bilateral CP. While it is still unclear how the two M1s interact in typical development, this second hypothesis supported by findings obtained from the IHI protocols should be interpreted with caution.

Understanding features of physiological MM helps researchers have a better standing ground to examine pathological MM. Two major features of MM are important in typical development. First, the force output in the involuntary hand increases in an exponential manner when the percentage of the maximal voluntary contraction (% MVC) increases in the voluntary hand (Todor & Lazarus, 1986). Therefore it is essential to control for a standardized voluntary force output. Second, the amount of MM can be task and age specific. For example, Connolly and Stratton (1968) showed that a clip-pinching task (Fog & Fog, 1963) was sensitive to induce MM for the ages between 5-13 years, and finger individuation was only sensitive for subjects until 8 years of age in TD children.

Compared to physiological MM in typical development, the amplitude of MM are more pronounced in children with USCP (Woods & Teuber, 1978). Kuhtz-Buschbeck, Sundholm, Eliasson, and Forssberg (2000) reported that children with USCP had 15 times stronger MM than TD children (measured by force transducer), and the intensity of MM did not decrease with age in children with USCP. Similar to the features of physiological MM, some factors were shown to influence the intensity of MM in children with USCP. Green (1967) reported that the manner of task performance and the muscles involved may influence the amount of MM in USCP. Specifically, MM in the biceps were more pronounced when the voluntary hand performed sustained contraction against

resistance, while those in the thenar muscles were more pronounced when the voluntary hand performed phasic or vigorous tasks. Other factors, such as the timing of brain lesion (stronger MM in congenital than those in acquired injury) (Sukal-Moulton et al., 2013; Woods & Teuber, 1978), the designated motor task (fist rotation induced the most pronounced MM among other tasks in Woods and Teuber (1978)), and the hand tested (stronger MM in the less-affected hand in Woods and Teuber (1978); whereas stronger MM in the more-affected hand in Cernacek (1961)) all influenced the amplitude of MM. These studied emphasized the research gap of the lack of systematic investigation of MM in children with USCP.

Excitingly, MM can be suppressed to some extent after given visual feedback in children with USCP (Kuhtz-Buschbeck et al., 2000). It was improved using a rTMS treatment protocol (repetitive TMS to inhibit the ipsilateral CST) in a 8-year-old boy with congenital MM (Kim et al., 2013), and was improved in a 15-year-old girl with congenital MM after training (Cincotta et al., 2003). These studies provide insights and invite possibility for rehabilitating MM in USCP once we understand the underlying neurophysiological mechanism. Given the impact of the CST connectivity on children's responsiveness to intensive hand therapy and the limitations of TMS and DTI, the primary aim of this study was to investigate **whether a simple clinical test could be used to determine the CST organization**. A clinical test can be applied to a wider range of children with USCP. Findings of this aim may facilitate stratifying patients prior to assigning the form of intensive hand therapy and can help researchers and clinicians determine locations for brain stimulation therapy. We hypothesized that a greater amount of MM will be associated with an ipsilateral CST connectivity controlling

the more-affected hand. As a second exploratory aim, we investigated whether MM could be ameliorated after three weeks of intensive hand therapy. This is an exploratory research question as our subjects consisted of a sample of convenience participating in a RCT of receiving either intensive unimanual or bimanual therapy. It is possible that MM may not change after intensive hand training because our training was not designed to reduce MM. Yet, it is also possible that the amount of MM could be reduced given the intensity of our training (90 hrs over 3 weeks).

II. METHODS

i. Participants

Participants were recruited from our website (http://www.tc.edu.centers.cit/), ClinicalTrials.gov, and online support forums. Children were a sample of convenience participating in our ongoing clinical trial that investigates the interaction between the CST connectivity and forms of intensive hand therapy. The inclusion criteria of the umbrella study were established based on our prior trials (Brandao et al., 2013; Gordon et al., 2011): 1) diagnosed with congenital USCP, 2) the ability to lift the more-affected arm 15 cm above a table surface and grasp light objects, 3) mainstreamed in school, 4) the ability to follow instructions during screening and complete the physical examination, and 5) the ability to comply with TMS and MRI procedures. Exclusion criteria included: 1) health problems unassociated with CP, 2) history of seizures after 2-year-old or currently on seizure medications, 3) visual problems, 4) severe spasticity at any joint (Modified Ashworth score>3.5), 5) orthopedic surgery on the more-affected hand within one year, and 6) botulinum toxin therapy in the upper extremity within the last six

months, 7) non-removable metallic objects, 8) claustrophobia, 9) family history of epilepsy. Informed assent/consent were obtained from all participants and their caregivers. This study was approved by the Institutional Review Boards of Teachers College.

ii. Study Design

This is a cross-sectional cohort study for Aim 1, and a prospective cohort study for Aim 2. To investigate Aim 1, children were assessed at one time point with the outcome measures (TMS, DTI, behavioral testing, grip force oscillations, and electromyography, details in section iv). To investigate Aim 2, children were assessed at two time points: one time prior to the beginning of camp (pre-test), and once immediately after camp (post-test) for all the outcome measures.

iii. Intervention Procedures

General intervention procedures. Three summer day camps (6hrs/day, 15 weekdays, 90 hours in total) were conducted at Teachers College from 2013-2015. Camp general procedures incorporate the principles of motor learning, such as repetitive practice, skill progression, whole-task and part-task practice, and positive reinforcement (Gordon & Magill, 2012). Children were randomly assigned to either unimanual or bimanual therapy based on their individual CST connectivity (determined by TMS) and their baseline unilateral dexterity (measured by Jebsen-Taylor Test of Hand Function). We adopted two intervention approaches: Constraint-induced movement therapy (CIMT) and Hand-Arm Bimanual Intensive Therapy (HABIT). These two approaches differ mainly in that children wore a cotton sling on the less-affected UE in CIMT, whereas children did not have any physical restraint in HABIT and used both hands.

Each camp was held in 2 separate rooms. Participants in one room received CIMT, and participants in the other room received HABIT. Children worked individually with trained interventionists (1:1 interventionist to participant ratio always maintained). Each room had its own supervisor to ensure the procedures of training were adhered to. Team meetings were conducted daily after camp to ensure the treatment strategy was applied based on individual impairment. Details of each therapeutic approach are below.

CIMT procedures. CIMT was modified to be child-friendly for children with USCP (Gordon, Charles, & Wolf, 2005; Gordon et al., 2011). Participant's less-affected UE was restrained in a cotton sling, and unimanual task practice was performed by the more-affected UE. The sling was snugly strapped to participants' trunk and was worn during the entire intervention except toileting breaks. Participants performed unimanual fine-motor and gross-motor functional and play activities using the more-affected UE. Activities were age-appropriate and targeted to individual motor skill level (e.g., supination). Interventionists provided assistance when needed (e.g., stabilizing the paper while the child draws on the paper).

HABIT procedures. Participants in HABIT were engaged in bimanual fine-motor and gross-motor functional and play activities (Charles & Gordon, 2006; Gordon et al., 2011). It was developed to be a child-friendly approach. Activities were chosen based on the role of the more-affected hand, progressing in complexity of motor skill from a non-dominant passive assist (e.g., stabilizing Playdoh® while cutting the dough) to active manipulator (e.g., moving Connect Four® pieces).

Both treatment approaches demonstrated comparable efficacy in children's unimanual and bimanual hand function, which was retained after a 6-month follow up period (Gordon et al., 2011; Sakzewski et al., 2011). The only difference in the efficacy was that children in the HABIT group had greater improvement in functional goals (measured by goal attainment scale, GAS) than those in the CIMT group, possibly due to the fact that most of the goals require the use of both hands and only children in the HABIT group were able to practice bimanual goals.

iv. Experimental Setup and Procedures

Magnetic Resonance Imaging (MRI). Each child received a structural MRI scan and a DTI scan at both pre-test and post-test. The structural scan was used in the TMS experiment to co-register stimulation sites with brain landmarks, using a stereotaxic system (Brainsight, Rogue Research, Montreal, Canada). The structural scan was also used for examining lesion type and location. DTI scan was used to reconstruct the contralateral CST by using Tractography (Kuo et al., 2016). This procedure serves two purposes: 1) verifying TMS-probed connectivity, and 2) determine the CST connectivity for those children without TMS responses.

T1-weighted MRI was performed at Columbia University Medical Center (CUMC) for 8 participants and at the Weill Cornell Medical College (WCMC) for 22 participants. We used 3T scanners (CUMC- Philips, Netherlands, WCMC- Siemens, Germany). Children were positioned head-first supine. For the structural scan, 165 slices were taken at the resolution of 256x256 pixels at CUMC, and 176 slices were taken at the same resolution at WCMC. For the DTI scan, 75 slices were taken at the resolution of 112x112 pixels for both sites. An echo-planar imaging (EPI) sequence was used

(TR=7638.99ms, TE=68.56ms for CUMC, TR=9000ms, TE=83ms for WCMC). A protocol of 55 diffusion directions was applied (*b* value=800s/mm^2) at CUMC, and 64 diffusion directions was applied (*b* value=1000s/mm^2) at WCMC.

Transcranial Magnetic Stimulation (TMS). To determine CST connectivity, TMS motor mapping was conducted at the Burke-Cornell Medical Research Institute or Teachers College using the same device. Frameless stereotaxy (Brainsight, Rogue Research, Montreal, Canada) allowed for online tracking of the position of the TMS coil relative to children's individual MRI structural scans. A six-channel EMG recording system (NeuroConn, Ilmenau, Germany) captured EMG data during TMS from surface electrodes over the FDI and wrist flexor muscles bilaterally. The TMS machine (Magstim Company Ltd, Wales, UK) triggered the recording of the EMG system, 100ms before and 400ms after each delivery of the TMS pulse. The position of each stimulation site was recorded in xyz coordinates. We probed the motor representation of the more-affected UE, starting from medial portion of the more-affected M1. The stimulation was gradually moved laterally and anteriorly/posteriorly until an MEP for the more-affected FDI was obtained. The same procedure was performed over the less-affected M1.

The following three measures, including behavioral MM assessment, involuntary grip force oscillations during contralateral hand pinching, and EMG recordings during contralateral hand pinching were used to quantify the amount or amplitude of involuntary MM. We used behavioral MM assessment as the primary measure to investigate Aim 1, given it is easy to administer clinically.

Behavioral Mirror Movements Assessment. Children performed unilateral movement tasks for the purpose of examining the involuntary MM in the contralateral

hand, including 1) whole hand opening and closing (Kuhtz-Buschbeck et al., 2000), 2) thumb-finger opposition (Woods & Teuber, 1978), 3) finger individuation (Kuhtz-Buschbeck et al., 2000), 4) index and middle finger "walking". During testing, participants sat comfortably at the chair with hip/knee joints at 90° flexion. They were instructed to perform the movements at the frequency of 1Hz (cued by a metronome). They started the task with the less-affected hand and then with the more-affected hand. Each task was performed for 5 trials. For the whole hand opening/closing task, they held up the UE in the air so that the shoulder joints are at 90° of flexion and elbow joints fully extended. Children were instructed to close the moving hand at the beat. For the thumb-finger opposition task, children rested their elbows on the table adjusted to a comfortable height with forearms straight up. During testing, they tapped each finger to thumb with the following order: index, middle, ring, little, index, middle, ring, little finger and so forth. For the finger individuation task, they rested both hands on the table with palms facing down (elbow at 90° flexion) for the starting position. During testing, they lifted and tapped each finger on the table surface as the following order: thumb, index, middle, ring, and little and so on. For the index and middle finger walking task, they started with resting one hand on the table with palm facing down, the moving hand was prepared for the task (participants either tucked the ring & little fingers with the thumb, or at least tried separating the index & middle fingers). During testing, they individuated and tapped the index finger and then middle finger on the table surface alternatively and "walked" the fingers forward (away from body). Task performance by either hand was videotaped. Inter- and intra-rater reliability were recently reported to be high for the first three tasks (ICC> 0.82) (Klingels et al., 2015).

Grip Force Oscillation during Repetitive Contralateral-Hand Pinch.

Participants pinched the transducer device (ATI Industrial, Apex, NC) using precision pinch of one hand while the other hand was gently holding another device. Force output of both hands was recorded using WinSC (Umeå University, Sweden) at the sampling frequency of 400Hz. For the starting position, children sat a table in front of their body so that the shoulders are at 0° flexion/extension on the side of the body; elbows are at 90° flexion, and the forearms resting on the table surface in neutral position. Participants began the task when holding the transducer discs gently in both hands. They were instructed to pinch the transducer disc repetitively using thumb and index fingers of one hand by following the tone programmed at 1 Hz (WinSC, Umeå University, Sweden) (Koerte et al., 2011). Children were first given a short practice session to familiarize themselves with the task. During testing, they were asked to perform 20 pinches with either hand (20 seconds each hand, first using the less-affected, then the more-affected). A short break after the 20 trials completed by one hand was allowed to avoid fatigue. Participants were measured for the maximal pinch force with either hand in the end of the testing, for the purpose of obtaining the percent of maximal voluntary contraction (%MVC).

Electromyography (EMG) Recordings. Similar to the GF oscillations paradigm, in order to quantify the intensity of MM, participants performed a precision pinching task with only one hand while the other hand was resting. Muscle activities were recorded using surface EMG. A four-channel recording system (NeuroConn, Ilmenau, Germany) recorded muscle activities in FDI and wrist flexor muscles bilaterally when children performed the task. Children were seated comfortably with arms and

hands supported in pillows/cushions prior to the start of the task. Investigators ensured the EMG signals were quiet and clean before recording. For the motor task, children pinched their unilateral index and thumb fingers by following a Powerpoint presentation. They were instructed to start pinching with their less-affected hand for 10 trials, followed by pinching with their more-affected hand for 10 trials. Each "pinch" slide (1 trial) lasted for 5 seconds (Seo, 2013), which followed by 7 seconds of a "relax" slide.

v. Data Analysis

TMS Data Analysis. EMG data during brain stimulation were imported into MATLAB (Mathworks, Natick, MA). A MATLAB script was written to show the recorded MEP for each muscle. Investigators identified the peak-to-peak MEP amplitude. A MEP amplitude ≥50μV is conventionally considered as a valid response to a TMS stimulus (Staudt et al., 2002). Finally, CST connectivity controlling the more-affected UE was determined by verifying valid MEP responses from findings of stimulating each M1 for each child. Children were categorized into ipsilateral, contralateral, or bilateral connectivity.

DTI Tractography. We used DTI Studio (Johns Hopkins University, Baltimore, MD) to reconstruct the contralateral CST of the more-affected UE for the DTIs obtained at CUMC. Similarly, we used Diffusion Toolkit and TrackVis (Massachusetts General Hospital, Boston, MA) to reconstruct the CST of the affected UE for the DTIs obtained at WCMC. Details of tractography using DTI Studio software can be found in Kuo et al. (2016) (Appendix E). Details of tractography using Diffusion Toolkit and TrackVis are the following. First, we corrected for movement artifacts with eddy current correction function using FSL (Analysis Group, Oxford, UK). We then used Diffusion Toolkit to

reconstruct the fibers and color maps by using the corrected diffusion weighted images as mask images. Subsequently we seeded a sphere-shaped region of interest (ROI) at the pyramidal tract at the lower pons level (blue coded areas) on an axial slice to reconstruct the CST by TrackVis (Thomas et al., 2005). A presence or absence of the CST projecting from the more-affected M1 was verified.

Behavioral Mirror Movements Analysis. A standardized score ranging from 0-4 was used to quantify the amount of visible mirror movements in the involuntary hand (Woods & Teuber, 1978). To illustrate, the following is how Woods & Teuber (1978) defined the scores: "score 0= no clearly imitative movement; 1= barely discernible repetitive movement; 2= either slight, but unsustained, repetitive movement, or stronger, but briefer, repetitive movement; 3= strong and sustained repetitive movement; and 4= movement equal to that expected for the intended hand."

All videos were scored by a physical therapist blinded to the CST connectivity findings, treatment allocation, and children's hand function scores. The mode of 5 trials was used for analysis. We explored the behavioral data by grouping subjects into different categories, such as presence (scores 1-4) versus absence (score 0) of MM, and stronger (either scores 3-4 or scores 2-4) versus weaker (scores 0-2 or scores 0-1) MM.

Grip Force Oscillations Analysis. Both the voluntary and involuntary finger grip force oscillations (2 separate channels) were collected and stored in a PC computer and extracted offline by using Winzoom (Umeå University, Sweden). The resulting mean grip force (GF) of the thumb (GF1) and index (GF2) fingers was calculated as (GF1+GF2)/2. The maximal and minimal GF oscillation of both hands, the amplitude of involuntary mirroring GF oscillations in the involuntary hand (maximal – minimal GF),

and the relative time difference between the two hands at the maximal oscillation time points (absolute time of mirroring- absolute time of voluntary movements) were the primary variables. To account for the differences in the individual voluntary GF, a ratio of involuntary/voluntary GF was also calculated.

EMG Data Analysis. EMG data were imported into MATLAB (Mathworks, Natick, MA). A MATLAB script was written to show the recordings from bilateral FDI and wrist flexors. The onset and offset of each pinch was identified visually. Similar to the GF oscillations analysis, a ratio representing the relative strength of MM was calculated by having mirroring amplitude divided by voluntary amplitude. EMG amplitude was defined as the root mean square of the power spectrum of the EMG signals. In addition, onset latency between the two hands was calculated.

vi. Behavioral Outcome Measures

The Jebsen-Taylor Test of Hand Function (JTTHF). The JTTHF is a standardized test quantifying unilateral dexterity as the movement time (seconds) to complete unimanual fine motor tasks (Jebsen, Taylor, Trieschmann, Trotter, & Howard, 1969). It consists of subtests including card flipping, small objects manipulation and placement, simulated eating, checker stacking, and empty and full can manipulation. JTTHF was modified as a child-friendly evaluation (the evaluator stops each subtest and records 180 seconds as the completion time when he/she perceived that a child was unable to complete the subtest, to prevent frustration and fatigue). Reliability is high for children with stable hand disability (0.95-0.99) (Taylor, Sand, & Jebsen, 1973).

The Assisting Hand Assessment (AHA). The AHA (version 4.3) quantifies the effectiveness of the more-affected hand use in bimanual play activities (Krumlinde-

Sundholm, Holmefur, Kottorp, & Eliasson, 2007). The AHA has excellent validity and reliability (inter-rater=0.97, intra-rater=0.99) (Krumlinde-Sundholm et al., 2007). The test was videotaped and scored off-site by an evaluator blinded to group allocation. Data is reported in 0-100 AHA units (Krumlinde-Sundholm, 2012).

Canadian Occupation Performance Measure (COPM). To evaluate children's functional goals, we interview the COPM with caregivers. The COPM identifies and measures changes in daily functional problems (Carswell et al., 2004). It has excellent validity and reliability (Verkerk, Wolf, Louwers, Meester-Delver, & Nollet, 2006). The functional goals to be practiced at camp were identified, ranked in importance, and rated on performance and satisfaction prior to the beginning of camp. Caregivers chose the goals (e.g., cutting food, dressing, using a keyboard) and rated the child's performance and level of satisfaction on a scale of 1-10, with 10 being the highest score.

vii. Statistical Analysis

Statistical analyses were performed using SPSS (IBM, NY, version 22). Non-parametric statistical analyses were performed to analyze ordinal variables (i.e., behavioral MM scores). Parametric statistical analyses were performed to analyze continuous variables (i.e., involuntary GF oscillations measured by force transducer, and involuntary muscle contraction measured by EMG).

To achieve Aim 1, two-tailed Fisher's exact tests were used to examine if a greater amount of MM was associated with CST connectivity assessed by TMS or DTI. Two by two contingency tables were used to compare TMS-probed CST connectivity with the quantification of MM. DTI-assessed connectivity was used as another assessment of CST connectivity to compare with the amplitude of MM.

To investigate Aim 2, Wilcoxon signed ranks test was used to test whether the changes in behavioral MM scores from the pretest to the posttest were significant. Two (intervention groups) by two (test sessions) repeated measure ANOVAs were used to examine if the intensity of MM measured with involuntary GF oscillations and EMG changed after intensive hand training and to examine if the CST connectivity impacts children's responsiveness to different forms of treatment.

To examine the direction of MM overflow, Wilcoxon singed rank test was performed to compare the behavioral MM scores between the two hands for each task. Paired t-tests were performed to compare the amplitude of MM measured with GF oscillations and EMG between the two hands. Linear regression models and correlational analyses (Spearman's rho for behavioral MM scores and Pearson's r for involuntary GF oscillations and EMG) were performed to examine how MM may impact hand function (i.e., JTTHF, AHA, COPM) and how age may affect MM in USCP. A Mann-Whitney U test was performed to examine whether the intensity of behavioral MM was affected by brain lesion type. One-way ANOVA was conducted to examine whether the intensity of GF and ratio of EMG were affected by brain lesion type. Finally, logistic regression models were performed to examine whether measures of MM were additive to predict CST connectivity. P-values < 0.05 were considered statistically significant.

viii. Sample Size Calculation

Sample size estimation was calculated from preliminary data of the first three cohorts (2012-2014). G*power version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007) was used to calculate the sample size. We first calculated the required sample size based on our preliminary data of behavioral MM scores (Woods & Teuber, 1978). To achieve

Aim 1, behavioral MM scores were put into a contingency table (Table 1, Appendix A). We used TMS-identified CST connectivity as the standardized outcome (ipsilateral versus contralateral/bilateral) and clinical testing scores (3-4: strong MM, 0-2: weak MM) as the secondary outcome. A Fisher's exact test with 2 independent groups was first used for sample size estimate. In our preliminary data (n= 11), $p1$= 0.6 (behavioral MM scores of 3-4 in the ipsilateral connectivity), $p2$= 0.17 (behavioral MM scores of 3-4 in the contralateral connectivity), α= 0.05 (type I error), and power= 0.8 (β= 0.2, type II error). This calculation yielded a sample size of 36 subjects to produce an actual power of 0.81. A similar calculation with $p1$= 0.6, $p2$= 0.17, α= 0.05, and only changing the power to 0.7 (β= 0.3, type II error) yielded a sample size of 32 subjects to produce an actual power of 0.72. A separate sample size calculation was performed based on the preliminary GF oscillations data (Table 2, Appendix A). Similarly, we used TMS as primary outcome and a criteria of GF >0.3N from the involuntary hand to determine the presence of mirror activities (Kuhtz-Buschbeck et al., 2000). In our preliminary data, $p1$= 1, $p2$= 0.333, α= 0.05, and power= 0.8. This yielded a sample size of 18 subjects. As the sample size estimation obtained from calculating the GF data was smaller than that from the behavioral MM scores, a total of 32 subjects would be sufficient to achieve Aim 1 for the primary measure.

III. RESULTS

i. Patient Flow

Patient flow is shown in the flow chart (Figure 1). During the study period (2013-2015), a total of 33 participants were recruited from the parent clinical trial. The inclusion and exclusion criteria of the parent study can be found in the paper in Appendix

F. Children were randomized to receive either CIMT (n= 16) or HABIT (n= 17). They were randomized offsite using concealed allocation stratified by age and JTTHF screening scores. Table 1 describes participants' demographic characteristics. There were no significant differences in children's age (independent t-test, p= 0.99) and in the baseline JTTHF (independent t-test, p= 0.78) between the two intervention groups.

ii. TMS-identified CST connectivity

Results of the TMS-probed connectivity, DTI-assessed connectivity, behavioral MM scores, and the presence of involuntary GF oscillations at baseline are shown in Table 2. For the TMS-probed CST connectivity, 3 participants out of 33 (9.1%) did not have TMS-induced motor evoked potentials (MEP) responses from EMG recordings on the more-affected UE when stimulating either the more-affected or the less-affected motor cortex (M1) (participant #19, 24, 27, Table 2). Of the remaining 30 participants with TMS-induced MEP responses, 17 had ipsilateral CST connectivity (51.5%), 9 had bilateral CST connectivity (27.3%), and 4 had contralateral CST connectivity (12.1%) probed by TMS.

Figure 1. Patient Flow Chart

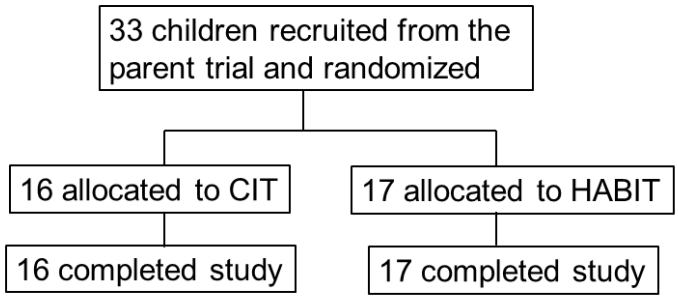


Table 1. Baseline Participant Characteristics

Characteristics	CIMT (n=16)	HABIT (n=17)
Mean Age (SD), years, months	9, 6 (2, 9)	9, 6 (3, 6)
Gender		
Male	11 (68.75%)	9 (52.94%)
Female	5 (31.25%)	8 (47.06%)
Paretic hand		
Right	7 (43.75%)	13 (76.47%)
Left	9 (56.25%)	4 (23.53%)
Lesion type		
CM	0 (0%)	1 (5.88%)
C/SC	1 (6.25%)[a]	2 (11.76%)
MCA	5 (31.25%)	7 (41.18%)
PVL	10 (62.5%)[a]	6 (35.29%)
NA	1 (6.25%)	1 (5.88%)
Race		
African American	1 (6.25%)	2 (11.76%)
Asian	1 (6.25%)	0 (0%)
Hispanic	1 (6.25%)	1 (5.88%)
Mixed	1 (6.25%)	0 (0%)
White	12 (75%)	14 (82.35%)
MACS		
I	2 (12.5%)	5 (29.41%)
II	9 (56.25%)	8 (47.06%)
III	5 (31.25%)	4 (23.53%)
Baseline JTTHF, mean (SD), s	440.96 (298.79)	411.8 (300.99)

Abbreviations: CIMT, constraint-induced movement therapy; HABIT, Hand-Arm Bimanual Intensive Therapy; SD, standard deviation; CM, brain malformation, C/SC, cortical/subcortical lesion, MCA, PVL, periventricular lesion; NA, not available; MACS, Manual Ability Classification System; JTTHF, Jebsen-Taylor Test of Hand Function.[a]One child had both C/SC and PVL.

Table 2. Baseline Results for TMS-probed Connectivity, DTI-assessed connectivity, Behavioral MM scores, involuntary Grip Force Oscillations

Participant #	TMS-probed CST Connectivity[a]	DTI-assessed CST Connectivity	Behavioral MM Scores[b]	Behavioral MM Scores[b]	Involuntary Grip Force Oscillations[c]	Involuntary Grip Force Oscillations[c]
		Presence or Absence of Contralateral CST	More-affected Hand	Less-affected Hand	More-affected Hand	Less-affected Hand
1	Ipsilateral	Absence	3	2	n/a	n/a
2	Ipsilateral	Presence	2	2	n/a	n/a
3	Ipsilateral	Absence	3	4	n/a	n/a
4	Ipsilateral	Absence	0	1	n/a	n/a
5	Ipsilateral	Presence	3	2	n/a	n/a
6	Ipsilateral	Absence	3	3	n/a	n/a
7	Ipsilateral	Presence	0	2	n/a	n/a
8	Ipsilateral	No DTI	0	1	n/a	n/a
9	Ipsilateral	Absence	4	2	n/a	n/a
10	Ipsilateral	Absence	0	3	n/a	n/a
11	Ipsilateral	Absence	0	1	+	+
12	Contralateral	Presence	2	2	-	-
13	Contralateral	Presence	1	1	-	-
14	Ipsilateral	Absence	3	4	+	+
15	Bilateral	Absence	2	2	+	+
16	Contralateral	Presence	1	1	-	-
17	Bilateral	Presence	3	2	+	+
18	Ipsilateral	Absence	3	2	+	+
19	No responses	Absence	2	1	+	+
20	Ipsilateral	Presence	0	0	+	-
21	Contralateral	Presence	1	0	-	-
22	Ipsilateral	Absence	3	2	u	u
23	Bilateral	No MRI	0	3	+	+
24	No responses	Absence	2	2	+	+

25	Bilateral	Presence	2	2	u	u
26	Bilateral	Presence	2	2	+	+
27	No responses	Absence	0	1	-	-
28	Bilateral	Absence	2	1	+	+
29	Bilateral	No MRI	0	0	u	u
30	Ipsilateral	Absence	0	1	+	+
31	Bilateral	Presence	1	2	n/c	n/c
32	Bilateral	Presence	2	2	+	+
33	Ipsilateral	No MRI	3	2	+	+

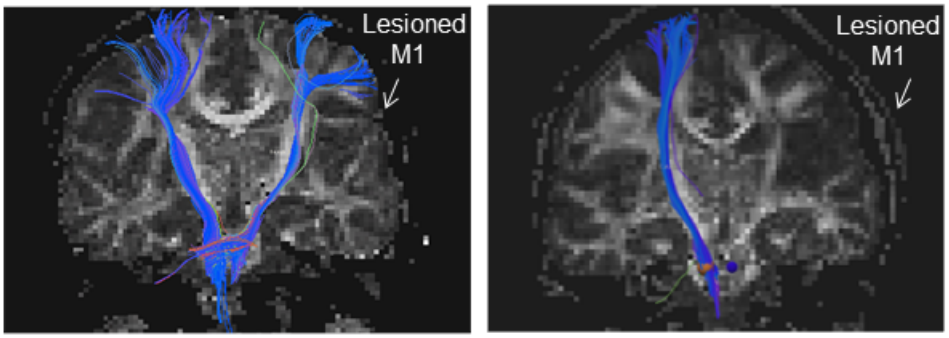
[a]: TMS connectivity: contralateral, presence of TMS-induced responses from EMG recordings on the more-affected UE by stimulating the more-affected motor cortex; ipsilateral, absence of TMS-induced responses from EMG recordings on the more-affected UE by stimulating the more-affected motor cortex, but presence of such responses when stimulating the less-affected motor cortex; bilateral, presence of TMS-induced responses from EMG recordings on the more-affected UE by stimulating both the more- and the less-affected motor cortex;
b: Mirror movements during hand opening/closing , scale score between 0-4, graded by Woods & Teuber criteria;
[c]: +, presence of involuntary GF (>0.3N), -: absence of involuntary GF (<0.3N); n/a: not available; u: participant unable to perform the task; s: unable to analyze data due to spasticity; n/c: participant was not compliant.

iii. DTI-identified CST connectivity

For the results of DTI-identified CST connectivity, we used either presence or absence of a preserved contralateral CST as it was technically difficult to decide which hand(s) the ipsilateral CST originating from the contralesional M1 projects to (see Table 2, also see paper in Appendix E). Four participants out of 33 (12.1%) did not have MRI data (1 child had a teeth brace affecting the DTI signals during data acquisition processes, 3 children were unwilling to participate in this part of the study). Of the remaining 29 participants, 16 children (48.5%) did not have a preserved contralateral CST projecting from the more-affected M1, and 13 children (39.4%) had a preserved contralateral CST (see Figure 2 for the corticospinal tracts reconstructed by DTI tractography of representative children).

Table 3 summarizes the comparison between TMS-probed and DTI-identified CST connectivity. Data from 9 children (patient #1-7, #9-10) had participated in our previous study investigating using DTI to determine the CST connectivity in children with USCP (Kuo et al., 2016) (Appendix E). In 26 children with available TMS mapping and DTI reconstruction results, Fisher's Exact Test showed that DTI may be used as an assessment to determine whether a contralateral CST was present (p= 0.02; sensitivity= 69.2%, specificity= 85%). Results from this study are consistent with those in Kuo et al. (2016).

Figure 2. Reconstructed Corticospinal Tracts of two Representative Subjects assessed by DTI Tractography



Representative child with a preserved contralateral CST

Representative child without a preserved contralateral CST

Table 3. Consistency between TMS-probed and DTI-assessed Contralateral Corticospinal Tract

		Contralateral Corticospinal Tracts detected by DTI		
		Yes[c]	No[d]	
TMS-evoked muscle responses by probing the more-affected M1	Yes[a]	9	2	11
	No[b]	4	11	15
		13	13	26

[a]: TMS yes: presence of TMS-induced MEP responses from EMG recordings on the more-affected UE,

[b]: TMS no: absence of TMS-induced MEP responses from EMG recordings on the more-affected UE,

[c]: DTI yes: presence of CST reconstructed by DTI tractography,

[d]: DTI no: absence of CST reconstructed by DTI tractography.

iv. Characteristics of Behavioral Mirror Movements Assessment Scores

Figure 3 shows the percentage distribution of the behavioral MM assessment scores during the performance of each task in either hand at baseline. Only one participant did not show MM in any task (3%). The remaining 32 participants showed at least minimal amount of discernable repetitive movements (score ≥ 1) during the performance of at least one of the tasks (hand opening/closing, finger opposition, individuation, finger walking).

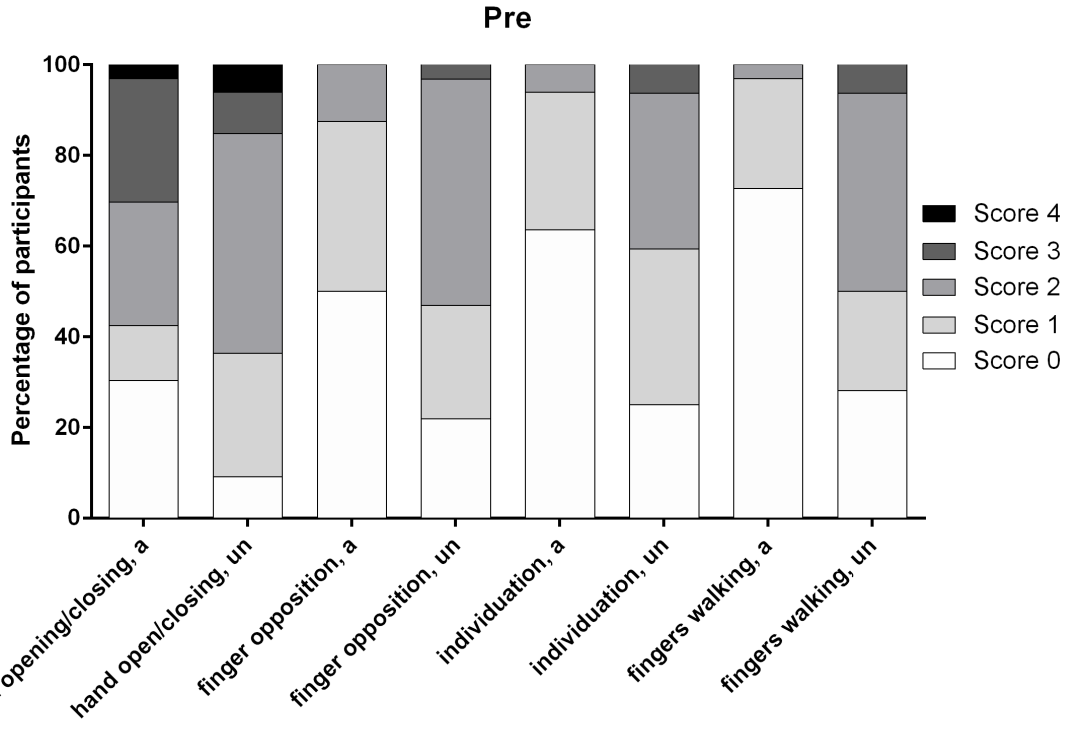
To investigate which task/hand may best capture the presence of MM, we first examined the percentage distribution between presence of MM (scores 1-4) and absence of MM (score 0) among all tasks. Performing all 4 designated tasks with the more-affected hand induced comparatively higher percentages of MM in the less-affected hand. Specifically, performing whole-hand opening/closing with the more-affected hand induced the highest percentage of the visible MM (score≥ 1) in the less-affected hand (n= 30, 90.9%, Figure 3). This incidence was followed by the percentages of the occurrence of MM in the less-affected hand when performing finger opposition, finger individuation, and index and middle finger walking with the more-affected hand (78.13%, 75.01%, 71.88% respectively). Performing all 4 tasks with the less-affected hand induced relatively lower percentages of MM in the more-affected hand during the same task. Performing whole-hand opening/closing, finger-thumb opposition, finger individuation, and index and middle finger walking with the less-affected hand induced < 70% of MM in the more-affected hand (69.69%, 50%, 36.36%, and 27.27%, respectively).

We subsequently investigated percentage distribution between scores 3-4 versus scores 0-2 to compare which task may induce stronger MM in children with USCP.

Results showed that performing whole-hand opening/closing with the less-affected hand induced the highest percentage of strong MM in the more-affected hand (n=10, 30.3% for score ≥ 3, see Figure 3). Mirror movements in the less-affected hand while performing the same task with the more-affected hand induced the second highest percentage of strong MM (n= 5, 15.15% for scores ≥ 3). Performing finger-thumb opposition, finger individuation, and fingers walking with the more-affected hand induced < 10% of strong MM in the less-affected hand (3.13%, 6.25, and 6.25% for scores ≥ 3 respectively). Finally, performing finger-thumb opposition, finger individuation, and fingers walking with the less-affected hand did not induce any occurrence of strong MM in the more-affected hand.

In order to examine the direction of movement overflow, we performed Wilcoxon signed rank test to compare the behavioral MM score differences within the same subject between the two hands. Results showed that a significant higher MM score occurred in the less-affected hand during the performance of finger-thumb opposition, finger individuation, and fingers walking (p= 0.001, p <0.001, p <0.001 respectively), but this side difference was not significant in the scores of hand opening/closing (p= 0.55).

Figure 3. Percentage Distribution of Behavioral Mirror Movement Assessment



a: more-affected hand

un: less-affected hand

v. Associations between Behavioral Mirror Movements Assessment Scores and CST Connectivity Assessed by TMS and DTI

In order to investigate whether a simple behavioral test can be used to identify the CST organization (Aim 1), we performed several Fisher's Exact Tests to examine the associations between TMS-identified CST connectivity and the amount of MM. Table 4 shows the contingency table testing the hypothesis that stronger MM were associated with an ipsilateral CST. Thirty participants were included in this analysis as 3 children did not have any MEP responses when probed with TMS. We combined children having

bilateral connections with those having a contralateral connection because only one child (out of 9) in the bilateral connectivity group had strong MM (see Figure 4). The remaining 8 children with bilateral connectivity had weaker MM, which was similar to those having a contralateral connectivity. While children with an ipsilateral connectivity presented with various amount of MM (Table 4, ipsilateral row, 52.9% with strong MM, 47.1% with weak MM), we found that 9 children out of 10 with strong MM had an ipsilateral pattern (Table 4, "strong MM scores" column). Fisher's Exact Test demonstrated that a stronger behavioral MM scores in the more-affected hand when opening/closing the less-affected hand were associated with an ipsilateral CST connectivity probed by TMS (p= 0.017). The sensitivity of using strong MM scores as a clinical test to identify the CST connectivity was 90%, and the specificity was 60%. There was no significant relationship between the CST connectivity and the behavioral MM scores in the less-affected hand while hand opening/closing (p= 0.35). Similarly, there was no significant relationship between the CST connectivity and the behavioral MM scores during the performance of the remaining three tasks with either hand (p> 0.49). We found no significant relationship between any of the tasks and the CST patterns by grouping behavioral MM scores as presence (scores 1-4) versus absence (score 0) ($p\geq$ 0.09).

We subsequently asked the question whether behavioral MM scores were additive to predict the CST patterns. To address this question, we examined the presence of MM (score $\geq$ 1) in each task and investigated whether a combination of any two, any three, or all four tasks correlated with the CST patterns. Results showed that any combinations of the presence of MM were not associated with the CST connectivity (Fisher's Exact Test,

any two tasks, $p > 0.13$ in the less-affected hand, $p > 0.69$ in the more-affected hand; any three tasks, $p > 0.43$ in the less-affected hand, $p > 0.63$ in the more-affected hand; all four tasks, $p = 0.71$ in the less-affected hand, $p = 0.63$ in the more-affected hand).

We further examined the associations between DTI-assessed CST connectivity and the amount of behavioral MM scores. No significant associations were found between any categorizations of behavioral MM scores as mentioned previously and DTI-assessed CST connectivity ($p > 0.2$ in the more-affected MM, $p > 0.1$ in the less-affected MM).

Figure 4. Distribution of Behavioral Mirror Movements Scores stratified by CST Connectivity

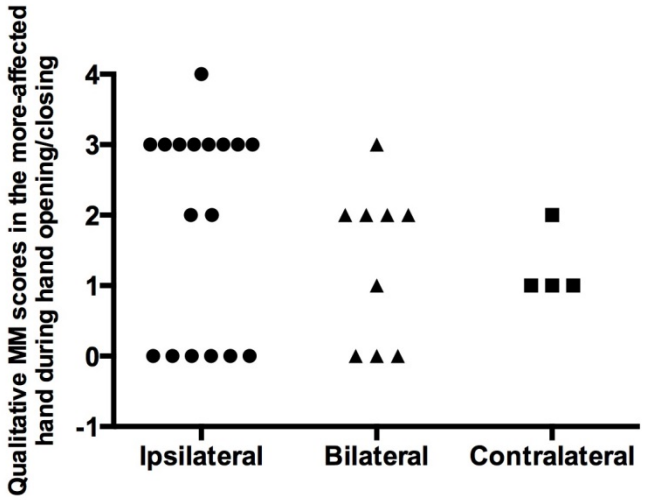


Table 4. Consistency between TMS-probed CST Connectivity & Behavioral MM Scores

		More-affected Hand		
		Strong[a]	Weak[b]	
TMS-measured CST Connectivity	Ipsilateral	9	8	17
	Bilateral or Contralateral	1	12	13
		10	20	30

[a]: Strong MM, scores 3-4 in the more-affected hand during the performance of whole-hand opening and closing

[b]: Weak MM, scores 0-2 in the more-affected hand during the performance of whole-hand opening and closing

vi. Characteristics of Involuntary Grip Force Oscillations during Repetitive Unilateral Pinch

The maximal and minimal grip force (GF) oscillations values and their respective absolute time during the performance of repetitive unilateral pinch were measured in a subset of 23 children. We used the amplitude of GF oscillations > 0.3N as our criteria for defining the occurrence of MM (Kuhtz-Buschbeck et al., 2000). One participant was not compliant; her data were therefore excluded (Table 2, participant #31). The task was too difficult for three other participants to perform, although testing was attempted (participant #22, #25, #29). Of the remaining 19 participants, 14 participants (73.7%) demonstrated involuntary GF oscillation (> 0.3N) in the more-affected hand when pinching with the less-affected hand. One participant out of those 14 only showed MM in the more-affected hand. The remaining 13 participants (68.4%) showed MM in both hands.

Figure 5 plots partial GF oscillations recordings over time of a representative participant with involuntary GF oscillations in the more-affected hand while repetitive pinching with the less-affected hand. The average involuntary grip force oscillations amplitude (maximal - minimal) and temporal characteristics of both hands in children with the occurrence of involuntary grip force oscillation are summarized in Table 5. In summary, performing repetitive unilateral pinch with the less-affected hand at 50.8±22.8 %MVC induced 3.7±2.6N of mirror movements in the more-affected hand (see Table 5, column "less-affected hand pinch"). This resulted in a ratio of 0.19±0.1, indicating that repetitively pinching with the less-affected hand induced ~19% of relative involuntary force oscillations amplitude in the more-affected hand. The time lag between the mirroring hand and voluntary hand was variable among participants. On average, there was a 41.2±39.4ms time lag in the absolute times of the peak GF between the two hands (voluntary GF oscillations in the less-affected hand preceded the mirroring GF oscillations in the more-affected hand by 41ms under the less-affected hand pinch condition). For the other condition, performing repetitive pinch with the more-affected hand at 41.9±22.6 %MVC induced 4.1±2.8N of MM in the less-affected hand. This resulted in a ratio of 1.0±0.8, indicating that repetitively pinching with the more-affected hand induced ~102% of relative mirroring GF oscillations in the less-affected hand. Similar to the previous condition, the onset latency under this condition varied among individuals. Interestingly, the average pattern of this variable was opposite to the latency pattern pertaining to the previous condition— involuntary grip force oscillations in the less-affected hand preceded voluntary force oscillations by 14.1±36.2ms.

Paired t-tests comparing the ratios of GF oscillations amplitude (involuntary/voluntary) between the two hands showed a significant higher ratio in the less-affected hand (less-affected hand MM/more-affected hand voluntary) as compared to that in the more-affected hand (n= 13, t= 3.79, p= 0.003). Significant difference was also found between the oscillations peak latency while pinching with the more- versus the less-affected hand (t= 3.83, p= 0.002). No significance was found when comparing the %MVC measured in the voluntary hand (t= 0.7, p= 0.5) or involuntary GF oscillations amplitude between the two hands (t= 0.25, p= 0.81).

Figure 5. Recordings of Grip Force Oscillations during Unilateral Repetitive Pinch in a Representative Participant with USCP

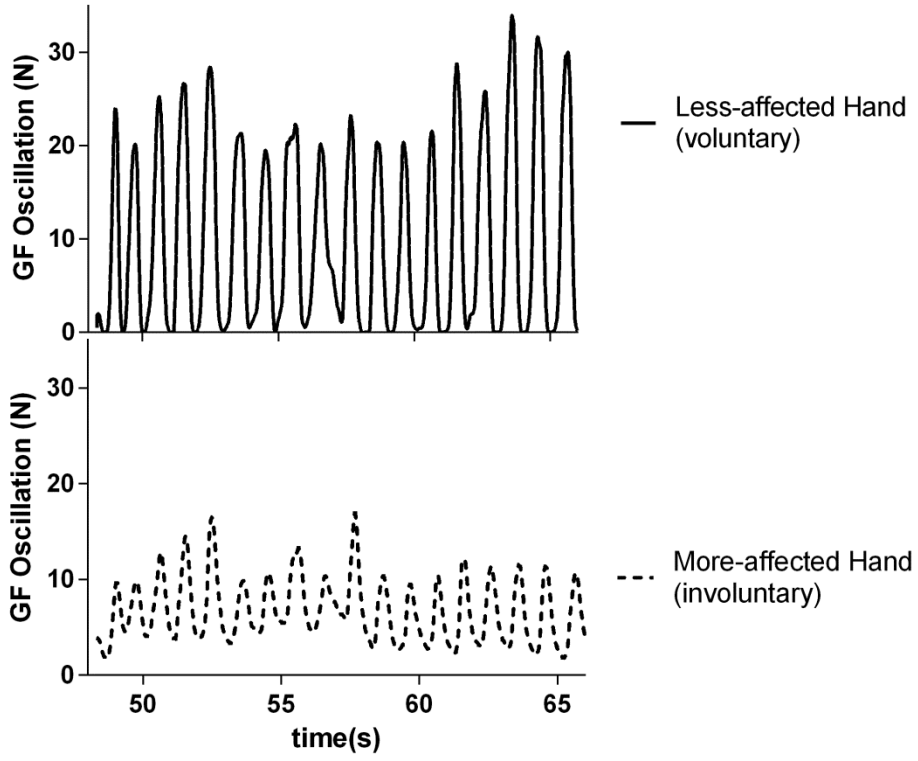


Table 5. Baseline Characteristics of Grip Force Oscillations

	Less-affected hand pinch[a] (n=14)	More-affected hand pinch[a] (n=13)
Voluntary Hand		
Mean GF Oscillations Amplitude (SD), N	19.21 (8.05)	5.07 (2.33)
% of MVC	50.77 (22.77)	41.90 (22.58)[b]
Involuntary Hand		
Mean GF Oscillations Amplitude (SD), N	3.65 (2.6)	4.07 (2.80)
Ratio (SD)[c]	0.19 (0.10)	1.02 (0.80)
Latency of Peak GF, ms[d]	41.24 (39.39)	-14.09 (36.17)

[a]: One column represents the voluntary and involuntary GF oscillations data under the specified condition; [b]: One participant did not follow instruction during performance of maximal grip force, this represents values for 12 subjects; [c]: Ratio=MM/voluntary; d: latency, +: voluntary movement preceded MM, -: MM preceded voluntary movement.

vii. Associations between Involuntary Grip Force Oscillations and CST Connectivity Assessed by TMS and DTI

In order to investigate whether involuntary GF oscillation, a secondary measure, may also be used to identify the CST organization, we performed Fisher's Exact Tests to examine the associations between TMS-probed CST connectivity and the presence or absence of involuntary GF oscillations. Table 6A shows the contingency table testing the hypothesis that the presence of MM measured by force transducer was associated with an ipsilateral CST organization. Sixteen participants were included in this analysis. All children categorized as having ipsilateral connectivity in this subset had involuntary GF oscillations (> 0.3N) in the more-affected hand (Table 6A, ipsilateral row, n= 6). Similar to the children with an ipsilateral pattern, all children with a bilateral connectivity had detectable involuntary GF oscillations (see Table 6B, n= 6). All children with a contralateral connectivity did not have involuntary mirror movements in their more-

affected hand (Table 6B, n= 4). Fisher's Exact Test showed that no significant association was found between the presence of involuntary GF oscillations in the more-affected hand and an ipsilateral CST connectivity probed by TMS (p= 0.23). The sensitivity of using involuntary GF oscillations (> 0.3N) in the more-affected hand as a measure to identify the TMS-probed CST connectivity was 50%, and the specificity was 100%. Likewise, there was no significant association found between the TMS-probed CST connectivity and the involuntary GF oscillations in the less-affected hand (Fisher's Exact Test, p= 0.59). The sensitivity of using mirroring GF oscillations in the less-affected hand as a measure to identify the CST connectivity was 45.45%, and specificity was 80%. No significant relationship was found even when we used the threshold of 1N to define the presence of mirroring GF oscillations (Fisher's Exact Test, more-affected hand, p= 0.31; less-affected hand, p= 0.6).

We further examined the associations between DTI-assessed CST connectivity and the presence or absence of involuntary GF oscillations. Although we had only 17 participants with available data for this comparison, we found significant relationship between the presence of involuntary GF oscillations in the less-affected hand and DTI-assessed CST (Table 7, Fisher's exact test, p= 0.0498). No significant relationship was found between the presence of involuntary GF oscillations in the more-affected hand and DTI-assessed CST (p= 0.13).

Table 6A. Consistency between TMS-identified CST Connectivity and Involuntary GF Oscillations

		More-affected Hand		
		MM Presence[a]	MM Absence[b]	
TMS-measured CST Connectivity	Ipsilateral	6	0	6
	Bilateral or Contralateral	6	4	10
		12	4	16

[a]: MM Presence: involuntary GF >0.3N in the more-affected hand during the less-affected hand pinch

[b]: MM Absence: involuntary GF <0.3N in the more-affected hand during the less-affected hand pinch

Table 6B. Details for Relationship between TMS-identified CST Connectivity and Involuntary GF Oscillations

		More-affected Hand		
		MM Presence[a]	MM Absence[b]	
TMS-measured CST Connectivity	Ipsilateral	6	0	6
	Bilateral	6	0	6
	Contralateral	0	4	4
		12	4	16

Table 7. Consistency between DTI-identified Contralateral CST and presence of Involuntary GF Oscillations

		Less-affected Hand		
		MM Presence[a]	MM Absence[b]	
DTI-identified Contralateral CST	Absence of Contralateral CST	8	1	9
	Presence of Contralateral CST	3	5	8
		11	6	17

viii. Characteristics of Involuntary Muscle Activities Measured by Electromyography during Unilateral Pinch

The EMG was measured in a subset of 32 children. Given that the baseline data for the cohort of 2013 were not available and that repeated-measure ANOVA showed the EMG ratios (involuntary amplitude/voluntary amplitude) did not change for the cohorts of 2014-2015 from the pre-test to the post-test (see Table 13, more-affected hand MM, $F= 1.31$, $p= 0.28$; less-affected hand MM, $F= 0.2$, $p= 0.67$), we used data obtained at the post-test to investigate the characteristics of this measure. Table 8 shows the distribution of the EMG activities in the specified hand. Five children showed spasticity in the more-affected hand during the less-affected hand pinched. One other participant showed spasticity in the more-affected hand while pinching with the less-affected hand. EMG recordings with the presence of spasticity were excluded because it affected the clarity of the baseline signals. The same child who was not compliant during testing using force transducer did not show reliable data during EMG testing either (participant #31); her EMG data were thus excluded. EMG data from 30 participants were available for data

analysis, regardless of hand. We defined the presence of MM when there were any visible and time-locked trials of above-baseline EMG signals in the FDI muscle of the involuntary hand when the voluntary hand pinched (Table 8, +). For the MM in the more-affected hand while the less-affected hand pinched, EMG recorded from 26 participants were analyzed. Twenty-two participants (78.8%) showed MM in the more-affected hand, and the remaining four participants (12.1%) did not show involuntary MM under the same condition (see Figure 6 for representative EMG data). The average ratio of involuntary EMG amplitude divided by voluntary EMG amplitude in those 22 children with visible MM was 0.53±0.49 (Table 9). Similar to the GF oscillations latency, the onset latency between the muscle contractions of the two hands was variable among individuals. The average onset latency measured by EMG was -169±1160ms (mirroring muscle contraction in the more-affected hand occurred before voluntary contraction). For the MM in the less-affected hand, EMG recording obtained from 28 participants were analyzed. Twenty-five participants (75.8%) showed involuntary MM in the less-affected hand, and the remaining three participants (9.1%) did not show MM under the same condition. The average ratio of involuntary/voluntary EMG amplitudes in these 25 children was 2.46±5.32 (Table 9). Similarly as the previous condition, onset latency between the muscle contractions of the two hands was variable. The average onset latency of the onset of two hands' EMG was 50±293ms (mirroring contraction in the less-affected hand occurred after the voluntary contraction).

Paired t-tests were performed to examine if the ratio was higher when one hand pinched versus the other. Results showed that there was no significant difference

between ratios of the two sides during unilateral precision pinch (n= 24, t= 1.81, *p*= 0.08), nor was the onset latency differed between the two hands (n= 20, t= 0.91, *p*= 0.38).

Figure 6. EMG Recordings of a Representative Participant with USCP

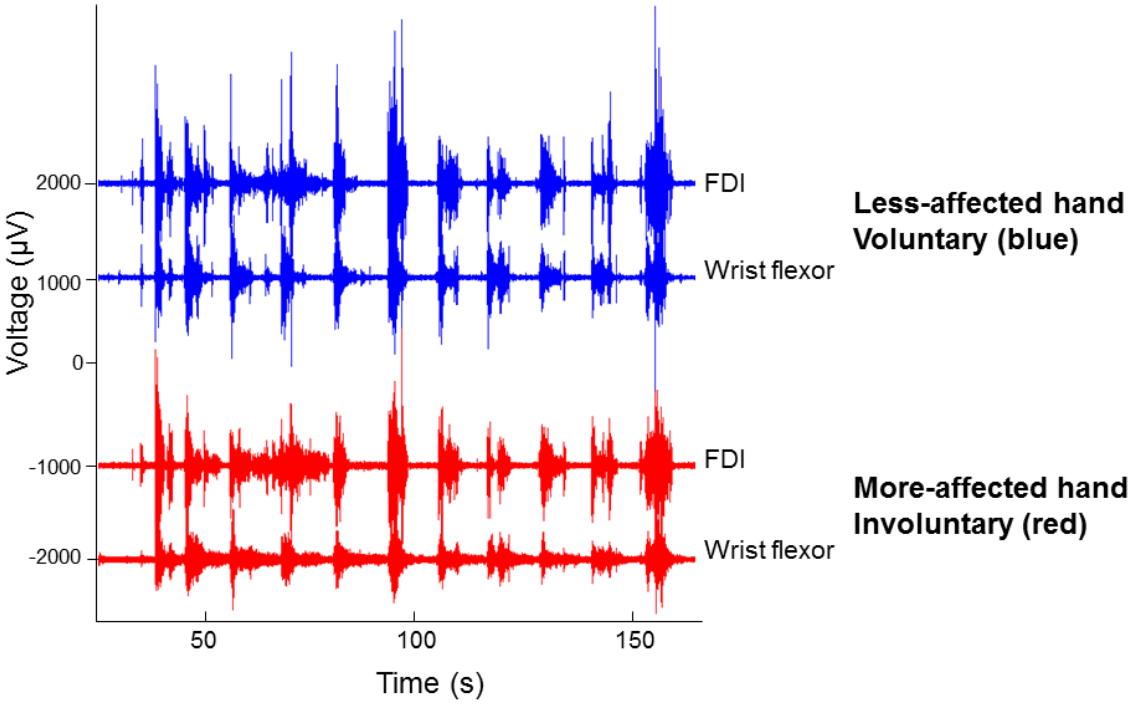


Table 8. Results of Involuntary EMG at Post-test

Participant #	TMS connectivity	EMG[a] More-affected hand	EMG[a] Less-affected hand
1	Ipsilateral	s	+
2	Bilateral	+	+
3	Ipsilateral	+	+
4	Ipsilateral	+	+
5	Ipsilateral	-	+
6	Ipsilateral	+	+
7	Bilateral	+	u
8	Ipsilateral	+	s
9	Ipsilateral	n/a	n/a
10	Ipsilateral	+	+
11	Ipsilateral	+	+
12	Bilateral	+	+
13	Contralateral	-	+
14	Ipsilateral	+	+
15	Bilateral	+	+
16	Contralateral	-	-
17	Bilateral	+	+
18	Ipsilateral	+	+
19	No responses	+	+
20	Ipsilateral	+	+
21	Contralateral	-	-
22	Bilateral	+	+
23	Bilateral	+	+
24	No responses	+	+
25	Bilateral	s	s
26	Bilateral	+	+
27	No responses	s	+
28	Bilateral	s	+
29	Bilateral	s	-
30	Ipsilateral	+	+
31	Bilateral	n/c	n/c
32	Bilateral	+	+
33	Ipsilateral	+	+

a: +, presence of EMG activities of involuntary hand, defined as > 0 % of successful trials,

-, absence of EMG activities of involuntary hand; n/a: not available; u: participant unable to perform the task; s: unable to analyze data due to spasticity;

n/c: participant was not compliant.

Table 9. Characteristics of EMG at Post-test

	Less-affected hand pinch (n=22)	More-affected hand pinch (n=25)
Ratio (SD)[a]	0.53 (0.49)	2.46 (5.32)
Onset latency, ms[b]	-169.19 (1160.31)	50.06 (292.91)

[a]: Ratio=MM/voluntary; [b]: latency, +: voluntary movement preceded MM,
-: MM preceded voluntary movement.

ix. Associations between Involuntary Muscle Activities Measured by EMG and CST Connectivity Assessed by TMS and DTI

In order to investigate whether involuntary mirroring muscle contractions measured by EMG may also be used to identify the CST connectivity, we performed Fisher's Exact Tests to examine the associations between CST connectivity and the presence or absence of involuntary muscle activities. Twenty-four children were included in the analysis comparing TMS-probed connectivity and involuntary EMG in the more-affected hand. Eleven children with an ipsilateral connectivity showed involuntary muscle activities recorded from the more-affected hand when pinching with the less-affected hand (Table 10A, the ipsilateral row). One child with an ipsilateral connectivity did not show involuntary muscle activities under the same condition. All children with a contralateral connection did not present with mirror activities in the more-affected hand. All children with a bilateral pattern showed involuntary muscle contractions in the more-affected hand (see Table 10B for details). Fisher's Exact Test showed that there was not a significant association between the presence of involuntary muscle activities in the more-affected hand and an ipsilateral CST probed by TMS (p= 0.59). The sensitivity of using mirroring EMG in the more-affected hand as a measure to

identify the CST connectivity was 45%, and the specificity was 75%. Similarly, there was no significant relationship between involuntary muscle contractions in the less-affected hand and TMS-probed connectivity. However, the specificity of using presence of involuntary EMG in the less-affected hand to identify a non-ipsilateral pattern probed by TMS was 100% (Table 10C, Fisher's Exact Test, p= 0.1; sensitivity= 59.1%, specificity= 100%).

Table 10A. Consistency between TMS-identified CST Connectivity and Involuntary EMG

		More-affected hand		
		MM Presence[a]	MM Absence[b]	
TMS-probed CST Connectivity	Ipsilateral	11	1	12
	Bilateral or Contralateral	9	3	12
		20	4	24

[a]: MM Presence: visible synchronizing muscle activities in the more-affected hand during less-affected hand pinch

[b]: MM Absence: invisible synchronizing muscle activities in the more-affected hand during less-affected hand pinch

Table 10B. Details for the relationship between TMS-identified CST Connectivity and Involuntary EMG

		More-affected hand	
		MM Presence[a]	MM Absence[b]
TMS-probed CST Connectivity	Ipsilateral	11	1
	Bilateral	9	0
	Contralateral	0	3

Table 10C. Consistency between TMS-identified CST Connectivity and Involuntary EMG

		Less-affected Hand		
		MM Presence[a]	MM Absence[b]	
TMS-probed CST Connectivity	Ipsilateral	13	0	13
	Bilateral or Contralateral	9	3	12
		22	3	25

A second analysis approach was performed to examine whether a higher EMG ratio was associated with an ipsilateral CST connectivity. For this second approach, we defined strong MM as when the EMG ratio (involuntary/voluntary amplitude) > 0.5; weak MM was defined when the EMG ratio < 0.5. Twenty-four children were included in the analysis comparing TMS-probed connectivity and EMG ratios. Five children with an ipsilateral connectivity showed strong MM in the more-affected hand when they voluntarily pinched with the less-affected hand (Table 11A, the ipsilateral row). Under this condition, seven children with an ipsilateral connectivity had weak MM. Two children with bilateral connectivity had strong MM, whereas the remaining seven children with bilateral connectivity had weak MM. All three children with a contralateral connection had weak MM. Fisher's Exact Test did not show a significant association between a strong MM (involuntary/voluntary ratio> 0.5) measured with EMG in the more-affected hand and an ipsilateral CST connectivity (p= 0.37; sensitivity= 71.4%, specificity= 58.8%). We did not find a significant association between strong EMG-measured MM in the less-affected hand and the TMS-probed CST connectivity (Table 11B, Fisher's exact, p= 0.14). When investigating strong EMG-measured MM in the

less-affected hand, twenty five children were included in the contingency table. Twelve children with an ipsilateral connectivity had strong MM, whereas 1 child with the same pattern had weak MM. Seven children with bilateral connections had strong MM, while 1 child with bilateral connections had weak MM. All three children with a contralateral pattern had weak MM. The sensitivity of using strong MM measured with EMG in the less-affected hand as a measure to identify the CST connectivity was 63.2%, and the specificity was 80%.

We further examined the associations between the presence of MM measured by EMG and DTI-assessed CST connectivity. Fisher's exact test showed that there was a significant association between the presence of MM measured by EMG in the more-affected hand and DTI-assessed connectivity (Table 12A, n= 23, p= 0.04, sensitivity= 63.2%, specificity= 100%). No significant association was found between the presence of EMG-measured MM in the less-affected hand and DTI-assessed connectivity (p= 0.15). We also found a significant association between strong MM in the less-affected hand measured by EMG and DTI-assessed connectivity (Table 12B, n=25, p= 0.02, sensitivity=71.4%, specificity=100%). No significant association was found between strong MM measured by EMG in the more-affected hand and DTI-assessed connectivity, however (p= 0.64).

Table 11A. Consistency between TMS-identified CST Connectivity and EMG ratios

		More-affected hand		
		Strong MM[a]	Weak MM[b]	
TMS-probed CST Connectivity	Ipsilateral	5	7	12
	Bilateral or Contralateral	2	10	12
		7	17	24

[a]Strong MM: the ratio of the involuntary EMG amplitude/voluntary EMG amplitude>0.5

[a]Weak MM: the ratio of the involuntary EMG amplitude/voluntary EMG amplitude<0.5

Table 11B. Consistency between TMS-identified CST Connectivity and EMG ratios

		Less-affected hand		
		Strong MM[a]	Weak MM[b]	
TMS-probed CST Connectivity	Ipsilateral	12	1	13
	Bilateral or Contralateral	7	4	11
		19	5	24

Table 12A. Consistency between DTI-identified CST and presence of Involuntary MM measured by EMG

		More-affected Hand		
		MM Presence[a]	MM Absence[b]	
DTI-identified Contralateral CST	Absence of Contralateral CST	12	0	12
	Presence of Contralateral CST	7	4	11
		19	4	23

[a]: MM Presence: presence of visible synchronizing muscle activities in the more-affected hand

[b]: MM Absence: absence of visible synchronizing muscle activities in the more-affected hand

Table 12B. Consistency between DTI-identified CST and strong MM measured by EMG

		Less-affected hand		
		Strong MM[a]	Weak MM[b]	
DTI-identified Contralateral CST	Absence of Contralateral CST	15	0	15
	Presence of Contralateral CST	6	4	10
		21	4	25

[a]: Strong MM: ratio (involuntary amplitude/voluntary amplitude)>0.5

[b]: Weak MM: ratio (involuntary amplitude/voluntary amplitude)<0.5

x. Relationship between Additive of Mirror Movements Measures and CST Connectivity

We further asked the question whether MM measures were additive to predict the CST connectivity. Logistic regression models were performed to examine whether a combination of MM measures (i.e., behavioral MM scores during whole-hand opening/closing, involuntary GF oscillations, involuntary EMG) was associated with TMS-probed CST patterns (ipsilateral versus contralateral/bilateral connectivity). The

best fitted regression model was found when we used dichotomous behavioral MM scores during hand opening/closing (strong versus weak) in the more-affected hand as a predictor and brain lesion type as a covariate to predict TMS-probed CST connectivity (p= 0.002). Likelihood ratio tests showed that both behavioral MM scores in the more-affected hand and brain lesion type were both significantly associated with TMS-probed CST connectivity (behavioral MM, p= 0.007; brain lesion type, p= 0.02). When we used MM scores during hand opening/closing (strong versus weak) in more-affected hand alone to predict TMS-probed CST patterns, behavioral MM scores was still significantly associated with CST connectivity (p= 0.006). No significant associations were found between any other combinations of MM measures and the TMS-derived CST patterns.

Similarly, logistic regression models were performed to examine whether a combination of MM measures was associated with DTI-assessed CST patterns. The best fitted regression model was found when we used the involuntary GF oscillations (> 0.3N or < 0.3 N), behavioral scores during hand opening/closing (strong versus weak), and strong MM measured by EMG (ratio> 0.5) in the less-affected hand as predictors and brain lesion type as a covariate to predict DTI-assessed CST connectivity (p= 0.007). Likelihood ratio tests indicated that lesion type was associated with DTI-assessed CST connectivity (p= 0.001), while the behavioral MM scores (p= 0.45), involuntary GF oscillations (p= 0.64), and strong MM measured by EMG (p= 0.25) did not contribute significantly to the DTI-assessed CST connectivity. When we took away lesion type and used behavioral MM scores, involuntary GF oscillations, and EMG-measured MM in the less-affected hand to predict DTI-assessed CST patterns, involuntary GF oscillations in the less-affected hand was significantly associated with DTI-assessed CST connectivity

(p= 0.005). No significant associations were found between DTI-probed CST connectivity and strong MM measured by EMG (p= 0.15) as well as between DTI-probed CST connectivity and strong behavioral MM (p= 0.47).

xi. Changes in Outcome Measures after Intervention

To investigate Aim 2 (exploratory aim), we examined whether the amount of MM changed after 3 weeks of intensive hand training. Table 13 summarizes the results for the changes in all outcome measures after intervention. For the behavioral MM scores, Wilcoxon signed rank test showed that the median of differences in the behavioral MM scores between the pre-test and post-test sessions distributed around 0 (p> 0.06). Similarly, statistical results obtained from the main effect of the repeated measure ANOVA showed that the changes from the pre-test to the post-test were not significant in the amplitude of involuntary GF oscillations (p> 0.33), in the ratios of GF oscillations amplitude (involuntary/voluntary) (p> 0.09), or in the ratios of EMG amplitude (involuntary/voluntary) (p> 0.28) in either hand.

Table 13. Changes in Behavioral MM Scores and GF Oscillations after 3-weeks of Intervention

Measures	n	Test Statistics[a]	*p*-value[a]
Behavioral MM Scores in the More-affected Hand			
Whole Hand Opening/Closing	33	-0.29	0.77
Finger-thumb Opposition	31	0	> 0.99
Finger Individuation	32	0	> 0.99
Index and Middle Finger Walking	32	0	> 0.99
Behavioral MM Scores in the Less-affected Hand			
Whole Hand Opening/Closing	32	-0.87	0.38
Finger-thumb Opposition	31	-1.9	0.06
Finger Individuation	32	-1.62	0.11
Index and Middle Finger Walking	32	-0.63	0.53
GF in the More-affected Hand[b]	14	1.05	0.33
GF in the Less-affected Hand[b]	16	0.38	0.55
GF ratio (more-affected MM/less-affected voluntary)[b]	14	3.37	0.09
GF ratio (less-affected MM/more-affected voluntary)[b]	16	0.02	0.9
EMG ratio (more-affected MM/less-affected voluntary)[b]	13	1.31	0.28
EMG ratio (less-affected MM/more-affected voluntary)[b]	14	0.2	0.67

[a]: statistics results were obtained from using Wilcoxon Signed Rank Test for qualitative MM scores (Z),
and from using repeated measure ANOVA for the GF and
EMG (F);
[b]: Data included only subjects with presence of mirror movements at both sessions.

xii. Relationship between the Amount of Mirror Movements and Hand Function

We performed linear regression models and correlation coefficients to examine whether the occurrence of MM affected UE functional performance. Linear regression models did not show significance when using behavioral MM scores to predict bimanual hand function measured by the AHA (Table 14, n= 21, $p \geq 0.18$ for all models). Yet linear regression models showed significant associations when using behavioral MM

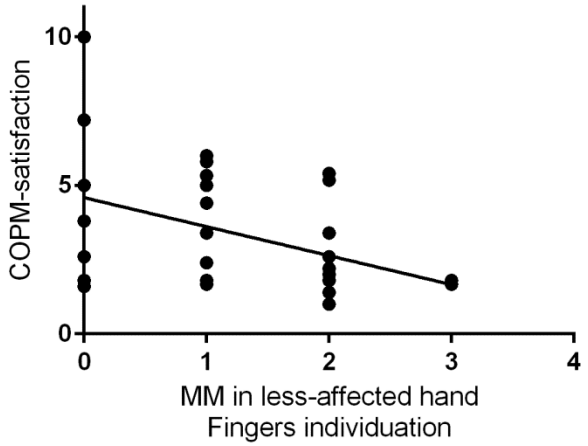
scores in the less-affected hand during finger individuation and finger walking to predict children's goal performance (measured by COPM satisfaction scores, Figure 7A & 7B; Table 14, finger individuation: F= 6.6, *p*= 0.016; finger walking: F= 6.7, *p*= 0.015). Specifically, higher MM scores in the less-affected hand during those two repetitive movements were fairly correlated with worse goal performance (finger individuation, Spearman's r= -0.36, *p*= 0.04; finger walking: Spearman's r= -0.39, *p*= 0.03). Interestingly, linear regression models demonstrated significant associations when we used the behavioral MM scores in the less-affected hand during finger opposition and finger walking to predict unimanual dexterity (n=32, finger-thumb opposition: F= 4.47, *p*= 0.04, Spearman's rho= -0.35, *p*= 0.048; finger walking: F= 13.01, *p*= 0.001, Spearman's rho= -0.5, *p*= 0.005). However, after removing the only one participant with a MM score of 3, the regression model did not show a significant association when using the behavioral MM scores in the less-affected hand during finger opposition to predict unimanual dexterity (F= 3.2, *p*= 0.08, Spearman's rho= -0.3, *p*= 0.1).

Table 15 shows results obtained using linear regression models and correlation analyses to examine the relationship between MM measured by involuntary GF oscillations and hand function. Although sample size was small for the statistical model, linear regression models demonstrated that involuntary GF oscillations in the more-affected hand and its corresponding ratio may predict children's bimanual performance measured by the AHA (more-affected hand GF, F= 7.4, *p*= 0.024, corresponding ratio, F= 6.96, *p*= 0.03). Pearson R also showed moderate correlations between involuntary GF oscillations amplitude in the more-affected hand and children's bimanual function (n= 11, GF oscillations in the more-affected hand and AHA, Pearson r= -0.67, *p*= 0.024). No

significant associations were found between involuntary GF oscillations and children's goal performance (measured by COPM, $p > 0.08$). Similarly, no significant associations were found between involuntary GF oscillations and children's unimanual dexterity (measured by JTTHF, $p > 0.16$).

Further correlational analyses were performed to examine the relationship between the strength of MM and children's improvements in hand function after 3 weeks of intensive therapy (see Table in Appendix B). No significant linear models or correlations were found between the behavioral MM scores in either hand and children's improvements in unimanual dexterity (linear regression models, $p > 0.64$; Spearman's rho between -0.03 to 0.12, $p > 0.52$) and bimanual hand function (AHA, linear regression models, $p > 0.17$; Spearman's rho between -0.22 to 0.41, $p > 0.07$). A significant linear regression was found when using behavioral MM scores in the less-affected hand during finger walking to predict improvements in goal performance (COPM satisfaction: $F=4.21$, $p = 0.05$; Spearman's rho= 0.35, $p = 0.052$). No other significant associations were found between the remaining behavioral MM scores and children's improvements in their goal performance (linear regression models, $p > 0.08$; Spearman's rho, $p > 0.14$). Similarly, no significant regression models or correlations were found between the involuntary GF oscillations or their corresponding ratios and children's improvements in unimanual dexterity (involuntary GF oscillations in either hand and percentage improvement in JTTHF, $p > 0.22$), bimanual hand function (involuntary GF oscillations in either hand and AHA, $p > 0.14$), or goal performance (GF oscillations in either hand and COPM, $p > 0.07$).

Figure 7A & 7B. Correlations between Behavioral Mirror Movements Scores and Goal Performance



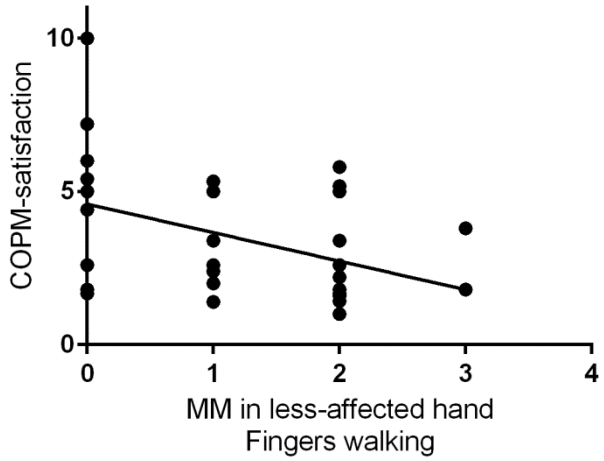


Table 14. Linear regression models and correlations between behavioral MM scores and functional outcome

	AHA (n=21)		COPM-satisfaction (n=32)		COPM-performance (n=32)		JTTHF (n=32)	
	F	Spearman's rho	F	Spearman's rho	F	Spearman's rho	F	Spearman's rho
More-affected hand								
Hand Opening/closing	0.86	0.19	0.46	-0.05	0.77	<0.001	4.33*	-0.26
Finger Opposition	0.37	0.01	1.17	-0.15	0	-0.05	2.11	-0.22
Finger Individuation	0.14	-0.18	1.91	-0.18	0.04	-0.23	0.4	-0.06
Finger Walking	0.04	-0.16	0.98	-0.11	0.02	-0.12	0.56	-0.18
Less-affected hand								
Hand Opening/closing	1.41	-0.19	3.41	-0.33	0.07	-0.17	0.12	0.09
Finger Opposition	0.09	-0.02	2.35	-0.2	0.29	-0.24	4.47*	-0.35*
Finger Individuation	0.13	0.03	6.55*	-0.36*	1.38	-0.31	2	-0.18
Finger Walking	1.92	0.22	6.7*	-0.39*	0.87	-0.3	13.01*	-0.5*

* indicates p-value < 0.05

Table 15. Linear regression and correlations between involuntary GF oscillations and functional outcome

	AHA (n=11)		COPM-satisfaction (n=21)		COPM-performance (n=21)		JTTHF (n=21)	
	F	Pearson's r	F	Pearson's r	F	Pearson's r	F	Pearson's r
More-affected hand								
GF amplitude	7.40*	-0.67*	0.21	-0.1	0.98	-0.22	0.31	0.13
Ratio (I/V)	6.96*	-0.66*	1.31	-0.17	1.32	-0.26	0.16	0.09
Less-affected hand								
GF amplitude	3.12	-0.51	1.31	-0.25	3.49	-0.39	0.96	0.22
Ratio (I/V)	2.09	-0.43	0.76	-0.2	3.12	-0.38	2.11	0.32

* indicates *p*-value <0.05

xiii. The Effects of Age on Mirror Movements in Children with USCP

We examined if age of children affected the intensity of mirror movements in children with USCP. Linear regression models and correlational analyses showed that there was no significant relationship between children's age and the intensity of MM measured by behavioral MM scores, force transducers, or EMG (linear regression model, $p > 0.1$, Spearman's rho and Pearson's R, $p > 0.15$).

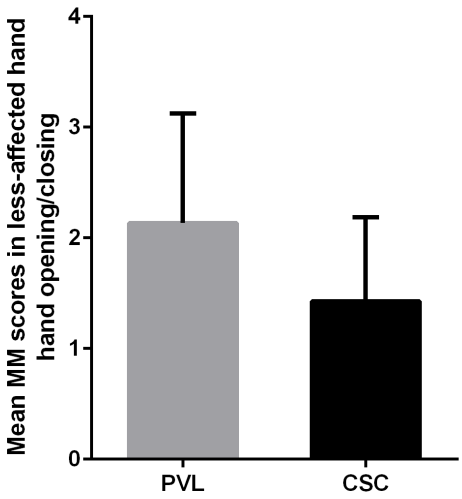
xiv. The Effects of Brain Lesion Type on Mirror Movements in Children with USCP

We subsequently examined whether brain lesion type influenced the amount of MM in children with USCP, providing further evidence that the timing of brain lesion may possibly affect the CST reorganization. There was only one child categorized as brain malformation, therefore we excluded his data in this analysis. In addition, one child was categorized as having both periventricular lesion (PVL) and cortical/subcortical lesion (CSC). Therefore her data were also excluded. Mann-Whitney U test showed that there were significant higher behavioral MM scores in PVL than those in CSC in the less-affected hand during hand opening/closing (Figure 8, $p = 0.04$). We did not find significant differences in the more-affected hand during the same task ($p = 0.08$). Similarly, there were no significant differences found in the remaining behavioral scores in the either hand between different lesion types ($p > 0.08$).

Independent t-tests showed that there were no significant differences in the involuntary GF oscillations amplitude and its corresponding ratio in either hand between different lesion types (involuntary GF oscillations in the more-affected hand, $t = -0.53$, $p = 0.6$; involuntary GF oscillations in the less-affected hand: $t = -0.54$, $p = 0.6$). Similarly,

independent t-tests showed that there were no significant differences in the EMG ratios in either hand between different lesion types (EMG ratio of more-affected/less-affected: t= -0.05, p= 0.96; EMG ratio of less-affected/more-affected: t= -1.5, p= 0.17).

Figure 8. Behavioral Mirror Movement Mean Scores within Periventricular Lesion and Cortical/Subcortical Lesion



PVL=periventricular lesion (early trimester lesion)

C/SC=cortical or subcortical lesion (late trimester lesion)

xv. Quantification of Cortical Control of the More-affected Hand and its Relationship with the Intensity of Mirror Movements

Finally, we investigated whether the amount of mirror movements may be related to the distribution of the corticospinal connections originating from either M1, providing further quantitative measurement of the CST connectivity. Instead of using the CST connectivity pattern, cortical control of the more-affected hand was quantified by the Laterality Index (LI). The LI was calculated with the following formula:

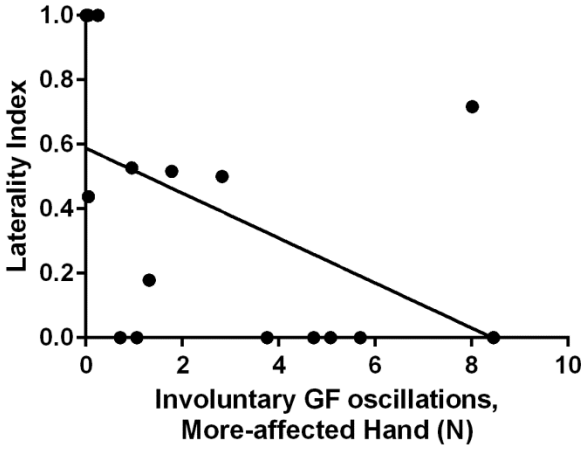
$$\text{LI} = \frac{\text{\# sites controlling the more-affected FDI originating from lesioned M1}}{\text{\# sites controlling the more-affected FDI from both M1s.}}$$

Although sample size was small (n=17), we found a trend when we used the amplitude of involuntary GF oscillations in the more-affected hand to regress the LI (Figure 9, linear regression, F= 4.38, p= 0.054; Pearson r= -0.48, p= 0.054). Specifically, stronger amplitude in the involuntary GF oscillations had a trend to be associated with a lower LI. A similar trend was found when we used the amplitude of involuntary GF oscillations in the less-affected hand to regress the LI (F=4.03, p= 0.063; Person r= -0.29, p= 0.27).

Similarly, we found a trend when we used the ratio of EMG in the more-affected hand (more-affected involuntary/less-affected hand voluntary) to regress the LI (n=23, linear regression, F=3.6, p= 0.07; Pearson r= -0.39, p= 0.08). There was still a trend in the same model even after we removed an outlier (n=22, linear regression, F= 3.2, p= 0.09; Person r= -0.37, p= 0.09).

Finally, we did not find significant linear regression models when we used any behavioral MM scores to predict the LI (n= 28, linear regression, p> 0.25; Spearman's rho, p> 0.29).

Figure 9. Correlation between Involuntary Grip Force Oscillations and Laterality Index



IV. DISCUSSION

This study investigated the relationship between mirror movements (MM) and corticospinal reorganization in children with unilateral spastic cerebral palsy (USCP) by examining with methods probing the CST connectivity and measures quantifying mirror movements. Our hypothesis that stronger MM were more associated with an ipsilateral corticospinal tract (CST) projection was supported by the association between behavioral MM scores in the more-affected hand during hand opening/closing and TMS-probed connectivity (Fisher's Exact Test, p= 0.02). Our hypothesis was also supported by the significant association between force transducer (FT)-measured MM in the less-affected hand and DTI-assessed connectivity (p= 0.0498), as well as supported by the significant associations between electromyography (EMG)-measured MM and DTI-derived connectivity (present of MM, more-affected hand, p= 0.04; strong MM, less-affected hand, p= 0.02). Similar findings were demonstrated in the logistic regression models, where we found that behavioral MM was a significant predictor of TMS-probed

connectivity (p= 0.006), and that FT-measured MM was a significant predictor of DTI-assessed connectivity (p= 0.005). Thus far, the amount of MM did not change after three weeks of intensive hand therapy (behavioral MM scores, p> 0.06; involuntary GF oscillations, p> 0.09; EMG-measured MM, p> 0.28). The occurrence of strong MM (score≥ 3) during hand opening/closing and the presence of involuntary GF oscillations are therefore both indicative of the CST connectivity.

i. Using a Simple Clinical Measure to identify the CST organization

Our data showed exciting results that a simple clinical measure may unfold the underlying motor system reorganization. This relationship had been demonstrated indirectly in a few studies (Carr et al., 1993; Farmer et al., 1991). However, Carr et al. (1999) included both congenital and acquired brain injuries, and Farmer et al. (1999) did not use behavioral MM assessment. Among the four tasks, hand opening/closing was the easiest task to perform by children with USCP with their hand impairment. It is possible that spasticity masked the intensity of MM during finger opposition, finger individuation, and finger walking due to the dexterity involved (Klingels et al., 2015; Reitz, 1998). Further, if MM precisely copied the other hand, the range of motion of whole-hand opening/closing in the voluntary hand was larger than the others. The unintentional movements were therefore easier to be discerned by the evaluator.

Our behavioral data showed that stronger MM in the more-affected hand were significantly associated with TMS-probed CST connectivity, but this association was not found when MM were measured in the less-affected hand. This finding is consistent with the findings of Staudt et al. (2004), who considered only MM present in the *more-affected hand* as an indicator of an ipsilateral CST pattern. Structurally, the ipsilateral

pathway connects the contralesional motor cortex (M1) and the more-affected hand. Therefore, unintentional movements occurring in the more-affected hand may conceivably be a direct representation of an ipsilateral projection. In this study, we also found a significant association between MM measured by force transducer in the *less-affected hand* and DTI-assessed CST (p= 0.0498). Meanwhile, we found significant associations between strong MM measured by EMG in the less-affected hand and DTI-assessed CST (p= 0.02). Collectively, our results showed that MM in either the more- or the less-affected hand may be predictive of the CST connectivity. To our knowledge, the associations between the occurrence of MM in the *less-affected hand* and CST connectivity has never been reported in the literature.

ii. Children with Bilateral Connectivity may Present with Characteristics of both Ipsilateral and Contralateral Connectivity

Interestingly, MM in the majority of children with bilateral CST organization behaved more similarly to children with a contralateral pattern when they were measured by behavioral assessment, whereas MM in the majority of children with bilateral connections behaved more similarly to children with an ipsilateral pattern when measured by force transducer and EMG (see Table 6B, Table 10B). These findings may result from the fact that children with a bilateral CST pattern still have an ipsilateral pathway, and that the detection of mirror movements can be measurement specific (Connolly & Stratton, 1968). Both force transducers and EMG may have the capacity to measure subclinical mirror activities that the clinical testing was not able to capture (Kuhtz-Buschbeck et al., 2000; Staudt et al., 2004).

Children with a bilateral or mixed CST patterns may present with the features of both ipsilateral and contralateral patterns, which makes the comparison challenging. Our primary aim was to investigate whether stronger MM were associated with an ipsilateral CST. Consequently it was logical to group children with bilateral and contralateral patterns together to answer this research question (as these two groups both preserved the contralateral CST). At the same time, we had a limited number of participants in each cell in our contingency table. Therefore we could only use a 2 (CST patterns) x 2 (strong versus weak MM) table (i.e., Fisher's Exact Tests) to compare the two methods, rather than a 3 (CST patterns) x 2 (strong vs. weak MM) table (i.e., Chi-square tests) (Agresti, 2013). Nevertheless, the high specificity of using the force transducer and EMG to identify those "non-ipsilateral patterns" can be useful when clinicians wish to assign appropriate therapy or determine the side for brain stimulation therapy and when the devices are available.

iii. Inconsistency between TMS-probed and DTI-identified CST patterns

Six children showed inconsistent findings when we probed the CST with two different methods: TMS and DTI (participants #2, 5, 7, 15, 20, 28). Four children did not have TMS-evoked muscle responses from stimulating the lesioned M1, but DTI reconstruction showed presence of a contralateral CST (participants #2, 5, 7, 20, Table 2). Interestingly, two of these children changed their connectivity from ipsilateral projection to bilateral projections after intensive training (participants #2, 7). While it is uncommon for the brain organization to change in this short period of time, it is possible that this change in the CST connectivity reflects the changes in the cortical excitability after

intensive hand training. The intensity to elicit a muscle response is usually high in children (Garvey & Mall, 2008; Rajapakse & Kirton, 2013). These two children (participants #2, 7) may have lowered the resting motor threshold in the more-affected M1 after training (i.e., increased the cortical excitability), which enabled the anatomically present contralateral CST to become "active" after training when stimulating with the same intensity of the TMS machine output. Whereas the functionality of these contralateral CST is unknown, the presence of contralateral CST detected by DTI tractography in these four children may elucidate some of the low behavioral MM scores (see Table 2, behavioral MM scores in the more-affected hand are 0 in participants #7, #20).

Two children (participants #15 and #28) showed bilateral connections as probed by TMS, yet they did not show a contralateral tract as measured by DTI. Our methods of DTI tractography strictly excluded those tracts not going through the cerebral peduncles as that was our region of interest (ROI) (Thomas et al., 2005). It is possible that the stimulation evoked muscle responses through pathways other than the corticospinal tract due to the aberrant motor system organization after early brain lesion (e.g., rubrospinal tracts) (see paper in Appendix E for discussion). In fact, participant #28 showed longer MEP onset latency (25.94ms) as compared to those in children with consistent TMS-probed and DTI-assessed bilateral connectivity (21.14ms) when we examined the contralateral CST with TMS (lesioned M1 projects to the more-affected FDI). It is possible that TMS activated indirect pathways projecting to the more-affected hand in participant #28 (more synapses), whereas our criteria of obtaining CST using DTI did not evaluate those indirect pathways.

Three children did not have MEP responses probed by TMS (#19, 24, 27). These children were relatively younger (6yrs 6 mos- 8yrs 4 mos) as compared to the remaining participants; their resting motor threshold could be higher. Therefore the minimal electrical current that was needed to elicit a motor response could be over the maximal output of the TMS machine. This is a limitation when we used TMS to probe the CST in children. And this is where DTI assessment can be useful when an anatomically present CST cannot be identified by using TMS.

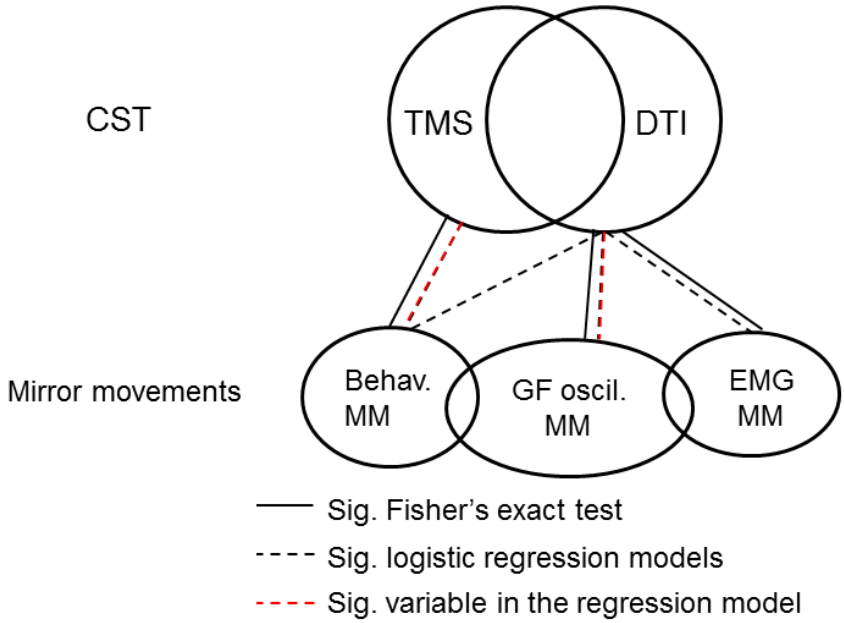
In summary, TMS and DTI may measure distinctive features of the CST. TMS measures functionally active motor pathway (i.e., the functional integrity), whereas DTI measures anatomically present motor pathway (i.e., the structural integrity). This may elucidate why TMS-derived connectivity correlated with clinical assessment (behavioral MM), whereas DTI-derived connectivity correlated with subclinical measures (involuntary GF oscillations and EMG) (see solid line in Figure 10). In other words, behavioral MM assessment may provide us information on "functional connectivity" (probed by TMS), whereas involuntary GF oscillations and EMG-measured MM may provide us information on "structural connectivity" (assessed by DTI).

iv. Using Additive Measures of MM to predict CST

Current results obtained from logistic regression models showed that the best fitted model was when we used the behavioral MM scores to predict TMS-probed CST, and the best predictor for DTI-assessed CST was the involuntary GF oscillations. These findings are in line with those results obtained from Fisher's exact tests. Combining MM measures improved the model fit when we predicted the DTI-assessed connectivity (Figure 10). This may result from that the three measures of MM detect distinctive

components of the corticospinal tracts, and that combining measures may increase the predictive strength. Note that results from the logistic regression models are still preliminary as we had only 17 subjects with available GF oscillations data. Figure 10 is still a working schematic diagram as our sample was limited.

Figure 10. Schematic Diagram of Current Findings

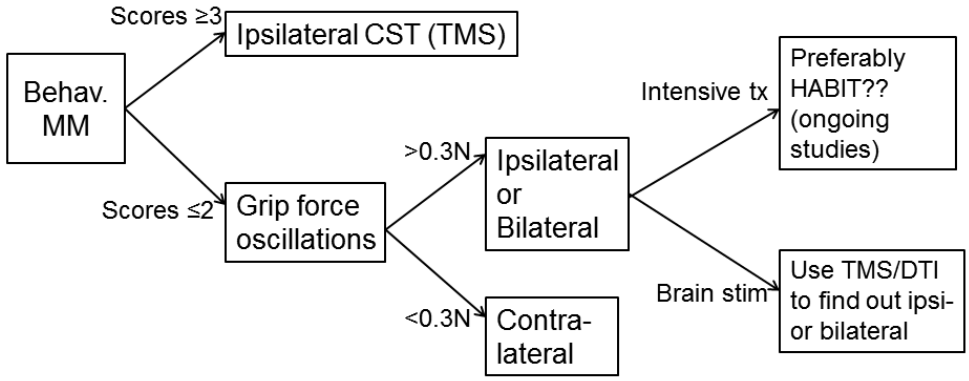


v. Proposed Clinical Implications

Based on findings of the current study, we propose a preliminary clinical assessment flow chart (Figure 11). While more clinical trials are still needed to understand the relationship between children's responsiveness to intensive hand therapy or brain stimulation and the CST patterns, this proposed flow chart may be useful when researchers or clinicians intend to apply these treatment approaches without available devices to probe the CST connectivity in children with USCP. For example, researchers may use the behavioral MM assessment (whole-hand opening/closing) as the first step to determine individual CST organization. Children with mirror movement scores ≥ 3 in the

more-affected hand would indicate they have at least an ipsilateral CST projection. If the behavioral MM scores ≤ 2, further evaluation would be needed to distinguish their CST patterns by examining with force transducers. Presence of involuntary GF oscillations (> 0.3N) examined with force transducers would be indicative of an ipsilateral or bilateral CST connectivity. Absence of involuntary oscillations (< 0.3N) would be indicative of a contralateral connectivity. From there, depending on what treatment may be available (intensive hand therapies or brain stimulation therapies), researchers or clinicians can further determine the next logical step. For example, intensive bimanual therapy may be a better treatment approach for children with ipsilateral connectivity (Friel et al., 2016). Meanwhile, brain stimulation on the contralesional M1 may be a better treatment approach for children with ipsilateral connectivity. Note that the impact of CST organization on children's responsiveness to intensive therapy is still under investigations.

Figure 11. Proposed Clinical Assessment Flowchart



vi. Impact of Brain Lesion Type on the Amount of Mirror Movements

Our findings of relatively higher MM scores in children with periventricular lesion (PVL) as compared to those with cortical/subcortical lesions (C/SC) are similar to

a few studies. Klingles et al. (2015) found this relationship only in MM in the *more-affected hand* during hand opening/closing. Whereas our results of higher MM scores in children with PVL than those with MCA are consistent with Klingles et al. (2015), this association was found in the less-affected hand in this study. This is another significant finding when mirror movements were measured in the less-affected hand. Whereas mirror movements in the less-affected hand were considered as "non-specific overflow" in Staudt et al. (2004), our results demonstrate that the timing of the lesion may also affect recovery processes of the CST controlling the less-affected hand.

vii. Direction of the Mirror Movements

As mentioned briefly in the Introduction, the direction of the movement overflow has been controversial in the literature. Our findings of higher behavioral mirror movements scores in the *less-affected hand* are consistent with a few studies (Klingels et al., 2015; Kuhtz-Buschbeck et al., 2000; Woods & Teuber, 1978). We also found a significantly higher GF oscillations ratio (involuntary/voluntary) in the less-affected hand than in the more-affected hand. Recently, Klingles et al. (2015) showed more "clear mirror movements" (score≥ 2) in the less-affected hand. In our study, the higher intensity of MM in the less-affected hand can possibly result from the underestimated intensity of MM in the more-affected hand, which was masked by spasticity.

viii. Lack of Changes after Three Weeks of Intervention

Despite the intensity of hand training that children received (90 hrs over 3 weeks), the amount of mirror movements did not change after three weeks of intervention. This is not surprising because our training was not designed to reduce the amount of mirror movements. Furthermore, if mirror movements are an indicator of the brain corticospinal

organization as shown in our results, it is unlikely for mirror movements to change given that they are indicative of the CST connections and that CST organization does not change commonly. Although we did have a few children who changed their CST connectivity immediately after intervention, as discussed earlier this change may be due to changes in the cortical excitability after intensive hand training rather than a structural change in the corticospinal system.

There are two movement tasks showing a trend of increase in the behavioral MM scores in the less-affected hand after 3 weeks of intervention: finger opposition and finger individuation (Table 13, finger opposition, p=0.06, individuation, p= 0.1). It is possible that this increase in the behavioral MM scores in the less-affected hand resulted from the improvements in dexterity in the more-affected hand after training.

The grip force oscillations ratio (more-affected MM/less-affected voluntary) showed a trend of decrease after intervention. To our knowledge, albeit not significant, this is the first study demonstrating a trend that intensive training, which was not focusing on reducing involuntary mirror movements, may potentially reduce a certain amount of mirror movements. It is possible that our sample size underpowered the results (n=14). The changes in the amount of MM after intensive training should be further explored in future studies.

ix. Temporal De-synchronization in the Grip Force Oscillations

The latency between the two hands measured by GF oscillations was partially consistent with the findings in Kuhtz-Buschbeck et al. (2000). The consistency between their and our findings was that the mirroring grip force oscillations in the less-affected hand preceded under the condition when the more-affected hand was voluntarily pinching

(-32ms in their Table II, -14ms in Table 5 here). It is possible that there are differential levels of intra-cortical inhibition modulating the more-affected hand and the less-affected, which results in this time lag between the voluntary and involuntary movements. Unfortunately, we did not test the intra-cortical inhibition modulating the less-affected hand. In addition, we had only 14 subjects for this measure and the latency was variable among participants. More research studying intra-cortical inhibition and/or facilitation and their effect on movement lateralization in children with USCP is needed to elucidate this de-synchronization between the two hands.

x. Relationship between Mirror Movements and Hand Function

We did not find significant correlations between bimanual skills and the intensity of mirror movements as in Kuhtz-Buschbeck et al. (2000). Yet the results of bimanual hand function (measured by AHA) from a third of our participants (n=11) are still not available. Thus, we still cannot draw a conclusion on the impact of MM on bimanual function measured by the AHA as of now. Nevertheless, MM appeared to disturb children's goal performance, as moderate correlations were found between the behavioral MM scores in the less-affected hand and COPM (Figures 7A and 7B, finger individuation and COPM, r= -0.36; finger walking and COPM, r= -0.39). Most of the goals are asymmetrical bimanual tasks (e.g., tying shoes, cutting paper with scissors, etc.). It is possible that the symmetrical nature of mirror movements hampered functional goal performance. The significant correlations we found between higher behavioral MM scores in the less-affected hand and unimanual dexterity measured by JTTHF may just reflect the better unimanual capacity in the more-affected hand to perform finger opposition and finger walking after training (i.e., children had better unimanual function

in the more-affected hand; more able to individuate their more-affected fingers, thus induced higher MM scores in the less-affected hand). The asymmetry of the two corticospinal tracts had been shown to be a good predictor of unimanual capacity (Duque et al., 2003; Friel, Kuo, et al., 2014). We conclude here that the initial severity of the corticospinal injury may better predict children's unimanual capacity, and we showed that mirror movements hampered functional goal performance in this study.

xi. The amount of Mirror Movements is not Age-dependent in Children with USCP

Our findings that the amount of MM was not age-dependent in children with USCP are consistent with several studies (e.g., Carr et al., 1993; Kuhtz-Buschbeck et al., 2000). This clearly distinguishes the differences between physiological MM and pathological MM and highlights different underlying mechanisms. Decrease in the amount of physiological MM in typical development depicts the maturation of the transcallosal pathways (increase the inhibition of those unintentional ipsilateral movements) (Mayston et al., 1999). The lack of significant associations between age and MM in this study suggested that the mechanisms underlying mirror movements are different in children with USCP and in typical development. In our study, we cannot rule out the possibility that the ipsilateral cortical control of the more-affected hand and decreased interhemispheric inhibition (from contralesional to lesioned M1) may both contribute to the occurrence of mirror movements in children with a bilateral connectivity. Further investigation using interhemispheric inhibition (IHI) or ipsilateral silent period (iSP) protocols to assess the inhibition capacity by using TMS may further elucidate the mechanism underlying MM in children with bilateral connectivity.

xii. Limitations and Future Considerations

As the first study to compare the CST connectivity with mirror movements using a comprehensive battery of measures, we have a few limitations. First, we did not control for voluntary grip force oscillations output. This may affect the amplitude of the involuntary output as Todor and Lazarus (1986) reported in typically developing children. Presumably, we could provide visual feedback for the amplitude of the voluntary GF output; however, it is challenging for children with USCP to control for a certain amount of force output, especially when pinching with the more-affected hand. The percentage of maximal voluntary contraction (% MVC) was not different between the two hands (p= 0.13), between the pre-test and the post-test (p> 0.15), and between the ipsilateral and the contralateral/bilateral groups (p= 0.06 in the less-affected hand, p=0.15 in the more-affected hand). Nevertheless, the uncontrolled voluntary GF oscillations amplitude (higher output in the ipsilateral group than that in the contralateral/bilateral group in either hand) may potentially account for certain variances between the ipsilateral and contralateral/bilateral groups, and this is our major limitation.

The second limitation was that we had a small number of subjects in the contralateral connectivity (n=4). Although sample size calculation indicated that 18 subjects would be sufficient for the grip force oscillations measure, the limited sample number in the contralateral connectivity may under-power the findings. This is a similar issue as when we compared TMS-probed connectivity with behavioral MM scores. As discussed earlier, the small number of participants also posed statistical challenges when we chose the statistical model (Fisher's exact rather than a Chi-square test).

Nevertheless, this limitation is hard to control for because approximately 50% of children had an ipsilateral pattern (Lotze et al., 2009).

The third limitation is that we did not randomize the task order of the behavioral MM assessment. It is possible that children showed less frequent MM in the last task (finger walking) due to fatigue.

Finally, we faced challenges when characterizing children with bilateral connectivity. As discussed, MM in children with bilateral CST connections sometimes behaved more similarly as children with a contralateral pattern (i.e., behavioral MM assessment), and they sometimes behaved more similarly as those with an ipsilateral pattern (i.e., involuntary GF oscillations and EMG). Although quantification of the cortical control of the more-affected UE was attempted (i.e., the Laterality Index), we only found trends rather than significant associations. Future studies may use other methods for quantifying cortical control of the more-affected hand. For example, researchers can take the amplitude of motor evoked potentials (MEP) obtained from stimulating both M1s into consideration.

In conclusion, we found mirror movements to be present (scores ≥1) in about 70% in the more-affected hands when measured with whole hand opening and closing in children with USCP. Similar incidence was found when MM was measured with the force transducer and EMG while children performed pinching with the less-affected hand (74%, 79% respectively). Strong MM (scores ≥ 3) in the more-affected hand assessed by hand opening/closing were associated with the TMS-probed CST connectivity. Force transducer measured MM in the less-affected hand and EMG-measured MM were significant associated with DTI-assessed connectivity. Brain lesion type may affect the

intensity of MM. Meanwhile, the amount of MM did not change after 3-weeks of intensive hand therapy. The associations revealed in this study may help researchers and clinicians understand the relationship between the CST connectivity and its behavioral manifestation (mirror movements) in children with USCP. Such information may further guide therapeutic strategies in a wider range of children with USCP.

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APPENDIX A
Pilot data

Table 1. A 2x2 contingency table summarizing TMS-identified CST connectivity and motor performance scores (Woods & Teuber, 1978) of the mirror movements.

	MM scores 3-4*	**MM scores 0-2**
TMS-identified ipsilateral connectivity	3	2
TMS-identified contralateral or bilateral connectivity	1	5

*MM scores are based on opening/closing hand in the more-affected (involuntary) hand

Table 2. A 2x2 contingency table summarizing TMS-identified connectivity and force amplitude of the mirror movements.

	GF >0.3N*	**GF <0.3N**
TMS-identified ipsilateral connectivity	4	0
TMS-identified contralateral or bilateral connectivity	2	4

*GF in the more-affected (involuntary) hand

APPENDIX B

Table. Regression and correlations between Behavioral Scores, Grip Force Oscillations, and Functional Improvements after Intervention

	AHA		COPM-satisfaction		COPM-performance		JTTHF	
	F	r[a]	F	r[a]	F	r[a]	F	r[a]
More-affected hand								
Hand Opening/closing	2.05	-0.21	0.45	0.09	0.08	-0.12	0.004	-0.07
Finger-thumb Opposition	0.61	-0.04	0.51	0.09	<0.01	-0.06	0.17	0.11
Finger Individuation	0.01	0.12	0.02	-0.02	1.37	-0.22	0.07	-0.01
Fingers Walking	1.69	0.41	1.67	-0.18	3.26	-0.27	0.01	0.02
Less-affected hand								
Hand Opening/closing	0.12	-0.22	1.3	0.08	0.12	-0.12	0.004	-0.03
Finger-thumb Opposition	0.29	0.07	0.91	0.14	0.06	-0.06	0.22	-0.03
Finger Individuation	0.09	0.02	1.73	0.15	0.28	0.03	<0.001	<0.01
Fingers Walking	0.03	0.11	4.21*	0.35	0.18	0.1	0.06	0.12
More-affected hand								
GF amplitude	0.26	-0.17	3.58	0.4	2.07	0.31	0.15	0.087
Ratio (I/V)	0.38	-0.2	1.96	0.31	0.96	0.22	0.15	0.089
Less-affected hand								
GF amplitude	2.02	-0.43	2.72	0.35	2	0.31	1.42	0.26
Ratio (I/V)	2.65	-0.48	0.59	0.17	1.27	0.25	1.6	0.28

[a]: Pearson correlation coefficient was performed for GF data and improvements, Spearman's rho was performed for behavioral MM scores.

APPENDIX C

Literature Review

I. Introduction

Successful performance of motor tasks requires not only proper movement planning and execution, but also the capacity of inhibiting unnecessary and involuntary movements (Lazarus & Todor, 1991). Mirror movements are defined as unintentional imitation or copy of voluntary movement of the contralateral limb (Woods & Teuber, 1978). Conceivably, it could negatively impact effective performance of asymmetrical bimanual activities (e.g., typing or unscrewing a bottle cap). Early studies in children with unilateral spastic cerebral palsy (USCP) focused on studying the quality of mirror movements and its relationship with the timing of the brain lesion (Woods & Teuber, 1978), the relationship between the degree of mirror movements and corticospinal reorganization (Carr, Harrison, Evans, & Stephens, 1993; Farmer, Harrison, Ingram, & Stephens, 1991), or aimed to quantify mirror movements using objective measurement such as force transducers (Kuhtz-Buschbeck, Sundholm, Eliasson, & Forssberg, 2000). These studies probed into the pathological mechanisms underlying mirror movements or sought to quantify mirror movements in children with early brain injury. Only until recently have there been studies investigating the impact of mirror movements on functional bimanual activities (Adler, Berweck, Lidzba, Becher, & Staudt, 2015; Islam, Gordon, Skold, Forssberg, & Eliasson, 2011; Klingels et al., 2015; Sukal-Moulton, Murray, & Dewald, 2013). In this review, I aim to first discuss two possible neurophysiological mechanisms of mirror movements, and then review studies focusing on the functional impact of mirror movements on unimanual and bimanual hand function. Finally, I will also discuss how the studies thus far may inform researchers and clinicians on therapeutic considerations for children with USCP.

II. Neurophysiological Mechanism of Mirror Movements

Two primary neurophysiological mechanisms have been proposed thus far in the literature to elucidate mirror movements in children with CP: a) an ipsilateral corticospinal tract (CST) projecting from the less-affected motor cortex (M1) to both upper extremities (UEs) (Carr et al., 1993; Farmer et al., 1991), and (b) co-activation of bilateral M1 resulting from deficiency of interhemispheric inhibition (IHI) (Koerte et al., 2011). These mechanisms are discussed in detail below.

An ipsilateral corticospinal pathway projecting to bilateral upper extremities (UEs) serves as the primary neurophysiological mechanism underlying the occurrence of mirror movements in children USCP. In their seminal study, Carr and his colleagues (1993) used Transcranial Magnetic Stimulation (TMS), electromyography (EMG), and cutaneomuscular reflexes to study the pattern of corticospinal tract reorganization and hand function outcome, including mirror movements. Their Group A consisted of patients with USCP with intense mirror movements (score 3 or 4 on a 0-4 scale, Woods & Teuber, 1978). The physiological findings probed by TMS (bilateral MEP responses from stimulating the unaffected M1), significant cross-correlation analysis from bilateral muscle recordings (which indicates presence of common descending motor volley to homologous FDIs), and concurrent cutaneomuscular reflex recordings from both hands (when stimulating the unaffected hand) all suggested that a branched ipsilateral CST innervating homologous hand muscles may underlie the presence of strong mirror movements in this group of patients (same EMG findings in Farmer et al., 1991). The rest of the participants (a combination of congenital or acquired brain injury) did not have this intensity of mirror movements (scores 0-2); nor did the neurophysiological findings demonstrate a branched ipsilateral pathway (no concurrent findings from homologous EMG or nerve reflexes). This

relationship between a branched ipsilateral motor pathway and bilateral upper extremity projections in early developmental processes was demonstrated in a feline model of USCP (Friel & Martin, 2007; Martin & Lee, 1999), and was further demonstrated in several neurophysiological studies in children with USCP probed by TMS (Eyre et al., 2007; Eyre et al., 2001; Staudt et al., 2004).

It was showed that the CST development is driven by activity-dependent competition (Friel & Martin, 2007; Martin & Lee, 1999) and motor experience (Martin et al., 2004) in the feline model of USCP. Perinatal brain injury in this model disrupts typical development of axonal connections. Projections from the injured M1 lose their competitive equality with the opposite M1. In contrast, projections from the undamaged M1 secure a competitive advantage over the injured M1. The axons projecting from the damaged M1 grow sparsely and have an aberrant distribution (synapsed with neurons in the dorsal zone in the gray matter, rather than at a typical intermediate zone), whereas the axons projecting from the undamaged M1 make functional connections with bilateral spinal cord gray matter. In a recent study, Serradj et al. (2014) showed that an ipsilateral projection and a common cortical origin for both forelimbs may result in atypical bilateral movements during exploratory behaviors in EphA4 (spinal cord midline axon repellent protein) knockout mice. This study creates as a great animal model for studying mirror movements in children with unilateral brain injury.

The mechanism underlying animal model of USCP described above delineates the CS reorganization processes in some children with USCP. By using TMS, Eyre and her colleagues (2001) showed that at ~6 months of age, stimulating the less-affected M1 in children with USCP often elicits motor evoked potentials (MEPs) on both UEs. Stimulating the more-affected M1 with TMS often elicits contralateral MEP with decreased amplitude or no MEP responses. By 24

months in children with USCP, probing the less-affected M1 induced bilateral MEPs with similar onset latencies, whereas stimulating the more-affected M1 induced no MEP responses. Approximately 50% of children with USCP have their more-affected UE controlled only by the ipsilateral CST projecting from the less-affected M1 (Lotze et al., 2009). Similar in methodology, Staudt and his colleagues showed that an ipsilateral CST reorganization may be associated with the presence of mirror movements (Staudt et al., 2004; Staudt et al., 2002). A refined hypothesis for this relationship by this group (Staudt et al., 2004) suggested that *only* mirror movements in the *more-affected hand* appeared to be a clinical sign of an ipsilateral pathway; whereas mirror movements in the *less-affected hand* may be found in children with all types of CST reorganizations (ipsilateral, contralateral, or bilateral). The direction of the movement overflow is more evident in the more- or less-affected hand is still controversial and may be sensitive to methodology (Kuhtz-Buschbeck et al., 2000; Staudt et al., 2004; Woods & Teuber, 1978). However an ipsilateral cortical origin innervating both UEs is a common mechanism discussed in the above studies.

A second potential hypothesis for the mechanism of mirror movements— co-activation of bilateral M1s— has been shown in physiological mirror movements in typically developing children (Mayston, Harrison, & Stephens, 1999), congenital mirror movements (Cohen et al., 1991), and in children with bilateral spastic cerebral palsy (CP) (Koerte et al., 2011). Physiological mirror movements are present in typically developing (TD) children and decline by ~ 8 years of age (Connolly & Stratton, 1968; Mayston et al., 1999; Wolff, Gunnoe, & Cohen, 1983). Mayston and her colleagues (1999) studied the mechanism of physiological mirror movements by means of EMG, cutaneomuscular reflexes, and TMS. Their results with no significant EMG cross-correlation analysis during bilateral fingers contraction rejected the

hypothesis that a common motor input is linked with the occurrence of mirror movements in TD children. Rather, the increased cortical activation in the M1 contralateral to the mirroring hand during voluntary index finger abduction of the opposite hand (measured by cutaneomuscular reflexes) and variable IHI findings (mostly lack of interhemispheric inhibition) in children suggested that bilateral M1s may be both active during unilateral movements. A limitation of this study is that the authors did not examine the cortical control of either hand (i.e., corticospinal connectivity) by using TMS. Therefore, they could not reject the possibility that both insufficient interhemispheric inhibition and a common ipsilateral motor representation may concurrently underlie physiological mirror movements in development. More recently, Koerte and her colleagues (2011) investigated mirror movements (measured by both behavioral testing and force testing), the interhemispheric inhibitory competence (measured by ipsilateral silent period, iSP, using TMS), and fiber tractography (measured by Diffusion Tensor Imaging, DTI) of the transcallosal and corticospinal pathways in children with bilateral spastic CP and healthy TD children. Although this is a small-scaled study (n=7 in bilateral spastic CP; n=12 in controls) and the difference in the iSP between the two groups did not reach statistical significance, they concluded that the decreased fiber integrity in the transcallosal pathways (but not the CST) and the weaker interhemispheric inhibition may possibly cause the increased intensity of mirror movements in children with bilateral CP. It is important to note that the underlying mechanism for the occurrence of mirror movements could be different in different subtypes of CP, however.

These two possible neurophysiological mechanisms may explain the presence or the intensity of mirror movements in children with USCP. Thus far, most studies of children with USCP favored the first hypothesis that an ipsilateral CST projection may be the pathophysiological origin for the occurrence of mirror movements. Future studies using a

combination of neuroimaging (such as DTI) and quantitative measurement (e.g., using a force transducer) may delve further into the anatomical structures of CST and its relationship with the intensity of mirror movements.

III. Functional impact of mirror movements in children with unilateral spastic cerebral palsy

It has been demonstrated that patients with mirror movements in the non-affected hand have worse hand function than those without (measured by Fugel-Meyer) in the adult stroke population, and the presence of mirror movements has been suggested to be associated with the processes of the neuroplastic recovery after stroke (Nelles, Cramer, Schaechter, Kaplan, & Finklestein, 1998). The incidence of mirror movements might not only reflect the motor system reorganization, but the behavioral manifestation in itself may also affect functional performance in USCP. I will discuss studies investigating the functional impact of mirror movements in children with USCP below.

The study by Kuhtz-Buschbeck and his colleagues (2000) suggested that mirror movements may disturb bimanual functional skills. They found significant correlations between the amount of mirror movements in either the more-affected or the less-affected hand (measured with force transducers) and the scores of bimanual function (measured by 6 bimanual tasks including carrying a tray, opening a bottle, cutting a sausage with a knife, holding and cutting paper with scissors, buttoning, and tying a knot; total scores range from 0-18). Of course this does not mean causation. In addition, for participants with very mild amplitude of mirror activity in that study, the distribution of the bimanual scores appears highly variable (scores ranged from 8-18 points for those children with mirror activity close to 0). Therefore, it is possible that other factors also contribute to the performance of those bimanual activities.

Islam and his colleagues (2011) were the first to directly test the impact of mirror movements on an asymmetric bimanual task in children with USCP. Participants were instructed to perform two tasks: 1) held grip devices in both hands and then placed the smaller device on top of the bigger device, and 2) held devices in both hands and then compressed a device with one hand repeatedly. Results from the first task showed that temporal coordination of bimanual grip force (GF) coordination was impaired in children with USCP as compared to TD children. Specifically, USCP decreased their GF in the releasing hand prior to an increase of GF in the holding hand, and the magnitude of GF increase in the holding hand in children with USCP was significantly smaller than that in TD children. Findings from the second task showed that there was only one subject (out of two with strong mirror movements that they conducted analysis for) decreased slightly in the grip force (GF) of the holding hand immediately after the decrease in the GF of the releasing hand. They concluded that there was insufficient impact of the presence of mirror movements on grip force modulation during the task. However, note that the primary goal for the study was to investigate the coordination of fingertip forces during the first bimanual task. Participants in their study were required to be able to perform the task successfully, and this criterion may have excluded those children with stronger mirror activities. Researchers should be cautious that the conclusion of this study that the presence of mirror movements having insufficient impact on force modulation could only be applied to that specific task and participant sample.

The study by Adler and her colleagues (2015) was the first study to directly investigate the impact of mirror movements on several hand function outcome measures. They showed that mirror movements have negative impact on the speed component of a new assessment developed in the study, Bimanual Activities Negatively Influenced by Mirror Movements (BANIMM).

This test assessed the time to complete five bimanual activities, including opening a chocolate bar, poking a straw into a drinking package, unwrapping a piece of candy, opening a bag of chips, and twisting off a cap of a full plastic bottle. These items were selected from findings of score differences on a questionnaire inquiring the speed and quality of children's performance on 33 activities of daily living (ADL) between children with and without mirror movements. Those five items were selected based on the magnitude of Z-score differences and easiness to measure the end of each task. Results from a multivariate analysis statistical model showed that mirror movements have significant negative impact on the time to complete the BANIMM, as well as a trend of a negative impact of mirror movements on the bimanual hand function scores measured by Assisting Hand Assessment (AHA). This study was the first to compare children with and without mirror movements using a timed functional measure. They were able to show that children with mirror movement demonstrated worse bimanual function (slower in the time to complete the BANIMM) than those without mirror movements. Future studies using quantitative measures of mirror movements (e.g., force transducers and/or EMG) may adopt similar statistical models to systematically examine the relationship between the amplitude or scale of mirror movements and its impact on hand function.

Klingles and colleagues (2015) investigated 78 children with USCP on how mirror movements may be correlated with children's hand function and brain lesion type. They found stronger mirror movements in the *less-affected hand* while performing fist opening/clenching (measured by Woods & Teuber criteria) were slightly to moderately correlated with worse muscle strength (measured by wrist strength, r= -0.35), worse unimanual dexterity (measured by Jebsen Taylor Test of Hand Function, r= 0.4), and worse bimanual hand function (measured by Assisting Hand Assessment, r= -0.4). In addition, more mirror movements in the *more-affected*

hand while fist opening/clenching were found in children with brain malformation and periventricular lesion, as compared to those having cortical-subcortical, and postnatally acquired brain lesions. Specifically, children with earlier brain lesion showed more intensive mirror movements. This study is the first to explore mirror movements and its relationship with hand function using an exhaustive list of assessments, including muscle strength, muscle tone, and standardized hand function measures. A major limitation of this study is its lack of using TMS to identify the CST organization, as a large component of the discussion was focused on the relationship between lesion time and CST reorganization. The impact of lesion time on the occurrence of mirror movements was already demonstrated by Woods and Teuber (1978) and Sukal-Moulton et al. (2013), and the relationship between lesion type (which implies lesion time) and the CST organization was previously demonstrated by Staudt et al. (2002). The correlations between mirror movements in the less-affected hand and hand function at different ICF levels (body and activity levels) reported in this study further supports the notion that mirror movements may negatively impact hand function in children with USCP.

IV. Conclusions and Future Considerations

In summary, two neurophysiological mechanisms have been proposed to underlie the occurrence of mirror movements: a) an ipsilateral CST projecting from the less-affected M1 that innervates bilateral UEs, and b) insufficient interhemispheric inhibition between the two M1s. The first hypothesis has more support in the literature in children with USCP (Carr et al., 1993; Farmer et al., 1991; Staudt et al., 2004) and is also supported by animal model of CP studies (Friel & Martin, 2007; Martin & Lee, 1999; Serradj et al., 2014).

Earlier studies focused on examining the relationship between CST and mirror movements and quantifying mirror movements using different measures, such as behavioral

measure (Woods & Teuber, 1978) and EMG (Carr et al., 1993; Farmer et al., 1991). They also aimed to establish the relationship among corticospinal tract reorganization, the timing of brain injury, and performance of mirror movements. They found that children with strong mirror movements had a branched ipsilateral CST (Carr et al., 1993), and the timing of brain injury would affect the intensity of mirror movements (Woods & Teuber, 1978). Recent studies sought to investigate the impact of mirror movements on either unimanual or bimanual hand function. Thus far, the reviewed studies showed negative impact of mirror movements on both unimanual and bimanual hand function measured with force transducers during task performance (Islam et al., 2011; Sukal-Moulton et al., 2013), and by performing correlation (Klingels et al., 2015; Kuhtz-Buschbeck et al., 2000) and multivariate analyses (Adler et al., 2015).

It was already shown that children could suppress mirror movements (suppressed to 35% of baseline level in the less-affected hand; 50% of baseline level in the more-affected hand) when they paid attention to the involuntary output (showed as visual signals), although force output was also reduced in the voluntary hand (Kuhtz-Buschbeck et al., 2000). If mirror movements indeed have negative functional impact as suggested by the reviewed studies, the next logical step would be to search for an appropriate treatment for reducing it without sacrificing the function of the voluntary hand. As the amount of mirror movements might be specific to outcome measures, future studies should consider using comprehensive measures, including TMS, DTI, EMG, force transducers, and clinical testing (such as motor tasks in Woods and Teuber, 1978) to thoroughly investigate the occurrence and the amplitude of mirror movements, and their relationship with CST reorganization in children with USCP.

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APPENDIX D

Original Article

Skilled Bimanual Training Drives Motor Cortex Plasticity in Children With Unilateral Cerebral Palsy

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Kathleen M. Friel, PhD[1,2,3], Hsing-Ching Kuo, MS[2], Jason Fuller, PhD[1,4], Claudio L. Ferre, PhD[2], Marina Brandão, PhD[5], Jason B. Carmel, MD, PhD[1,3], Yannick Bleyenheuft, PhD[6], Jaimie L. Gowatsky, MA[7], Arielle D. Stanford, MD[8], Stefan B. Rowny, MD[7], Bruce Luber, PhD[9], Bruce Bassi, MD[7], David L. K. Murphy[7], Sarah H. Lisanby, MD[10], and Andrew M. Gordon, PhD[2,7]

Abstract

Background. Intensive bimanual therapy can improve hand function in children with unilateral spastic cerebral palsy (USCP). We compared the effects of structured bimanual skill training versus unstructured bimanual practice on motor outcomes and motor map plasticity in children with USCP. *Objective.* We hypothesized that structured skill training would produce greater motor map plasticity than unstructured practice. *Methods.* Twenty children with USCP (average age 9.5; 12 males) received therapy in a day camp setting, 6 h/day, 5 days/week, for 3 weeks. In structured skill training (n = 10), children performed progressively more difficult movements and practiced functional goals. In unstructured practice (n = 10), children engaged in bimanual activities but did not practice skillful movements or functional goals. We used the Assisting Hand Assessment (AHA), Jebsen-Taylor Test of Hand Function (JTTHF), and Canadian Occupational Performance Measure (COPM) to measure hand function. We used single-pulse transcranial magnetic stimulation to map the representation of first dorsal interosseous and flexor carpi radialis muscles bilaterally. *Results.* Both groups showed significant improvements in bimanual hand use (AHA; $P < .05$) and hand dexterity (JTTHF; $P < .001$). However, only the structured skill group showed increases in the size of the affected hand motor map and amplitudes of motor evoked potentials ($P < .01$). Most children who showed the most functional improvements (COPM) had the largest changes in map size. *Conclusions.* These findings uncover a dichotomy of plasticity: the unstructured practice group improved hand function but did not show changes in motor maps. Skill training is important for driving motor cortex plasticity in children with USCP.

Keywords

rehabilitation, pediatric, transcranial magnetic stimulation, neuroplasticity, hemiplegia

Introduction

Unilateral spastic cerebral palsy (USCP) is caused by damage to the developing brain within the first 2 years of life. USCP results in weakness and motor deficits on one side of the body. Improving hand function is a main goal for most children with USCP.[1] Intensive bimanual therapy improves hand function in children with USCP.[2-6] In our intensive bimanual training model (Hand-Arm Bimanual Intensive Therapy; HABIT), children spend 3 weeks using both upper extremities in play-based activities, 6 h/day.[4] Task difficulty systematically increases as motor proficiency progresses, further enhancing improvement.[7] We recently showed that HABIT improves unimanual skill, bimanual hand use, and functional hand use.[8] Given the functional impact of training, it is important to determine how training affects the brain.

[1]Burke-Cornell Medical Research Institute, White Plains, NY, USA
[2]Teachers College, Columbia University, New York, NY, USA
[3]Weill Cornell Medical College, New York, NY, USA
[4]New York University, New York, NY, USA
[5]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[6]Université Catholique de Louvain, Brussels, Belgium
[7]Columbia University Medical Center, New York, NY, USA
[8]Alkermes, Inc, Waltham, MA, USA
[9]Duke University, Durham, NC, USA
[10]Division of Translational Research, National Institutes of Health, Bethesda, MD, USA

Supplementary material for this article is available on the *Neurorehabilitation & Neural Repair* website at http://nnr.sagepub.com/content/by/supplemental-data.

Corresponding Author:
Kathleen M. Friel, Burke-Cornell Medical Research Institute, 785 Mamaroneck Avenue, White Plains, NY 10605, USA.
Email: kfriel@burke.org

Skill training drives plasticity of the motor system.[9-12] Skill training involves progressively greater task difficulty, whereas unskilled use of the hand involves repeating a movement that can be done without learning and does not increase in difficulty.[7] Animal models of USCP[2] and stroke[13-15] have demonstrated the importance of skill training in rehabilitation. Skill training improves motor outcomes and expands the motor map of the affected extremity, while repetitive unskilled use of the affected extremity does not produce map changes or motor recovery.[2,11] The brain circuits that underlie motor control in USCP have been well-characterized in an animal model.[16] Specifically, work in the USCP model demonstrated that skill training, but not unskilled motor use, drives plasticity of cortical motor maps and corticospinal connections.[2] This study leverages our understanding of critical ingredients in USCP rehabilitation in the animal model to test whether skill training changes motor maps and hand function in children with USCP. Several studies have shown that constraint-induced movement therapy produces changes in motor cortex physiology in children with CP[17-19] and stroke patients.[20-22] Although several studies have examined the effects of constraint and bimanual therapies in children with USCP,[6,23-26] to our knowledge, this study is the first to specifically examine the critical ingredients that drive motor cortex plasticity associated with *bimanual* training in children with USCP.

We compared the effects of bimanual therapy that incorporates structured skill training versus unstructured play-like hand use on manual skill in children with USCP. Each group received 90 hours of therapy, 6 hours/day, 3 weeks. During training, children actively used both hands in play-based activities. Structured skill training incorporated 3 key components: (*a*) progression of task difficulty, (*b*) repeated practice of isolated movements that are components of a more complex task, and (*c*) repetition of functional goals, such as tying shoes. Children in the unstructured practice group performed bimanual movements during play, but did not progress skill of activities, nor practice isolated movements or functional goals. In the structured skill training group, task difficulty was increased by imposing greater spatial or temporal constraints, or by providing tasks that required increased skilled use and problem solving.[7,8] An example is placing a game board farther away from the child, to encourage a farther reach as a child's arm extension increased. In the unstructured practice group, no constraints were placed on how a child completed an activity (eg, game board kept in close position).

We used single-pulse transcranial magnetic stimulation (TMS) to assess topography and excitability of the motor cortex map of the affected hand before, immediately after, and 6 months after training. We hypothesized that structured skill training, but not unstructured practice, would drive changes in size and excitability of the motor map.

Methods

Participants

We recruited participants from regional clinics, our website (http://www.tc.edu/centers/cit/), ClinicalTrials.gov (NCT00305006), and online communities. Inclusion criteria were the following: (*a*) congenital USCP, (*b*) ability to lift arms 15 cm above table surface and grasp light objects, and (*c*) cognition similar to their age-specific peers in school. Exclusion criteria were the following: (*a*) health problems that would interfere with participation, (*b*) history of seizures after 2 years of age with active use of antiseizure medications, (*c*) uncorrected visual problems, (*d*) severe spasticity (Ashworth ≥3), (*e*) surgery on more-affected hand within 1 year, (*f*) botulinum toxin in upper extremity within 6 months, and (*g*) nonremovable metallic objects in body. Informed assent was obtained from participants and consent from caregivers. Procedures were approved by the institutional review boards of Teachers College, Columbia University, where hand training was performed, and the New York State Psychiatric Institute, where brain imaging and motor mapping were acquired.

Interventions

Day camps were conducted at Teachers College, Columbia University, during summers 2010 to 2012. Eighteen children were randomized to either structured HABIT (n = 8, structured skill group) or unstructured bimanual play (n = 10, unstructured practice group), performed in separate rooms. Motor outcomes from these children have been published in a larger randomized clinical trial that included 22 children.[8] Eighteen of the children in the randomized clinical trial also participated in this TMS mapping study. Two teenagers (ages 15, 17) were assigned to the structured skill group because they were self-motivated to maximize skill demand of activities. This balanced group size at 10 each. Clinical characteristics did not differ between groups (Table 1; χ^2 $P > .05$). Details of materials, methods, randomization, location, and adherence are presented in Supplementary Materials.

Intervention Description

Procedures. All interventionists, parents, children, and motor skill assessors were blinded to therapy group and study hypotheses. Children were randomized to receive structured or unstructured HABIT. Participants engaged in age-appropriate bimanual training 6 hours/day for 15 days (90 hours). In both groups, activities were chosen that required use of both hands. If a child stopped using one hand during therapy, interventionists immediately reminded the child to use both hands.

Table 1. Baseline Participant Characteristics.

Characteristics	Structured (n = 10)	Unstructured (n = 10)
Mean age in years, months (SD)	9.11 (3.5)	8.11 (2.5)
Gender		
Male	6 (60%)	6 (60%)
Female	4 (40%)	4 (40%)
Paretic upper extremity		
Right	6 (60%)	6 (60%)
Left	4 (40%)	4 (40%)
Lesion location		
Right	4	4
Left	6	6
Laterality of motor map by TMS		
Contralateral	1 (10%)	3 (30%)
Bilateral	5 (50%)	1 (10%)
Ipsilateral	4 (40%)	6 (60%)
Race		
Asian	0	1 (10%)
Caucasian	9 (90%)	7 (70%)
Hispanic	1 (10%)	1 (10%)
Mixed	0	1 (10%)
Manual Ability Classification System		
I	2 (20%)	2 (20%)
II	5 (50%)	6 (60%)
III	3 (30%)	2 (20%)
Baseline AHA, mean (SD), logits	58.5 (8.3)	63.3 (11.5)
Baseline JTTHF, mean (SD), seconds	286.7 (239)	225.2 (166)
Baseline COPM Performance, mean (SD)	3.6 (1.8)	3.1 (0.9)
Baseline COPM Satisfaction, mean (SD)	3.2 (2.2)	3.6 (1.5)

Abbreviations: SD, standard deviation; AHA, Assisting Hand Assessment; JTTHF, Jebsen-Taylor Test of Hand Function; COPM, Canadian Occupational Performance Measure.

Table 2. Similarities and Differences Between Treatment Groups.

Treatment Characteristic	Structured	Unstructured
Treatment duration	90 hours	90 hours
Ratio interventionist to child	At least 1:1	At least 1:1
Homework (1 hour/day) during training and for 6 months after training	Yes	Yes
Focus on bimanual practice	Yes	Yes
Skill progression during therapy	Yes	No
Practiced shaping of movements	Yes	No
Practiced functional goals	Yes	No

Structured HABIT. This group consisted of 10 children. Structured HABIT[4,5,27] differed from unstructured bimanual training in 3 ways (Table 2): (*a*) progression of task difficulty, (*b*) repeated practice of isolated movements, and (*c*) practice of functional goals.

Progression of task difficulty. Task difficulty was graded by either increasing the temporal and spatial complexity of the movements or by increasing the complexity of how the affected hand was used.

Repeated practice of isolated movements. Part-task practice (shaping) emphasized practice of a single movement component. Task performance, time on task, and number of repetitions were logged.

Practice of functional goals. Play goals, such as dribbling a basketball, and activity of daily living goals, such as tying shoes, were determined by the participant and their family before training. Goals were practiced during training.

Unstructured Bimanual Training. Children (n = 10) were engaged in intensive use of both hands, without focus on skill. Bimanual activities were selected according to the child's interest. Interventionists were trained only to provide activities that required use of both hands in a playful context. They were told that the emphasis was on having fun rather than rehabilitation. The supervisor ensured interventionists provided no increase in task complexity, no guidance on how to use the affected hand, and no gradation of task demands. The participants did not practice functional goals or part-task practice of movement components.

Behavioral Measures

Participants were evaluated prior to treatment, within 2 days after treatment, and 6 months later by a physical therapist blinded to group allocation. Three outcome measures were used to quantify unimanual capacity, bimanual performance, and functional goals, based on the International Classification of Functioning, Disability, and Health.

We quantified unilateral dexterity using the Jebsen-Taylor Test of Hand Function (JTTHF).[28] Participants use one hand to perform functional movements, including flipping cards, manipulating/placing small objects, simulated eating, checker stacking, and manipulating cans. The outcome is the time (seconds) to perform movements.

To quantify how the hands function together, we employed the Assisting Hand Assessment (AHA).[29,30] The AHA quantifies the effectiveness of assisting hand use in performing bimanual activities in children with unilateral upper limb disabilities. The AHA has excellent validity, reliability (0.97-0.99), and responsiveness to change.[8,29] The test was videotaped and scored off-site by an experienced blinded evaluator. Scores were computed as transformed logits (AHA units).

To measure performance and satisfaction levels in functional goals, we employed the Canadian Occupational Performance Measure (COPM).[31] The COPM is a standardized measure that identifies goals and detects changes in self-care, productivity, and leisure performance areas. Through interview, the caregiver identified the child's functional goals and ranked their importance. They rated satisfaction and performance of each goal (maximum 5). Mean performance and satisfaction scores were analyzed. Both groups of children set goals, but only the structured group practiced goals during the intervention.

TMS Motor Mapping

We used single-pulse TMS to evoke movements of selected digit and wrist muscles of the affected hand to address whether training changed the motor map. We measured motor responses with surface electromyography (EMG) of the first dorsal interosseous (FDI) and flexor carpi radialis (FCR) muscles bilaterally. TMS details are presented in Supplementary Materials.

Each child's TMS map was colocalized to their structural magnetic resonance imaging (MRI), to allow motor mapping that was consistent between each time point. Details are presented in Supplementary Materials.

TMS-evoked motor responses were recorded with surface EMG electrodes. Electrodes were connected to a Brainvision ExG amplifier (NeuroConn, Ilmenau, Germany). TMS pulses were first delivered to the affected hemisphere to search for an EMG response (motor evoked potential [MEP]) of the affected FDI. If an MEP of the affected FDI could not be elicited in the affected hemisphere, the uninjured hemisphere was probed for MEPs of the affected FDI. In all cases, if an MEP of the affected FDI or FCR was not found in the injured hemisphere, it was found in the uninjured hemisphere.

We determined the threshold for provoking an MEP. The TMS coil was held at the location that provoked the largest FDI response ("hotspot"). MEPs were evoked beginning at a suprathreshold stimulus intensity. Stimulator output was lowered at 2% increments. The lowest stimulator output at which MEP responses of the affected FDI could be elicited from 5 of 10 pulses was defined as the motor threshold (MT). An MEP was categorized as a response if the latency between the TMS pulse and MEP onset was less than 40 ms, and if the amplitude of the MEP was at least 50 μV.

After the MT was determined, a circular grid was superimposed onto the child's MRI using Brainsight. The grid was centered at the affected FDI hotspot. Grid spacing was 1 cm. The grid consisted of 5 concentric rings, resulting in a grid with a 5 cm radius (81 grid points). Single TMS pulses (3-6 per site when an MEP was found, 1-2 per site when no MEP was found) were delivered to each grid point, starting at the hotspot and moving concentrically along the grid, ending at the outermost ring. Stimuli were delivered at an intensity of 110% affected FDI MT, frequency <0.1 Hz. This intensity has been used previously to map stroke patients after constraint therapy.[32] Average MEPs per site were calculated using peak-to-peak amplitude.

TMS maps were done before training, within 2 days of the end of training, and 6 months after training. For each child, the same intensity of stimulation (110% pretraining resting MT) was used in all TMS sessions. TMS data analyses methods are presented in Supplementary Materials.

Statistics

Statistics were performed using SPSS (IBM, V21). Intention-to-treat analyses were conducted. Missing data (one 6-month follow-up, unstructured group) were interpolated based on the group average for the 6-month time point. A 2 (group) × 3 (time) ANOVA with repeated measures was performed on all measures. We performed post hoc analyses

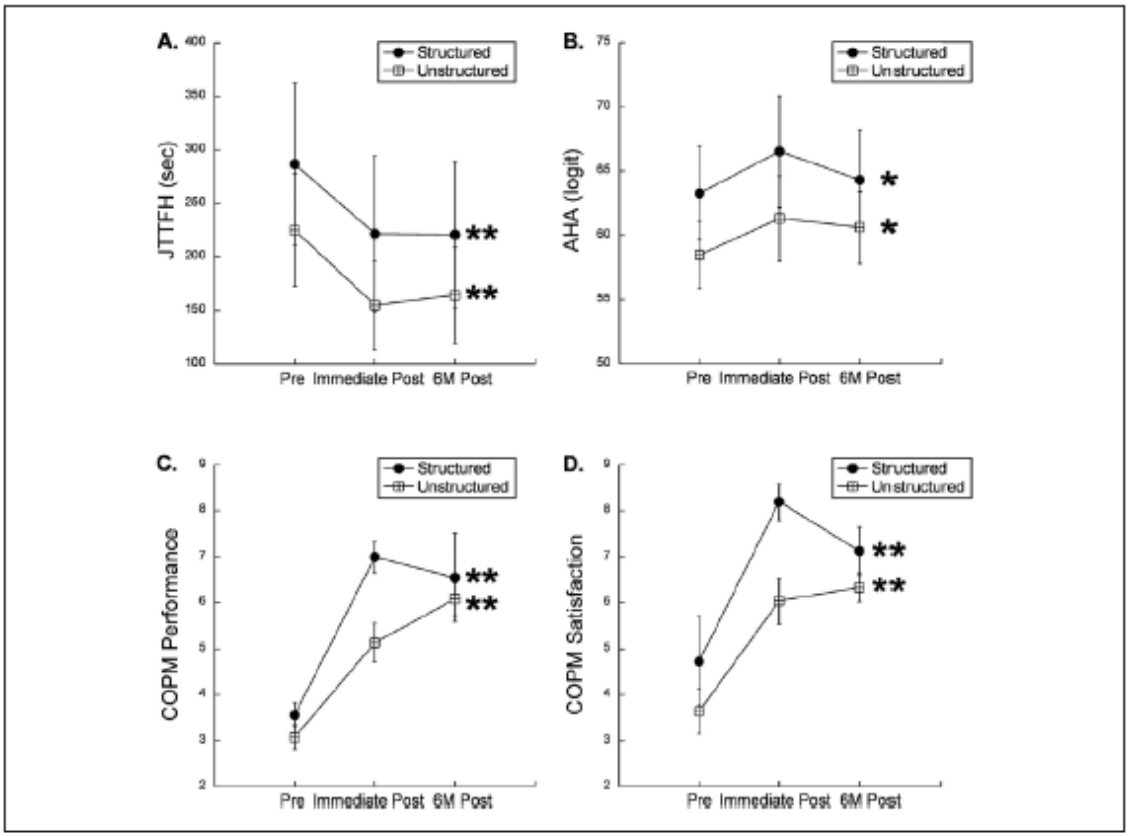

Figure 1. Changes in skill of the affected hand after intensive bimanual training.
Structured and unstructured groups improved equally well in bimanual use of the affected hand (A), movement speed of the affected hand (B), performance of functional goals (C), and satisfaction of performance of functional goals (D). Asterisks indicate within-group differences from baseline to postintervention time points. *$P < .05$, **$P < .01$.

when a main ANOVA effect was found, correcting for multiple comparisons (Bonferroni). The group × test session interaction effect tested if improvements along test sessions differed between groups. Linear regression examined associations between behavior and TMS measures.

Results

Changes in Hand Function After Bimanual Training

Hand function data from the 18 children randomized to group are published as part of a randomized clinical trial.[8] Changes in bimanual hand use were measured with the AHA (Figure 1A). AHA improved after training ($F[2, 17] = 4.03$, $P = .037$, 1.9-unit improvement pre-post training, retained at 6 months), though not a clinically meaningful difference. There was no interaction between training type and AHA improvement ($F[2, 17] = 0.57$, $P = .57$). Both groups improved equally well in bimanual use of the affected hand.Changes in unimanual dexterity were assessed with the JTTHF. There was an overall improvement in the JTTHF in the affected hand after training across all subjects a clinically meaningful amount (Figure 1B; $F[2, 17] = 8.74$, $P < .001$, 17% improvement pre-post training, retained at 6 months). There was no interaction between training type and JTTHF improvement ($F[2, 17] = 0.08$, $P = .92$). Both groups improved equally well in unimanual performance with the affected hand.

Improvements on functional goals were measured with the COPM. There was an overall improvement in COPM-Performance (Figure 1C; $F[2, 17] = 42.19$, $P < .001$). There was a trend toward a significant interaction between COPM-Performance and training type ($F[2, 17] = 3.2$, $P = .068$). The structured skill group trended toward greater improvement than the unstructured group in functional use of the affected

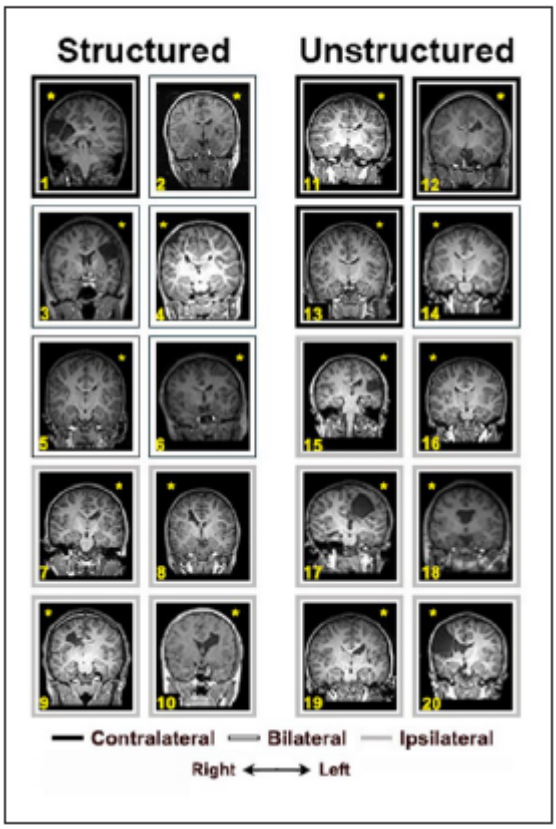

Figure 2. Coronal MRI slices showing a cross-section of each child's lesion.
The color of the frame surrounding the MRI indicates the CST laterality of the child (black = contralateral, white = bilateral, gray = ipsilateral). The yellow asterisk indicates the side of injury.

hand. There was also an overall improvement in COPM-Satisfaction (Figure 1D; $F[2, 17] = 15.77$, $P < .001$) but no interaction between COPM-Satisfaction and training type ($F[2, 17] = 1.76$, $P = .20$). Both groups improved a clinically meaningful amount in COPM-Performance and Satisfaction.

Improvements in all measures were maintained 6 months after therapy, as there were no statistically significant changes between the immediate posttraining and 6 months posttraining measures for either group.

Laterality of Motor Map Controlling the Impaired Hand

We determined the location of the motor map of the affected hand (Table 1, Figure 2). Children were categorized as contralateral if 100% of TMS responses in the affected hand resided in the same hemisphere as the lesion. Children were categorized as ipsilateral if 100% of TMS responses in the affected hand resided in the opposite hemisphere as the lesion. There was no difference in the distribution of hand map laterality (contralateral or ipsilateral to affected hand) between the 2 groups (Table 1, Fisher's exact, $P = .85$). Latency of MEP response was not different for contralateral or ipsilateral responses across all subjects ($P > .3$).

Six children (5 structured, 1 unstructured) had motor maps of the affected hand in both hemispheres—that is, bilateral motor maps. There was a strong asymmetry in map size of the 2 sides. In 5 of the 6 cases (4 structured, 1 unstructured), the map in the injured hemisphere was approximately double the size of the map in the other hemisphere (ratio, injured–uninjured hemispheres = 1.95, SD = 0.11). For these cases, we used the map in the injured hemisphere for analysis. In the remaining child with bilateral maps (structured group), the map of the affected FDI and FCR was 4.75× larger in the uninjured hemisphere than the injured hemisphere, and the map in the uninjured hemisphere was used for analyses.

Changes in Motor Maps After Bimanual Training

We examined changes in motor maps after structured skill training or unstructured practice. Figure 3 shows representative motor maps from one child per group. Map size increased in the structured (1A-1C), but not the unstructured group (2A-C).

After training, there was a significant interaction between map size (FDI + FCR) of the affected hand and training type ($F[2, 17] = 4.7$, $P = .036$). There was a significant increase in map size of the affected hand in the structured skill group (pre-post training $P = .009$, 23.3% increase; pretraining to 6 months posttraining $P = .047$, 34.9% increase) but not in the unstructured practice group ($P > .3$; <10% increase; Figure 3D-E). Map size of the affected FDI increased significantly in the structured skill group (pre-post training $P = .046$, 27.4% increase; pretraining to 6 months posttraining $P = .05$, 34.2% increase), but not in the unstructured group ($P > .2$). The map size for the affected FCR did not change significantly in the structured skill group immediately after training ($P = .85$, 6.1% increase) but was significantly greater than baseline 6 months after training ($P = .049$, 42.2% increase). In the unstructured group, FCR map size did not change after training ($P > .3$).

Amplitude of motor responses to TMS increased significantly (Figure 4) after structured skill training but not unstructured practice (Figure 4D-E). There was a significant interaction between average MEP of the affected FDI and treatment group ($F[2, 17] = 4.21$, $P = .033$). The structured skill group showed a significant increase in FDI MEP size from pretraining to 6 months posttraining ($P < .0001$, 19% increase), though not from pretraining to immediately following or immediately following to 6 months later. There was no significant change in FDI MEP size in the unstructured practice group ($P > .8$). There was a significant

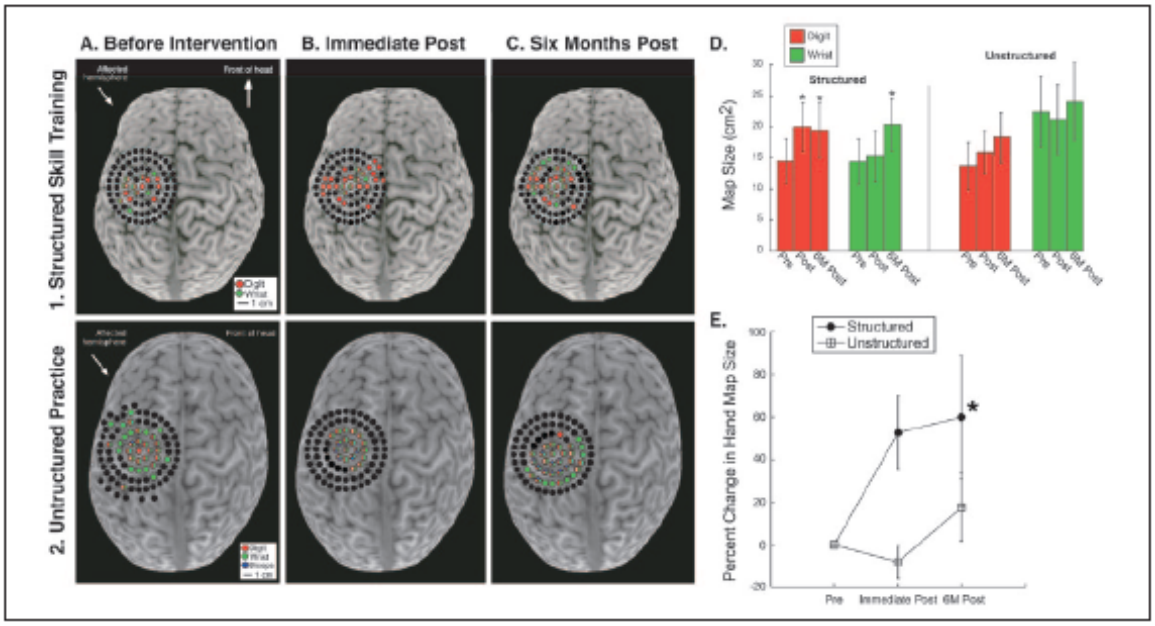

Figure 3. Changes in motor maps after training.
Colored dots represent movement evoked by TMS at that site. (A1-C1) Structured skill training, representative maps of the affected hand from one child. (A2-C2) Unstructured practice, representative maps of the affected hand from one child. (D) Quantification of map changes. The size of the motor map of the affected hand increased significantly only in the structured training group (*$P < .05$).

interaction between average MEP of the affected FCR and group ($F[2, 17] = 4.96$, $P = .019$). The structured skill group showed a significant increase in FCR MEP size from pretraining to 6 months posttraining ($P < .0001$, 78.1% increase) but not pretraining to immediate posttraining. There was no significant change in FCR MEP size in the unstructured practice group ($P > .9$).

Plasticity of Ipsilateral Versus Contralateral Maps

We determined the location of the motor map of the affected hand (Table 1). There was no difference in the distribution of hand map laterality (contralateral or ipsilateral to affected hand) between groups (Fisher's, $P = .85$). Representative maps are shown in Figure 2 (contralateral) and Figure 3 (ipsilateral). Both ipsilateral and contralateral maps expanded after structured skill HABIT ($F[2, 8] = 4.6$, $P = .048$). The magnitude of map changes were not different in children with an ipsilateral versus contralateral CST ($F[2, 7] = 0.32$, $P = .74$).

Associations Between Map Changes and Hand Function Changes

Children in the structured skill group who showed the most improvement in COPM-Performance also had the largest changes in hand map size (Supplemental Figure 1, $F[1, 8] = 7.5$, $P = .013$, $r = .54$, $r^2 = .30$). Children with larger improvements in JTTHF showed larger expansions of the hand motor map ($F[1, 8] = 5.6$, $P = .045$, $r = .64$, $r^2 = .41$). There was not a significant association between hand map change and improvement in the AHA ($P = .95$, $r = .02$). In the unstructured group, there were no significant associations between map changes and JTTHF, AHA, or COPM changes ($P > .3$, $r < .35$).

Importantly, baseline hand function and amount of recovery was not related to whether the child's map of the impaired hand was located in the injured or uninjured hemisphere (JTTHF $F[1, 18] = 4.82$, $P = .10$; AHA, $F[1, 18] = 1.4$, $P = .24$; COPM Performance, $F[1, 18] = 0.42$, $P = .48$, no interactions).

Stability of Motor Maps in the Absence of Intervention

We show the stability of motor maps in the absence of intervention (Supplementary Materials).

TMS Safety Outcomes

Of the 70 TMS sessions conducted in this study, 2 participants reported mild headache after 4 (5.7%) of the sessions, 3 participants reported discomfort in the headband used for neuronavigation after 5 (7.1%) of the sessions, and 2 participants reported discomfort with sitting for an extended

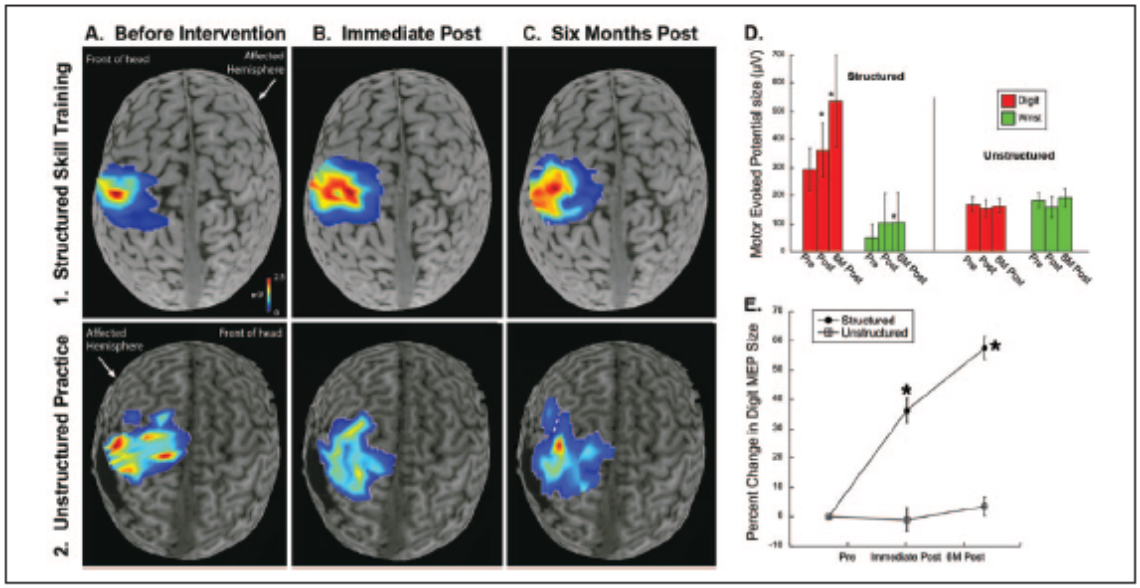

Figure 4. Changes in magnitude of motor evoked potentials in TMS maps after structured skill training.
(A1-C1) Maps of the affected hand located contralateral to the affected hemisphere in a representative case. Red color indicates stronger MEP response. (A2-C2) Representative maps of the affected hand in a representative case from the unstructured practice group. (D-E) MEP amplitude of the representation of the affected FDI increased significantly after structured but not unstructured training (*P < .05).

period of time after 3 (4.3%) sessions. All side effects resolved without treatment within 1 hour after TMS. No serious adverse events occurred.

Discussion

This is the first study, to our knowledge, to examine the critical ingredients of bimanual training that drive changes in motor cortex physiology in children with USCP. We compared bimanual structured skill training and unstructured practice. The study uncovered 3 main findings: (*a*) skill training increased the size and strength of the motor map of the affected hand, (*b*) unimanual skill of the affected hand and performance of functional goals were correlated with increased map size, and (*c*) Motor maps were plastic whether they resided in the hemisphere contralateral or ipsilateral to the affected hand.

Our findings are consistent with studies showing that repetitive practice of a specific motor *skill* drives map plasticity.[2,11,12,33-35] In animal models of stroke and USCP, and in human stroke patients, constraint of the unaffected forelimb, plus skill training of the affected forelimb, can improve motor skill and expand the motor map of the affected limb.[21,22,36-40] In human stroke patients, the magnitude of map expansion was correlated with improvements in skill.[41] Training has also been shown to increase fMRI activations in secondary motor and cerebellar regions.[42] Importantly, constraint alone, without skill training, does not change motor maps.[2,11]

Though further work is needed to uncover the mechanisms of cortical plasticity associated with skill-based neurorehabilitation, work in animal models has determined that skill training, but not unskilled motor activity, increases densities of synapses, dendritic arbors, and spines in the motor networks.[43] These changes are associated with increases in neurotrophin expression[44] and increased density of spinal interneurons.[2]

We found that structured and unstructured therapy both resulted in equal improvements in unimanual movement speed and bimanual use. It is possible that the 2 groups had differences in movement kinematics or other more detailed measures of movement quality, since the clinical outcome measures we used do not quantify the quality of movements. Further studies should examine kinematics of motor recovery in children with USCP.

We found that functional gains and changes in cortical plasticity were independent of hemisphere of control, that is, ipsilateral or contralateral to the affected hand. Constraint-induced movement therapy studies in children with USCP suggested that in children with ipsilateral control of the affected hand, improvements in movement speed[45] and changes in M1 excitability[17] were less robust than for children with a contralateral CST. However, we found that ipsilateral control of the affected limb showed

similar amounts of plasticity as contralateral control. Ipsilateral pathways have the capacity to be adaptive, functional, and plastic,[46-48] though a larger study is needed to better understand differences in plasticity of different hemispheres of control.

This study uncovers a dichotomy of neuroplasticity. The unstructured practice group, which improved hand function, did not show motor cortex plasticity in TMS maps. The high dose (90 hours) may have washed out group differences. Lower dosages could result in differences between groups. While we did find a positive association between functional gains and cortical plasticity, it is possible that with lower doses, associations between motor outcomes and plasticity would be more apparent in the structured skill group than the unstructured group.

Changes in motor function can occur without causing changes in motor maps.[2,9] Some improvements in motor skill can be driven by existing motor networks, while more robust changes in motor skill are associated with rewiring of motor pathways.[49] It is also likely that plasticity occurred in brain regions other than M1, such as secondary motor areas, sensory networks,[50-52] subcortical brain structures,[53] and spinal interneuronal systems.[2,35,54] It is likely that plasticity in these systems underlies the motor improvements seen in the unstructured practice group. Further studies, specifically those that can measure plasticity in different brain regions, are needed to examine plasticity in other systems during rehabilitation.

The relative timing of motor map changes and improvements in motor skill has been studied in animal models of stroke and USCP. Behavioral improvements after infarct plateaued before changes in motor maps could be detected.[55,56] In contrast, in USCP, map changes were found after pharmacotherapy[57] or constraint-induced movement therapy[2] that preceded motor recovery. It is possible that intensity of the structured skill group was sufficient to drive motor map plasticity, but that a longer duration of unstructured practice may have been needed to change motor maps.

The current findings show that skill training is a critical ingredient for driving motor cortical plasticity in children with USCP. While both groups improved in clinical outcomes, the structured skill training group showed the most improvement in functional gains. This work and other evidence[58] indicate that skill training is an important ingredient in neurorehabilitation strategies.

Limitations

This study has several limitations. The relatively low number of participants limits generalizability. While we did match groups on baseline JTTHF and age, there were differences between groups in the distribution of CST projection patterns. Second, this study only measured M1 plasticity. Methods that examine cortical plasticity throughout the brain, such as functional MRI and electroencephalography, can determine other locations of plasticity associated with hand rehabilitation. We discuss challenges of applying our methods to children with USCP in Supplemental Materials. Finally, clinical outcomes reported here measure indirect aspect of movement quality. Further study of movement quality is needed to explore the relationship between cortical plasticity and rehabilitation outcomes.

Conclusions

Structured and unstructured bimanual training improved hand function in children with USCP. Skill training produced stronger improvements in functional goals. There was a dichotomy between these improvements and cortical plasticity. Only skilled training induced motor map plasticity. Further work is needed to examine the interplay between cortical physiology and motor skill improvements.

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APPENDIX E

TITLE PAGE

Using Diffusion Tensor Imaging to Identify Corticospinal Tract Projection Patterns in Children with Unilateral Spastic Cerebral Palsy

Hsing-Ching Kuo[1], Claudio L. Ferre[1], Jason B. Carmel[2,3], Jaimie L. Gowatsky[4], Arielle D. Stanford[5], Stefan B. Rowny[4], Sarah H. Lisanby[6], Andrew M. Gordon[1], Kathleen M. Friel[2].

[1]. Department of Biobehavioral Sciences, Teachers College, Columbia University, New York, NY 10027
[2]. Burke-Cornell Medical Research Institute, White Plains, NY 10605, and Brain Mind Research Institute and Department of Neurology, Weill Cornell Medical College, New York, NY 10021
[3]. Department of Pediatrics, Weill Cornell Medical College, New York, NY 10021
[4]. New York State Psychiatric Institute, Columbia University, New York, NY 10032
[5]. Clinical Science, Alkermes Inc, Waltham, MA 02451
[6]. Division of Translational Research, National Institutes of Health, Rockville, MD 20852

Corresponding Author:
Kathleen Friel, PhD
Burke-Cornell Medical Research Institute, White Plains, NY 10605
785 Mamaroneck Avenue
White Plains, NY, 10605
Phone: 914-368-3116
Email: kaf3001@med.cornell.edu

ABSTRACT

Aim: The aim of this study was to determine whether diffusion tensor imaging (DTI) can be an accurate assessment for identifying the corticospinal tract (CST) projecting from the more-affected motor cortex in children with unilateral spastic cerebral palsy (USCP). ***Method***: Twenty children with USCP participated in this study (16 males, 4 females; mean age= 9y, 2mo (SD= 3y, 2mo), MACS level= I-III). We used DTI tractography to reconstruct the CST projecting from the more-affected motor cortex (M1). We mapped the motor representation of the more-affected hand by stimulating the more- and the less-affected M1 measured with single-pulse Transcranial Magnetic Stimulation (TMS). We then verified the presence or absence of the contralateral CST by comparing the TMS map and DTI tractography. Fisher's exact test was used to determine the consistency between findings of TMS and DTI. ***Results***: DTI tractography successfully identified the CST controlling the more-affected hand (sensitivity=82%, specificity=78%). ***Interpretation***: Contralateral CST projecting from the lesioned M1 assessed by DTI is consistent with findings of TMS mapping. Since CST connectivity may be predictive of response to certain upper extremity treatments, DTI-identified CST connectivity may potentially be valuable for determining such connectivity where TMS is unavailable or inadvisable for children with seizures.

What this paper adds:

- DTI can be used to accurately identify CST projections from the lesioned M1 in children with USCP
- DTI potentially increases availability of methods to determine CST organization in children with USCP.

Running foot: DTI in children with hemiplegia

Cerebral palsy (CP) is the most common cause of motor deficits in children. Unilateral spastic cerebral palsy (USCP), the most common subtype of CP,[1] is characterized by motor deficits lateralized to one side of the body. Studies using magnetic resonance imaging (MRI) showed that the etiology may include middle cerebral artery occlusion or hemorrhage, hypoxic-ischemic encephalopathy, brain malformation, and periventricular leukomalacia.[2, 3] USCP may also result from other causes of brain abnormality, such as prematurity[4] or sinovenous thrombosis[5]. Damage can affect the cerebral cortex, subcortical structures,[3, 4] and the descending corticospinal tract (CST).[6, 7] The CST is the primary motor pathway descending from the motor cortex (M1) innervating muscles controlling skilled voluntary movement.[6, 7]

In typical developing infants, the CST axons project bilaterally from M1 to the spinal cord prenatally.[8] Throughout the first 6 months of life, the ipsilateral CST is pruned, reinforcing the crossed contralateral CST sprouting into the spinal cord. A typical contralateral CST connection is established by 1-2 years of age.[8-10] Perinatal brain injury can disrupt this typical course of activity-dependent refinement. In children with USCP, damage to the more-affected M1 weakens the contralateral/crossed projection.[11] The ipsilateral CST projecting from the less-affected M1 is hence strengthened.[8, 9] Transcranial Magnetic Stimulation (TMS) can be used to assess this cortical control of the upper extremity (UE).[8, 9, 12] Approximately fifty percent of children with USCP have their more-affected UE controlled by the ipsilateral CST.[13] Consequently, the less-affected M1 controls bilateral movements whereas the more-affected M1 controls no movement in these children. Stimulating the less-affected M1 with TMS in this group of children with USCP often elicits muscle responses on both UEs, while no responses are observed when stimulating the more-affected M1.[8]

Constraint-Induced Movement Therapy (CIMT) has been shown to improve hand function and can produce long-term improvements in children with USCP.[14, 15] However, it is costly (thousands of dollars per child) and time consuming (60-90 hours).[16] It is therefore imperative to target CIMT to children who are most likely to benefit. CST connectivity or "rewiring" (i.e., which M1 controls the more-affected UE) in children with USCP may be used as a biomarker to stratify patients before prescribing particular therapies.[17] Kuhnke and colleagues[13] demonstrated that children with an ipsilateral CST (absence of a contralateral CST) responded less than children with a preserved contralateral CST in the speed component of the Wolf Motor Function Test after CIMT. A recent review proposed using individual CST rewiring as a method to predict hand function and treatment outcome in children with USCP.[17] These studies suggested CST connectivity should be carefully examined for targeting treatments based on individual pathology. TMS is a traditional neurophysiological method to examine CST connectivity and its function in children with USCP.[8, 9, 12] Unfortunately, TMS poses a risk in children with seizure disorder, a highly concomitant disorder (35%) in children with CP.[1] DTI tractography is a neuroimaging method of reconstructing white matter tracts and allowing further investigation of pathway integrity.[18-20] The benefit of using DTI to assess a preserved contralateral CST is that it typically takes a short period of time to administer (~10 minutes) and does not pose a risk to children with seizure disorders. The aim of this study was to investigate whether DTI can be an accurate assessment to identify the presence or absence of a contralateral CST in children with USCP. We hypothesized that DTI can be an accurate assessment for identifying the contralateral CST projecting from the more-affected M1 to the more-affected UE.

METHOD

Participants

We recruited participants from our website (www.tc.edu/centers/cit/), ClinicalTrials.gov (NCT00305006), and online support groups. Participants were a convenience sample participating in our clinical trials.[21] The inclusion criteria for the parent trial were established based on our prior trials[14]: 1) diagnosed with congenital USCP, 2) the ability to lift the more-affected arm 15 cm above a table surface and grasp light objects, 3) mainstreamed in school, 4) the ability to follow instructions during screening and complete the physical examination. Exclusion criteria of the parent study included: 1) health problems unassociated with CP, 2) current/untreated seizures, 3) visual problems, 4) severe spasticity at any joint (Modified Ashworth score >3.5), 5) orthopedic surgery on the more-affected UE within the previous year, and 6) botulinum toxin therapy in the UE within the last six months. Children who met the following additional criteria were recruited for this study: 1) aged between 6-17 years, 2) ability to comply with TMS and MRI procedures. Additional exclusion criteria were: 1) history of seizures after 2-years-old, 2) non-removable metallic objects in the body, 3) claustrophobia, and 4) family history of epilepsy. Informed assent/consent was obtained from all participants and their caregivers. The study was approved by the Institutional Review Boards of Teachers College, Columbia University, the New York State Psychiatric Institute, and Burke Medical Research Institute.

Procedures

Magnetic Resonance Imaging Protocols

Each child received a structural MRI scan and a DTI scan (in the same session). The structural scan was used in the TMS experiment to co-register stimulation sites with brain

landmarks, using a stereotaxic system (Brainsight, Rogue Research, Montreal, Canada). The structural scan was also used for examining lesion location.[22] The DTI scan was used to reconstruct the CST and to obtain fiber characteristics.

T1-weighted MRI was performed on a 3T scanner (Philips, Netherlands) in the Columbia University Medical Center. Children were positioned head-first supine. For the structural scan, 165 slices were taken at the resolution of 256x256 pixels. For the DTI scan, 75 slices were taken at the resolution of 112x112 pixels. An echo-planar imaging (EPI) sequence was used (TR= 7638.99ms, TE= 68.56ms). A protocol of 55 diffusion directions was applied (*b* value= 800s/mm^2).

DTI Tractography

We used DTI Studio (Johns Hopkins University, Baltimore, MD) to reconstruct the contralateral CST of the more-affected UE. We first created an image to mask the background noise at the threshold of 30dB, by using standard linear-regression for tensor calculation. We then excluded noisy images containing movement artifacts for every child by visually inspecting the original images using the Apparent Diffusion Constant (ADC) function.[23] An average of 457 slices (SD= 192, 11.1% of the number of images taken for each child) were excluded. We used Fiber Assignment by Continuous Tracking (FACT) method for fiber reconstruction. Fiber tracking started with the Fractional Anisotropy >0.3 and stopped with the FA<0.25 or if tract turning angle >70º.

We placed the first seed at pre- and post-central gyri of the more-affected hemisphere (a circular region of interest (ROI), 25cm^2, centered at the central fissure) to reconstruct the contralateral CST on the DTI color map (same size/location for each child, Figure1A1, "OR"

function in DTI Studio).[24] The size of this ROI was determined based on our recent findings [25]. The seeded slice was always in the axial plane (average= 12.4 slices, SD= 2.1 slices below the first axial slice that showed visible cortex), examined in a cranial-caudal direction. Since central fissure was not always easily identified on a DTI color map of the lesioned M1, we cross-referenced with the FA map for precisely localizing the seed. An example of the obtained fiber after seeding the first ROI can be found in Figure1B1. Then we placed a second seed at the pyramidal tract at the lower pons level on the more-affected side (see Figure1A2, an anterior blue-coded area where the CST typically passes through, "AND" function in DTI Studio).[6, 24, 26] An example of DTI tractography result after combining the two seeds can be found in Figure1B2. Our criteria strictly excluded fibers that do not pertain to a "conventional" CST. This first tractography approach was independent of TMS findings.

Transcranial Magnetic Stimulation Motor Mapping

TMS experiments were conducted at the New York State Psychiatric Institute. We used single pulse TMS (Magstim 200 stimulator, 70 mm figure-of-eight coil) to assess the cortical control of the more-affected UE. Frameless stereotaxy (Brainsight, Rogue Research, Montreal, Canada) allowed for on-line tracking of the position of the TMS coil relative to a child's MRI. We used an electromyography (EMG) recording system (Brainvision, Morrisville, NC) during TMS stimulation for simultaneously recording bilateral muscle responses using surface electrodes over the first dorsal interosseous (FDI) and flexor carpi radialis (FCR) muscles.[7] We mapped the motor representation of the more-affected FDI and FCR by probing the more- and the less-affected M1 (as in Figure 2). Details of TMS mapping procedures are presented in Online Only materials.

TMS Data Analysis

TMS-induced EMG data were imported into MATLAB (Mathworks, Natick, MA). A MATLAB script was written to measure the MEP amplitude for each muscle. Investigators identified the onset and offset of the MEP. For each grid point in the map, the average MEP strength was calculated. Each grid point was categorized as a digit (FDI), wrist (FCR), or a combination of the two muscles by the presence or absence of an MEP at that site.

CST Fiber Characteristics Assessed by DTI

We obtained CST fiber characteristics by using a second approach (independent of the first approach). We first seeded at TMS-derived motor area on the DTI color map. We then excluded fibers that do not pertain to the CSTs (e.g., fibers passing through corpus callosum, cerebellum, and medial lemniscus at the pons). This second approach allowed us to obtain fiber characteristics more precisely by using individual motor maps. Figure 2 shows examples of using individual TMS-derived motor map to reconstruct CSTs for studying corticospinal fiber characteristics.

Statistical Design

We used SPSS (version 19, IBM, NY, USA) for statistical analysis. Two-sided Fisher's Exact Test was used to determine the consistency of TMS and the first DTI tractography approach for determining the presence or absence of a contralateral CST. We calculated the sensitivity and specificity of using DTI as an assessment to identify the contralateral CST. We used paired t-tests to determine if the DTI measures of the more-affected CST were significantly

different from those of the less-affected CST. P-values <0.05 were considered statistically significant for the study.

RESULTS

Twenty children with USCP (age range= 6y,1mo-17y,1mo, mean age= 9y, 2mo, SD= 3y, 2mo) who met inclusion criteria participated in the study. Clinical characteristics of all participants are summarized in Table I. In addition, we show the stratification of participants by TMS-identified connectivity: 9 children (45%) had CST projecting from the less-affected M1 (ipsilateral connectivity), 2 children (10%) had CST projecting from the more-affected M1 (contralateral connectivity), and 9 children (45%) had CST projecting from both M1s (bilateral connectivity).

DTI is an accurate assessment of contralateral CST connectivity

Table II summarizes the consistency between the two methods under study: TMS vs. DTI. Each participant was categorized into one of the four categories by verifying whether there were TMS-derived muscle responses by probing the more-affected M1, and by examining the presence or absence of the CST projecting from the more-affected M1 using DTI tractography. Fisher's Exact Test showed that DTI is an accurate assessment of CST connectivity (p=0.02). When using TMS as the standardized assessment, the sensitivity of using DTI for assessing CST connectivity was 81.8%, and the specificity was 77.8%. Two participants with bilateral connectivity (examined by TMS) showed discrepancy between the two methods in their contralateral CST (Table II, upper right cell, TMS "yes", DTI "no"). Stimulating the more-affected M1 using TMS-induced motor evoked potentials (MEP) in the more-affected UE in

these two participants. However, their CST passed through the medial lemniscus at the pons after we placed the first seed on the M1 (see figure in Online Only Material).[24, 26] Therefore we considered these as tracts other than the CST for these two participants. Two other participants did not have a TMS-evoked MEP from stimulating the more-affected M1, yet a visible CST projecting from the more-affected M1 was visible (Table II, TMS "no", DTI "yes").

Integrity of the more-affected CST is compromised

We investigated the differences in CST fiber integrity between the more- and less-affected sides in children with bilateral connectivity using the second tractography approach. Significant differences between bilateral tracts could provide neuroanatomical evidence of a compromised CST descending from the more-affected M1 in the mild to moderate form of children with USCP in this study (MACS level I-III). The DTI measures of nine children with bilateral connectivity were used to investigate this comparison. The DTI measures examined included FA, fiber volumes (mean number of fibers/voxel), Radial Diffusivity (DR, $(\lambda 2+\lambda 3)/2)$), and Mean Diffusivity (MD, $(\lambda 1+\lambda 2+\lambda 3)/3)$) (Table III). Two-tailed paired t-tests demonstrated that the DTI measures of the more-affected CST were significantly different from those of the less-affected CST. Specifically, the values of the ipsilateral FA and volume were higher (indicating better integrity) than those of the contralateral side ($p<0.01$). In addition, the ipsilateral DR and MD were lower than those of the contralateral side (both $p<0.01$).

DISCUSSION

DTI Tractography can Identify the Contralateral CST in USCP

The primary aim of this study was to investigate whether DTI tractography can accurately and independently identify a preserved contralateral CST in children with USCP. Identifying the presence/absence of a contralateral CST controlling the more-affected UE may help in clinical decision making regarding treatment outcome and determining the location for brain stimulation treatments (e.g., stimulating the more-affected M1 for children with a preserved contralateral CST; stimulating the less-affected M1 for the absence of a contralateral CST). Compared to the findings of TMS mapping, DTI tractography is sensitive (81.8%) and specific (77.8%) to identify the contralateral CST in children with USCP. Although two DTI tractography approaches were used, we compared findings of TMS mapping with the presence/absence of the contralateral CST derived from the first approach. Our finding of the consistency between the two methods suggests that DTI can be used to assess a contralateral CST, especially for children who cannot receive TMS. We propose that DTI tractography can be used clinically as a tool for determining the CST connectivity in children with USCP.

Two participants (bilateral connectivity measured by TMS) showed inconsistency between DTI and TMS in the contralateral CST (Table II, TMS "yes", DTI "no"). DTI tractography showed that these fibers originating from the more-affected M1 passed through the medial lemniscus, but not the anterior pyramidal tract at the lower pons.[24] The clinical characteristics of these two cases cannot explain their disorganized CST (case 1: age= 8y11mo, MACS= level III, lesion type= cortical/subcortical, case 2: age= 17y1mo, MACS= level II, lesion type= cortical/subcortical). Nor did their TMS results show any discrepancy in MEP onset latency as compared to other children with bilateral connectivity (independent t-test, t= 0.05, p= 0.966). The inconsistency between TMS and DTI in these two participants suggests that reorganization in the motor system may be variable and not always be measured by a single method. An

example case showing incongruence of TMS and DTI outcomes is shown in Online-Only materials.

Two other participants (ipsilateral connectivity measured by TMS) did not have TMS-evoked MEP responses from stimulating the more-affected M1, yet a visible CST projecting from the more-affected M1 was reconstructed (Table II, TMS "no", DTI "yes"). These two cases both had brain lesion type categorized as periventricular leukolamacia (PVL) with MACS level II (case 3: age=10y, 1m, case 4: age=14y, 1mo), although this combination (PVL and MACS level II) consisted of 35% of our participants. Children's tolerance for high intensity stimulation is sometimes low, and the motor threshold for the more-affected M1 is typically high in children with USCP.[9] It is possible that the stimulation intensity was not strong enough to activate their CSTs projecting from the more-affected M1 (tested up to 85% TMS device output; stopped due to children's intolerance), despite the CSTs being anatomically present.

Fiber Integrity in the More- vs. Less-affected CST

Our results comparing the fiber integrity of the more- vs. the less-affected CSTs showed differential characteristics of the ipsilateral and the contralateral CSTs in children with bilateral connectivity (t-tests, $p<0.05$). Previous studies[6, 7] used MRI cross-sectional areas of the cerebral peduncles to compare the integrity of two CSTs and one study[26] used DTI tractography to directly compare the two CSTs in children with USCP. Our study added evidence that the two CSTs present differential fiber integrity projecting from bilateral M1s in children with USCP with MACS level I-III. This second approach allowed precisely seeding individualized ROIs since motor map location can be variable in children with USCP.

Limitations

The results of this study may not be generalizable in the clinical setting, as variability exists in DTI acquisition protocols. Our imaging protocols contained 55 diffusion directions. However, this level of precision may not be achievable in every MRI facility, particularly in the clinical setting. Second, we did not include the CSTs originating from the less-affected M1when comparing the two methods, given it was challenging to determine where those fibers descend to (could control either hand). This is a technical limitation of using brain DTI as it only captures images caudally to the junction of medulla and cervical spinal cord. Even if DTI of the cervical spine is available, tractography can be challenging when fibers are crossing.[24] In addition, our sample encompassed children with mild to moderate levels of hand function impairments (MACS level I- III). Conceivably, children with more severe impairment (e.g., MACS IV or V) may demonstrate a more disorganized CST, making DTI maps more difficult to define. Whereas the CST in severely affected children may be present, highly disorganized fibers would be more difficult to reconstruct. It would be ideal to compare our data to typically developing children or children whose lesions were postnatal. Lastly, due to the small number of the available contralateral CST in our participants, we were unable to perform correlation analysis between DTI-derived fiber characteristics and hand function measures. We propose further studies to recruit a larger sample and age-matched controls to study this relationship.

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Tables

Table I. Partcipant Demographic and Clinical Characteristics

Mean age (SD) (years, months)	9,2 (3,2)
Gender	
Male	16
Female	4
Paretic hand	
Right	10
Left	10
Lesion type	
Right	10(0[a], 5[b], 5[c])
Left	10(0[a], 6[b], 4[c])
Bilateral	0
Race	
White	11
Hispanic	4
Mixed	3
African American	1
Asian	1
MACS[d]	
I	3
II	14
III	3
CST Connectivity of the more-affected UE[e]	
Ipsilateral	9
Contralateral	2
Bilateral	9

[a]brain malformation, [b]abnormality of periventricular white matter,
[c]cortical/subcortical lesion, [d]Manual Ability Classification System,
[d]identified by TMS motor mapping.

Table II. 2x2 Contingency Table Summarizing Consistency between TMS & DTI

		Contralateral Corticospinal Tracts detected by: **DTI**		
		Yes[c]	No[d]	
TMS-evoked muscle responses by probing the more-affected M1	Yes[a]	9	2	11
	No[b]	2	7	9
		11	9	20

Table III. Comparison of DTI measures of participants (n=9) with bilateral connectivity

	Ipsilateral CST (SD)	Contralateral CST (SD)	t-value	*p* value[b]
Fractional Anisotropy (FA)	0.533 (0.031)	0.495 (0.017)	4.121	0.003*
Volume (mean of fibers/voxel)	12.193 (2.289)	8.058 (2.490)	4.303	0.003*
Mean Diffusivity (MD)[a]	0.781 (0.011)	0.838 (0.051)	-3.646	0.007*
Radial Diffusivity (DR)[a]	0.518 (0.023)	0.583 (0.034)	-4.813	0.001*

a: unit=10-3 mm^2/s, b: 2-tailed paired t-test, * indicates *p* value <0.05

Figures

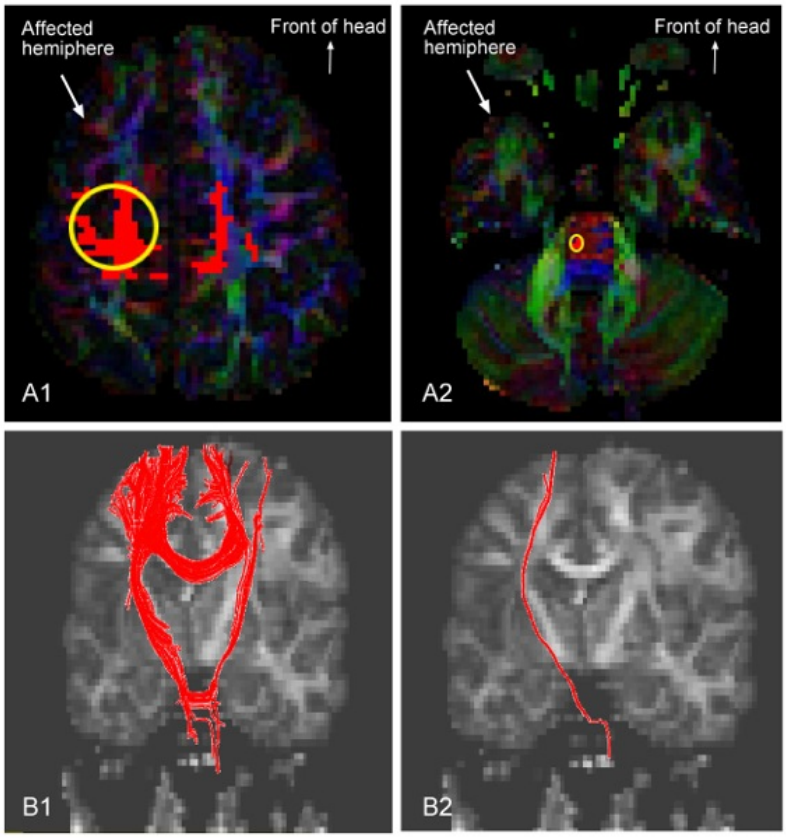

Figure 1. A1-A2: Yellow circled regions show the two seeds in DTI color map, axial slices. A1: ROI1: 25cm^2 circular region seeded at pre-central and post-central gyri of the more-affected M1. A2: ROI2: seeded at pyramidal tract at the pons. B1-B2: Reconstructed tracts (red fibers) after seeding the ROIs. B1: Reconstructed tracts after seeding ROI1. B2: Reconstructed tracts after combining ROI1 & ROI2.

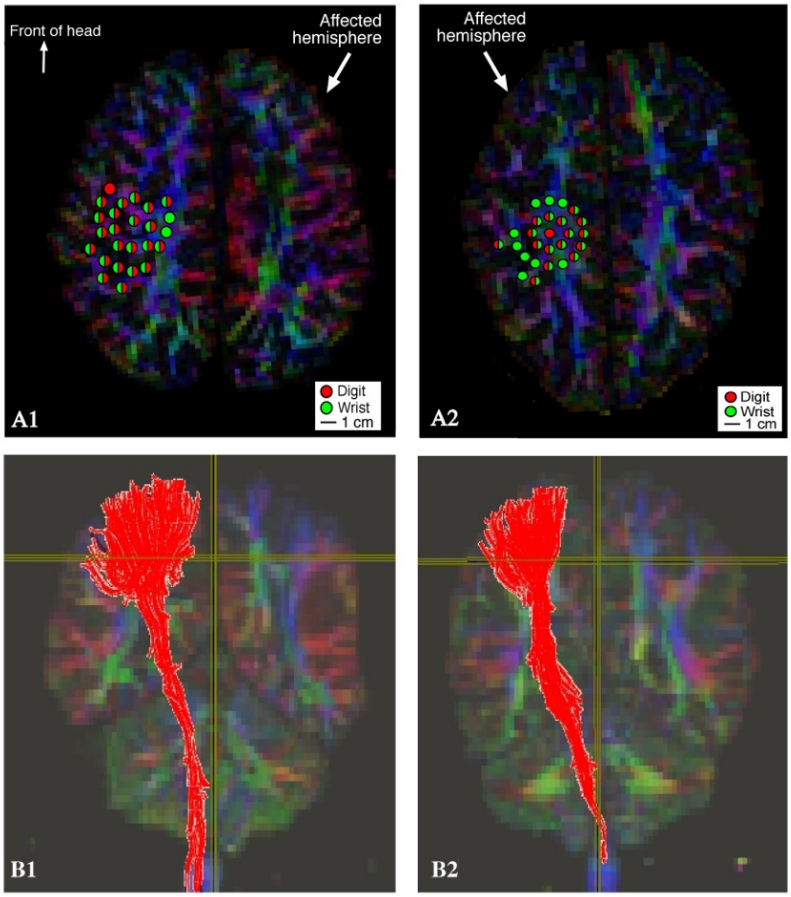

Figure 2. A1-A2: Color dots represent TMS motor map of the more-affected hand (red- FDI, green- FCR). A1: A participant with TMS motor map (color dots) on the less-affected M1. B1: An ipsilateral corticospinal tract (red fibers) after DTI tractography by seeding TMS motor map on the less-affected M1 (ROI seeded based on the area of color dots in A1). A2: A participant with TMS motor map on the more-affected M1. B2: A contralateral corticospinal tract (red fibers) after DTI tractography by seeding TMS motor map on the more-affected M1 (ROI seeded based on the area of color dots in A2).

APPENDIX F

Clinical Research Article

Comparison of Structured Skill and Unstructured Practice During Intensive Bimanual Training in Children With Unilateral Spastic Cerebral Palsy

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Marina B. Brandão, PhD[1], Claudio Ferre, MA[2], Hsing-Ching Kuo, MS[2], Eugene A. A. Rameckers, PhD[3-5], Yannick Bleyenheuft, PhD[6], Ya-Ching Hung, EdD[7], Kathleen Friel, PhD[8], and Andrew M. Gordon, PhD[2]

Abstract

Background. High-intensity training aims to improve hand function in children with unilateral spastic cerebral palsy (USCP). However, the extent to which skill training is required is not known. *Objectives.* To compare the effects of intensive bimanual training with and without structured progression of skill difficulty, on manual dexterity, bimanual hand use, daily functioning, and functional goals in children with USCP. *Method.* Twenty-two children were randomized to structured practice group (SPG) or unstructured practice group (UPG), and received 6 h/d training during 15 days. Children from the SPG were engaged in fine and gross motor bimanual activities, with skill progression and goal training. Children from UPG performed the same activities without skill progression or goal training. Participants were evaluated before, immediately and 6 months after training by a physical therapist blinded to group allocation. The primary outcomes were the Jebsen-Taylor Test of Hand Function (JTTHF) and Assisting Hand Assessment (AHA). Secondary outcomes included the Canadian Occupational Performance Measure (COPM), Pediatric Evaluation of Disability Inventory (PEDI), and ABILHAND-Kids. *Results.* Both groups showed similar improvements in the JTTHF, AHA, ABILHAND-Kids, COPM-satisfaction, and PEDI ($P < .05$). A significant interaction in the COPM-performance scale ($P = .03$) showed superior improvements of the SPG immediately, but not 6 months, after the intervention. Conclusions: Children from both groups demonstrated improvements in dexterity and functional hand use. This suggests that for intensive bimanual approaches, intensive training at such high doses may not require structured practice to elicit improvements. However, there may be immediate added benefit of including goal training.

Keywords

upper extremity, hand, hemiplegia, cerebral palsy, bimanual training, intensive rehabilitation, training ingredients, constraint-induced movement therapy, goal practice, hand-arm bimanual intensive training (HABIT)

Unilateral spastic cerebral palsy (USCP) is characterized by motor impairments mainly lateralized to one side of the body, with the resulting impaired hand function affecting functional activity and participation. Increasing evidence supports the use of 2 treatment approaches based on motor learning principles, constraint-induced movement therapy (CIMT) and intensive bimanual training. CIMT involves physical restraint of the less-affected upper extremity (UE) along with intensive, skilled practice of the more-affected hand.[1-10] Bimanual training involves practice of functional and play activities requiring the use of both hands.[10-20] Hand-arm bimanual intensive training (HABIT) is a highly structured form of bimanual training.[11,13,19,21] Both CIMT and HABIT were designed with the idea that *intensity of training*[22,23] and *progression of skill* difficulty[24] are essential to improve motor function. They include part and whole practice, and modifying tasks to elicit desired movements and to ensure successful use of the more-affected hand.[21,24,25]

[1]Faculdade de Ciências Médicas de Minas Gerais, Belo Horizonte, Brazil
[2]Teachers College, Columbia University, New York, NY, USA
[3]Maastricht University, Maastricht, Netherlands
[4]Adelante Center of Expertise in Rehabilitation and Audiology, Hoensbroek, Netherlands
[5]University for Professionals for Pediatric Physical Therapy, AVANSplus, Breda, Netherlands
[6]Université Catholique de Louvain, Brussels, Belgium
[7]Queens College, City University of New York, Flushing, NY, USA
[8]Burke-Cornell Medical Research Institute, White Plains, NY, USA

Corresponding Author:
Andrew M. Gordon, Department of Biobehavioral Sciences, Teachers College, Columbia University, Box 93, New York, NY 10027, USA.
Email: ag275@columbia.edu

Recently, several randomized trials have shown both approaches result in improvements in unimanual and bimanual measures.[12-18,20,26,27]

There is evidence to suggest that the behavioral demands of the tasks and motor skill training may elicit cortical reorganization[28] underlying concurrent functional outcomes. For optimal efficacy, it is believed that training must be challenging, involve progressive increase of behavioral demands and active participation. Studies of skilled training in animals show plasticity of UE cortical representation, whereas those animals receiving unskilled training (repetition only) did not.[28,29] Friel et al[30] investigated mechanisms for restoring motor function after unilateral corticospinal system damage in cats during early postnatal development. They compared skilled practice of the affected limb in a reaching task along with restraint of the unaffected forelimb limb in one group of cats (analogous to CIMT) with restraint only (analogous to forced use[31]) in another group. The skilled training group had improvements in motor skills of the affected limb along with expansion of the motor map, whereas the restraint-only group did not demonstrate plastic changes. Thus, skilled training is an important element in animal models of rehabilitation.

In humans, the extent to which skill training is required during intensive training is not well-understood. Uswatte et al[32] found similar outcomes of shaping and task-practice without skill progression during CIMT in adult stroke patients' hand function. The extent to which skilled practice is required for pediatric UE rehabilitation approaches is not known. One precision grip study in children with USCP demonstrated that task repetitions alone could lead to better motor performance of this task.[22] It is conceivable that intensity alone may be the key element to improvements in hand function; that is, at such high doses, specific components may not be important.

In the present study, we compare the effects of intensive bimanual training with and without structured progression of skill difficulty, on manual dexterity (Jebsen-Taylor Test of Hand Function, JTTHF) bimanual hand use (Assisting Hand Assessment, AHA), daily functioning (ABILHAND-Kids, Pediatric Evaluation of Disability Inventory, PEDI) and functional goals (Canadian Occupational Performance Measure, COPM) in children with USCP. We hypothesized that children in the structured practice group (SPG) would have greater improvements compared to children in the unstructured practice group (UPG) on all measures.

Method

Participants

Participants were recruited from area clinics, our Web site (http://www.tc.edu/centers/cit/), and online support groups. Potential participants were first screened via e-mail/telephone. Those between the ages of 6 and 13 years with congenital hemiplegia were invited to receive an on-site physical examination or an examination videotaped by their physical/occupational therapist. The inclusion criteria were established based on prior HABIT trials[11-13]: (1) the ability to lift the more-affected arm 15 cm above a table and grasp light objects, (2) mainstreamed in school, and (3) demonstrated ability to follow instructions during screening/testing. Exclusion criteria included (1) health problems unassociated with cerebral palsy, (2) current/untreated seizures, (3) visual problems interfering with treatment/testing, (4) severe muscle tone at any joint (Modified Ashworth score >3.5), (5) orthopedic surgery on the more-affected hand within 1 year, and (6) botulinum toxin in the more-affected UE within the past 6 months or intended treatment within the study period. Informed consent was obtained from participants and caregivers. The study was approved by the Teachers College Institutional Review Board.

Procedures

General Procedures. Three bimanual training day-camps were conducted at the university from July 2010 to July 2012. Participants (6-8 each camp) were randomized offsite using concealed allocation stratified by age and JTTHF screening score, into an SPG and UPG. Each camp had 3 to 4 children per group, each group separated in different rooms.

Participants in each group were engaged in treatment 6 h/d for 15 consecutive weekdays (90 hours) during school recess by trained interventionists. These included graduate students in kinesiology/neuroscience, speech pathology, and psychology, and undergraduates. The interventionists were not aware of the study hypotheses or that different procedures would be carried out in each room. The pretreatment training, administered by the supervisors, was standardized during a 2-hour session based on established manual of procedures, before interventionists were assigned to a group and child. Group training focused on procedures common to the 2 groups, strategies to engage children in use of hands, safety and data logging procedures. Additional training was provided specific to each group during the interventions and daily team meetings. Interventionists and children in each room were kept partitioned at all times, and interventionists were instructed not to discuss the camp with each other outside of treatment hours. The 2 treatment rooms had supervisors, who were not blinded to the study hypotheses, and were responsible for ensuring treatment fidelity. The SPG was supervised by a physical therapist who modeled and ensured uniformity of treatment. The UPG was supervised by a nonclinician who ensured that interventionists did not provide structured practice or graduation of task complexity. Emphasis in this group was to engage children to use the more-affected hand in play

activities. Both groups were monitored by an occupational therapist and a nonclinician who followed all the activities, to avoid possible confounds in the specificities of each training modality (ie, skill progression).

Room design permitted participants to work individually with their interventionist or in groups (1:1 interventionist:participant ratio). Interventionists were paired with children prior to randomization based on age, gender, and caregiver input. Interventionists avoided verbal prodding to use the more-affected hand, and instead provided tasks necessitating the use of both hands and established rules prior to each activity, allowing the child to choose which hand to use for different components of a bimanual activity. Caregivers were instructed to engage participants in bimanual activities for 1 h/d during and for 6 months following the intervention and document practice using home activity logs. No information regarding training of functional goals at home or skill progression was provided for either group.

Structured Practice Procedures. Children from the SPG were engaged in age-appropriate fine and gross motor bimanual activities using motor learning approaches consistent with HABIT.[23] Activities were selected by considering the role of the more-affected hand increasing in complexity from a nondominant passive assist (eg, stabilizing paper while drawing) to active manipulator (eg, reorienting paper while cutting) using increasingly complex bimanual coordination and participants' interests. Task demands were graded and participants were engaged in active problem solving.

Children participated in whole and part task practice. Whole task practice involved sequencing successive movements within the context of tasks (eg, card games). The activities were performed continuously for at least 15 to 20 minutes. The spatial and temporal coordination of targeted movements were practiced within the context of completing the task. Part task practice[21,25] required breaking down motor skills into smaller components (eg, playing-card turning to promote forearm supination), while increasing repetitions and skill requirements. This served to provide specificity/intensity of treatment by requiring as many targeted repetitions as possible over repeated 30-second intervals. Part practice included both bilateral symmetrical (eg, reaching toward object(s) with both hands) and asymmetrical (eg, pulling apart objects) movements. Task difficulty was graded by varying the spatial and temporal constraints, or by providing tasks that require progressive skilled use of the more-affected hand as performance improved. Task- and age-specific knowledge of results were provided for encouragement.[23] Supervisors instructed interventionists regarding the main focus of the treatment (eg, reaching) for each child based on his or her pronounced movement deficits, interests, and potential for improvement. Activity logs were collected, which included activities performed, time on task including part/whole practice, the number of repetitions, task performance and skill progressions.

Practice of functional goals established by caregivers was based on children's interests and abilities. Goal training was performed up to 30 min/d during camp. With the exception of goals, tested task items were never trained.

Unstructured Practice Procedures. Children from the UPG were engaged in age-appropriate fine and gross motor bimanual play activities (with the exception of functional goal practice) *with the same activities* without any adaptations or progression. As in the SPG, rules were established requiring use of their more-affected hand. Activities were selected from the same battery of choices as that of the SPG, according to child's interest and willingness to use the more-affected hand. Interventionists were trained only to provide activities that require the use of both hands in a playful and enjoyable context. Thus, the aim of the unstructured training was to provide children opportunities to use their more-affected hand in bimanual play activities, without considering how this hand was used or how use could be improved or challenged. Interventionists were specifically told "these children receive excessive therapy during the year, and that our purpose was to provide a fun, supportive environment to avoid frustration or perception that activities were therapeutic." Thus, the focus of training was only to keep the more-affected hand involved in the performance of the activities. Supervisors ensured that no increase in task complexity, verbal prompts how to use the more-affected hand or gradation of tasks demands were provided. Such supervision happened throughout the intervention and in daily meetings with interventionists. Activity logs were collected, which included activities performed and the time spent in these activities.

Measures

Participants were evaluated directly prior to treatment (pretest), within 2 days ("immediate") and 6 months after treatment by a physical therapist blinded to group allocation (verified following testing). Two primary outcome measures were used to quantify bimanual and unimanual outcomes under the International Classification of Functioning and Health (ICF) "activity and participation" domain.[33]

The AHA version 4.3[34] quantifies the effectiveness with which a child with unilateral disability uses his or her affected (assisting) hand in *bimanual activity*. The AHA has excellent validity/reliability.[35] The test was videotaped and scored offsite by an experienced evaluator blind to group assignment. Data were reported in logit-based units (AHA-units).

The JTTHF is a standardized test of simulated functional tasks quantifying the time to complete a battery of unimanual activities.[36] The activities include flipping index cards,

object placement, simulated eating, stacking checkers, and manipulating empty and full cans. Reliability for children with nonprogressive hand disabilities is high.[37]

Four secondary measures were also used (ICF "activity and participation domain").

To establish/evaluate children's functional goals we conducted the COPM with caregivers. The COPM identifies and measures changes in functional problems considered relevant by clients through interview, and is valid/reliable.[38-40] The most relevant functional goals to be accomplished are defined, ranked in importance, and rated on performance and satisfaction.[38-40] In this study, caregivers selected the goals and rated the child's performance and level of satisfaction since these are abstract concepts for children of this age.

The ABILHAND-Kids is a valid/reliable questionnaire assessing manual ability of children.[41] The test comprises a list of manual activities in which the caregivers score the amount of difficulty children with cerebral palsy may experience during their performance in activities of daily living that required hand use. Data were reported in logit-based units.

To assess children's daily functioning, caregivers were interviewed using the PEDI, a valid/reliable test[42] focusing on child's functioning in daily living activities at home.[43] Children's self-care functional skills and caregiver assistance were assessed.

A subset of 13 children (7 in SPG, 6 in UPG) wore an activity monitor (Manufacturing Technology Inc, Fort Walton Beach, FL, #7164; 5.1 cm × 2.6 cm × 1.5 cm, 42.9 g) on each wrist during a continuous 3-hour period in camp on the seventh or eighth intervention day to determine how much the children in each group moved. The units sample (10 Hz) and store summed values in memory, and data are downloaded to a personal computer. The number of accelerations is measured as activity counts (0.01664 g for an acceleration of 2.13 g directed parallel to the *x*-axis with a frequency of 0.75 Hz), which were used to determine the percentage of time each hand was used.[34]

Statistical Design

Sample size calculations were performed based on JTTHF scores derived from an earlier HABIT trial.[13] A mean decrease from 380.84 to 249.58 seconds (131.25 ± 121.86 seconds) for the HABIT group was reported. With significance level = .05, $1 - \beta = 0.80$, $\mu 1 - \mu 2 = 131.25$, and $\sigma = 121.86$, and estimated 10% dropout, 11 participants in each group were required. Intention-to-treat principles were employed.

A 2 (group) × 3 (test sessions) analysis of variance with repeated measures on test sessions was performed using SPSS 15 for all measures except the COPM. Since goal performance may be influenced by development and practice in ongoing care (follow-up), a 2 (group) × 2 (pre/post) analysis of performance was performed on the COPM. The overall group-by-test session *interaction* tested whether the pattern of change between sessions varied across groups. Newman-Keuls post hoc tests were used to compare pretest and each of the posttests.

Results

Patient Flow

Patient flow is shown in the CONSORT diagram (Figure 1). During recruitment (June 2010-2012), 86 individuals were screened. Ultimately, 22 qualified individuals agreed to participate and were randomized into the SPG and UPG (see Figure 1 legend for details). One child from the UPG proved to have developmental dyspraxia and was not provided the intended treatment (ie, requiring sequential instructions, analogous to part practice) and the AHA was not usable. The child and the matched pair from the SPG were excluded. Thus, 20 participants (10 per group) completed the study. Table 1 describes participant characteristics. There were no significant group differences in baseline scores for any measure.

Treatment Characteristics

Activity logs showed both groups spent more than 90% of time engaged in activities that required the use of the more-affected hand (SPG, 94.3%; UPG, 98.9%). In the SPG, whole task practice accounted for 87.8% of the time, and the remaining 12.2% comprised part task practice. Only whole practice occurred for the UPG. The *accelerometry data* for the subset of children who wore it indicated that on average the less-affected hand moved 79% and the more-affected hand moved 74% of the time for the SPG, and the less-affected hand moved 82% and the more-affected hand moved 68% for the UPG ($P > .05$, paired *t* test between the SPG and UPG). Thus, there was no difference in the amount of more-affected UE use between groups.

On average, activities for the SPG progressed in difficulty 55.4 times (standard deviation [SD] = 35.4, range = 11-117) throughout the intervention. Skill progression was not reported or observed for the UPG.

Manual Dexterity and More-Affected Hand Use in Bimanual Activities

For the JTTHF, there was a 65.9-second (28.1%) and a 77.4-second (33.2%) decrease in time for the SPG and UPG, respectively (Table 2, Figure 2A). Newman-Keuls post hoc tests revealed a significant improvement between the pretest and immediate posttest that was maintained at 6 months. For the AHA, there was an increase of 2.3 and 2.8

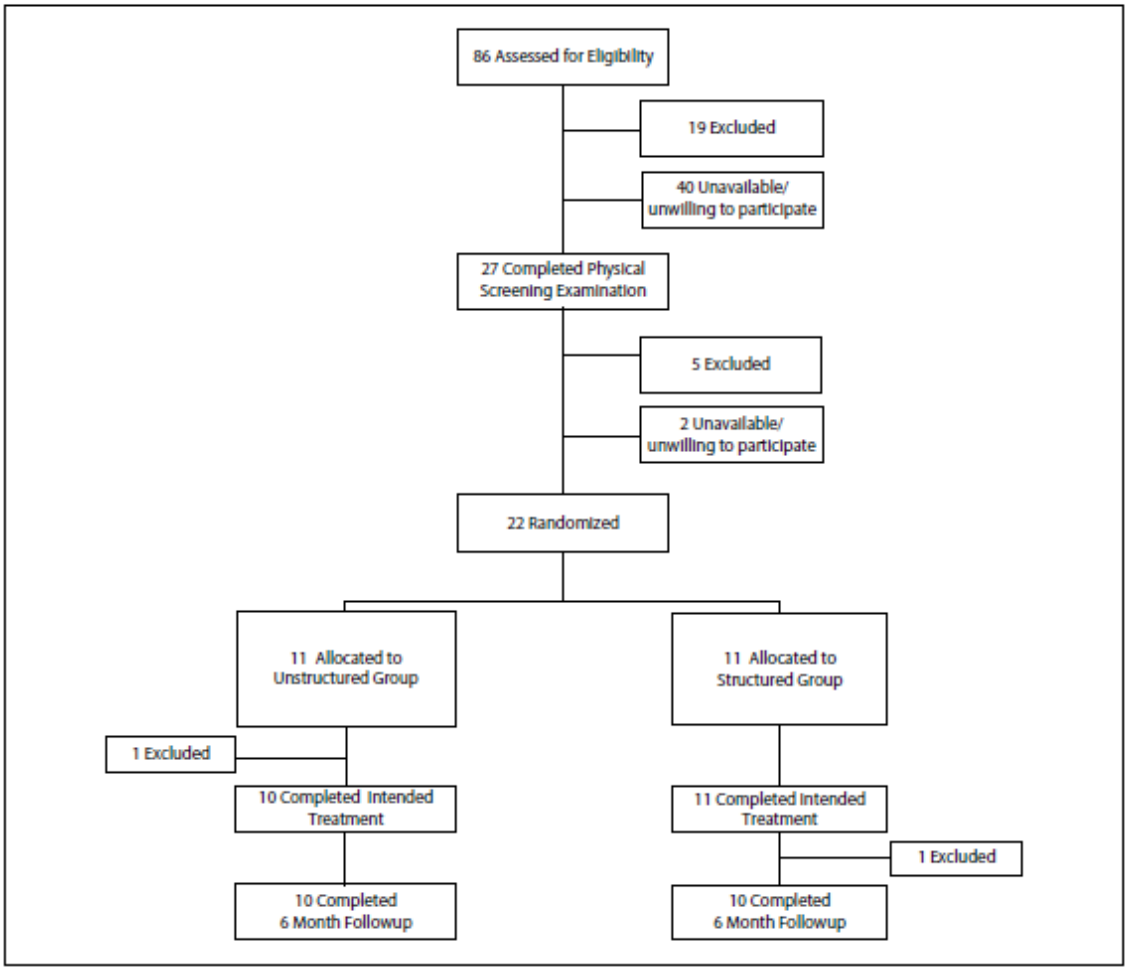

Figure 1. CONSORT flow diagram showing progress through the stages of the study, including flow of participants, withdrawals, and inclusion in analyses. A total of 86 individuals potentially met the study criteria and were invited to undergo physical screening. Twenty-two children qualified for the study and were randomized into the structured practice group (SPG) or unstructured practice group (UPG). One child from the UPG proved to have apraxia and was not provided the intended treatment. The child and the matched pair from the SPG were excluded.

AHA-units for the SPG and UPG, respectively (Figure 2B, Table 2). Post hoc tests revealed a significant improvement between the pretest and immediate posttest, but a return to baseline levels at 6 months, which was uncorrelated with age. There were no group × test session interactions for either measure.

Functional Goals and Daily Functioning

The majority of goals were bimanual (remaining goals were unimanual with the more-affected hand). Most of the goals comprised self-care activities (eg, dressing, grooming, and eating), followed by play (eg, ball activities). At camp, the SPG spent on average 370 minutes practicing goals whereas the UPG did not practice goals. At home, children spent 165 and 184 minutes for the SPG and UPG, respectively, practicing goals. Both groups presented significant improvements after the intervention on satisfaction and on performance, but there was a significant group × test session interaction, with greater improvement for the SPG (Table 2). This difference was no longer present at 6 months (paired *t* test). There was no correlation (Pearson) between the amount of home practice of goals and COPM improvement.

Table 1. Baseline Participant Characteristics.

Characteristics	SPG (n = 10)	UPG (n = 10)
Mean age (SD) years, months	8, 6 (1, 5)	8, 3 (1, 5)
Gender, n (%)		
Male	6 (60)	6 (60)
Female	4 (40)	4 (40)
More affected hand, n (%)		
Right	7 (70)	7 (70)
Left	3 (30)	3 (30)
Lesion location (type)		
Right	3 (0[a], 2[b], 1[c])	3 (0[a], 1[b], 2[c])
Left	7 (1[a], 5[b], 1[c])	7 (0[a], 6[b], 1[c])
Race, n (%)		
Caucasian	8 (80)	7 (70)
African American	1 (10)	0 (0)
Hispanic	1 (10)	2 (20)
Mixed	0 (0)	1 (10)
MACS		
I	2	1
II	6	7
III	2	2
Baseline JTTHF, mean (SD), seconds	233 (159)	234 (168)
Therapy		
PT	10	7
OT	10	9

Abbreviations: SPG, structured practice group; UPG, unstructured practice group; SD, standard deviation; MACS, Manual Ability Classification System; JTTHF, Jebsen-Taylor Test of Hand Function; PT, physical therapy received off-site (number of individuals); OT, occupational therapy received off-site (number of individuals).
[a]Disorder of cellular migration.
[b]Abnormality of periventricular white matter.
[c]Cortical/subcortical lesion.

For the ABILHAND-Kids, both groups presented significant improvement with no group × test session interactions. Both groups improved on the functional self-care skills and caregiver assistance scale of the PEDI, with no significant interaction. Post hoc tests indicated significant improvements for the functional self-care skills at immediate posttest that were maintained at 6 months. However, the caregiver assistance scale was only significantly higher at the 6-month follow-up.

Control Group

It is conceivable that the similar improvements were simply because of repeating the tests over a 3-week period. To determine whether that was likely the case, we subsequently conducted the tests twice, 3 weeks apart, in a group of 10 (nonrandomized) children with USCP (mean age = 8.9 years, 5 males, 5 females) who did not receive treatment. There were no significant changes in the AHA (test 1 = 65.5 AHA-units [sd = 12.4]; test 2 = 64.8 AHA-units [SD = 14.3]), JTTHF (test 1 = 496.1 seconds [SD = 306.6]; test 2 = 465.8 seconds [SD = 315.9]), ABILHAND-Kids (test 1 = 1.6 logits [SD = 1.3]; test 2 = 1.6 logits [SD = 1.1]), PEDI functional skills self-care (test 1 = 61 [SD = 7.57]; test 2 = 63.3 [SD = 6.93]), or PEDI caregiver assistance (test 1 = 35.7 [SD = 3.2], test 2 = 36.7 [sd = 5.7]).

Discussion

To our knowledge, this study is the first to compare components of pediatric intensive bimanual rehabilitation protocols. Children from both the SPG and UPG demonstrated improvements in dexterity and functional use of the hands. This suggests that at least for such intensive bimanual approaches, training may not require structured practice to elicit improvements in clinical measures, and that the emphasis can be placed on fun activities that require use of both hands. However, considering that the SPG showed superior improvements in functional goals, there may be added benefit of including goal training. These findings are discussed in relation to other treatment approaches and neuroplasticity in animal and humans.

Similar dexterity improvements for both groups did not support our hypothesis of greater improvements for the SPG, and are not in agreement with animal model studies that point to the importance of skilled training in the acquisition of motor skills.[28-30] However, in a study in which squirrel monkeys were exposed to a reaching task that did not involve skilled training, the animals showed improvements in the number of pellet retrievals and increase in speed to retrieve them.[28] Thus, it is possible that even without skilled training, intensive use of the more-affected hand leads to improvements in the speed to perform unimanual tasks.[28] Improvements in both groups are analogous to the similar improvements in the amount and quality of more-affected hand use between a group of adults with hemiparetic stroke submitted to CIMT regardless of whether shaping was used.[28] However, as acknowledged by the authors, it is not possible to conclude that skilled training is not relevant as the task practice group was submitted to some elements of shaping, such as verbal feedback and information related to the individual's performance.[32] In the present study, we carefully monitored children's activities during play activities from both groups. However, it was not possible to monitor every interaction or to avoid verbal feedback and encouragement.

This study suggests that bimanual structured practice may not be essential for improvements. The structure of intensive protocols is not well described in the literature. Some authors describe protocols comprising 1 interventionist to 2 children,[14-16] or combining caregivers' and therapists actions,[44] which may reduce the amount of skilled training. Nevertheless, these studies report improvements in hand

Table 2. Results.

	Pretest	Immediate Follow-up	6-Month Follow-up	Change Score	Test Session Effect *P* Value (η^2)	Interaction *P* Value (η^2)
AHA (AHA-units)						
SPG	61.9 (55.4, 68.4)	64.2 (56.0, 72.4)	62.4 (55.4, 69.4)	2.3 (−0.9, 5.5)	—	—
UPG	63.8 (57.3, 70.3)	66.6 (58.4, 74.8)	63.0 (56.0, 70.0)	2.8 (−0.4, 6.0)	—	—
Mean	62.9 (58.2, 67.5)	65.4 (59.6, 71.2)	62.7 (57.7, 67.7)	2.5 (0.4, 4.7)	*P* < .05 (.217)	*P* = .48 (.020)
JTTHF						
SPG	234.8 (126.0, 343.6)	168.9 (82.7, 255.1)	182.6 (94.7, 270.4)	−65.9 (−108.5, −23.3)	—	—
UPG	233.4 (124.7, 342.2)	156.0 (69.8, 242.2)	162.4 (74.5, 250.2)	−77.4 (−120.0, −34.8)	—	—
Mean	234.2 (157.2, 311.0)	162.5 (101.5, 223.4)	172.5 (110.4, 234.6)	−71.7 (−101.0, −42.3)	*P* < .001 (.462)	*P* = .792 (.007)
COPM-Performance						
SPG	3.6 (2.7, 4.4)	6.9 (6.1, 7.7)	6.8 (6.0, 7.5)	3.3 (2.4, 4.2)	—	—
UPG	3.2 (2.4, 4.1)	5.1 (4.3, 5.9)	6 (5.3, 6.8)	1.9 (1.0, 2.8)	—	—
Mean	3.4 (22.8, 4.0)	6.0 (5.4, 6.6)	6.4 (5.9, 6.9)	2.6 (1.9, 3.3)	*P* < .001 (.928)	*P* = .031 (.067)
COPM-Satisfaction						
SPG	4.7 (3.4, 6.0)	8.2 (7.3, 9.1)	7 (6.2, 7.7)	3.5 (2.2, 4.8)	—	—
UPG	3.8 (2.5, 5.0)	5.9 (4.9, 6.8)	6.2 (5.5, 7.0)	2.1 (0.8, 3.4)	—	—
Mean	4.2 (3.3, 5.1)	7 (6.4, 7.7)	6.6 (6.1, 7.1)	2.8 (1.9, 3.7)	*P* < .001 (.928)	*P* = .131 (.022)
ABILHAND-Kids						
SPG	1.7 (0.8, 2.5)	2.5 (11.5, 3.5)	3.0 (22.0, 3.9)	0.8 (0.0, 1.6)	—	—
UPG	1.4 (0.5, 2.2)	1.9 (0.9, 2.9)	2.1 (11.2, 3.1)	0.5 (−0.3, 1.3)	—	—
Mean	1.5 (0.9, 2.1)	2.2 (11.5,2.9)	2.6 (11.9, 3.2)	0.7 (0.1, 1.2)	*P* < .01 (.246)	*P* = .667 (.017)
PEDI: Self-care						
SPG	65 (61.2, 68.8)	69.5 (66.2, 72.8)	70.3 (67.0, 73.6)	4.5 (2.78, 6.2)	—	—
UPG	61.0 (57.2, 64.8)	63.9 (60.6, 67.2)	67.2 (64.0, 70.5)	2.9 (1.2, 4.6)	—	—
Mean	63.0 (60.3, 65.7)	66.7 (64.3, 69.1)	68.8 (66.4, 71.1)	3.7 (2.5, 4.9)	*P* < .001 (.540)	*P* = .358 (.025)
PEDI: Caregiver assistance						
SPG	33.9 (30.2, 37.6)	33.7 (30.0, 37.4)	37.1 (34.3, 39.9)	−0.2 (−2.5, 2.1)	—	—
UPG	28.9 (25.2, 32.6)	31.2 (27.5, 3439)	34.4 (31.6, 37.2)	2.3 (0.0, 4.6)	—	—
Mean	31.4 (28.8, 34.0)	32.5 (29.8, 35.1)	35.8 (33.8, 37.8)	0.9 (−0.7, 2.8)	*P* < .001 (.449)	*P* = .240 (.042)

Abbreviations: SPG, structured practice group; UPG, unstructured practice group; JTTHF, Jebsen-Taylor Test of Hand Function; AHA, Assisting Hand Assessment; COPM, Canadian Occupational Performance Measure (performance and satisfaction scales); PEDI, Pediatric Evaluation of Disability Inventory (functional skills and caregiver assistance scales).

function.[15,44] The high intensity in both groups seems to be the main contributor to the similar improvements in dexterity as the (albeit nonrandomized) post hoc control group did not demonstrate changes in the same measures. Only part practice, skill progression, and goal training differed. Intensity is considered an important ingredient in motor learning, as it leads to repetitions of movements and the development of new motor strategies.[23] Thus, in intensive protocols the amount of practice is seemingly more important than what is practiced. However, these results may be dose dependent, as it is possible that at lower intensity, other components (eg, specificity of training) may be more important.[10] The low number of movement repetitions resulting from usual and customary schedules of physical/occupational therapy may partly explain the lack of evidence for these approaches.[45] Thus, it is possible that skilled practice is important in such low dose interventions.

One unexpected result was the lack of maintenance of AHA increases at the 6-month follow-up. Although it seems contrary to previous studies documenting persistent changes on this outcome following CIMT or HABIT,[3,13,15] the lack of retention may be because of the age of the participants, who were generally older than those from other studies. Eliasson et al[46] reported no change in AHA following CIMT in 8- to 17-year-old children. We concur with their suggestion that changes in bimanual function are harder to achieve in older children.[46] It is possible that at older ages, dexterity (eg, JTTHF) and functional use (eg, COPM, PEDI, ABILHAND-Kids) may improve and be maintained, but children may have well established their own strategies to use the more-affected hand in the performance of bimanual activities, and that this strategy is more difficult to change. Goal performance was the only measure to improve differentially, that is, greater initial gains for the SPG. These

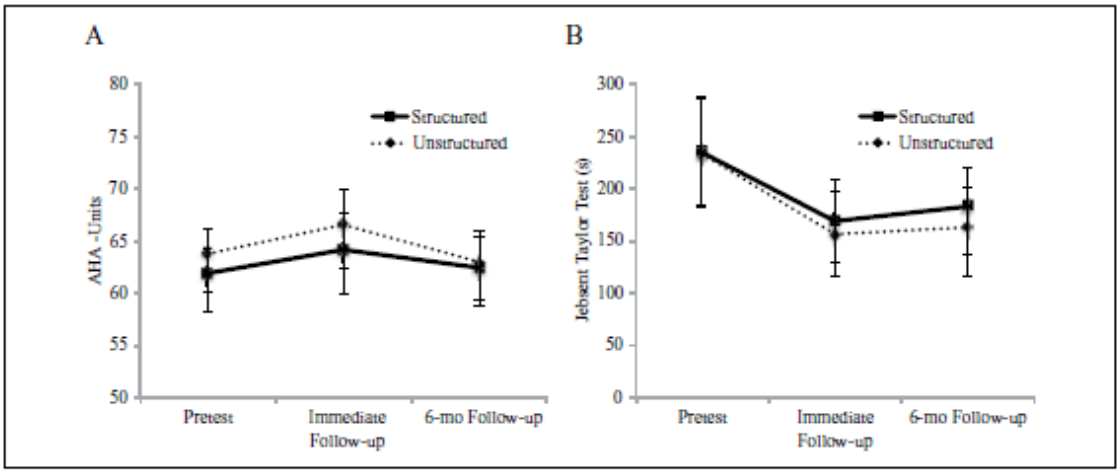

Figure 2. (A) Mean ± standard error of the mean (SEM) scaled logit scores on the Assisting Hand Assessment (AHA); higher scores represent better performance. (B) Mean ± SEM time to complete the 6 timed items (writing excluded) of the Jebsen-Taylor Test of Hand Function. Faster times correspond to better performance. The potential maximum allowable time to complete each item was 180 seconds, resulting in a maximum score of 1080 seconds.

results corroborate studies that found superior improvements on goal performance when goals are practiced.[13,47] If the 6-month follow-up data are compared, the differences were no longer significant. As children from the SPG had the opportunity to practice some of the goals and this practice involved skill progression, it is possible that structured training contributed to the development of strategies to perform relevant functional goals.[47] Such information confirms the need of specific and direct training for the accomplishment of goals related to the use of hands by children with USCP, but the end result over a longer period may not differ.

Limitations

Although the interventions were administered in separate rooms and the groups and interventionists were blinded to the study questions, as mentioned above it is not possible to ensure that skill progression did not occur in the UPG. It is possible that children from either group could have been self-motivated to challenge themselves. However, the children, interventionists and families were not aware of the differences in the protocols provided for each group.

It is also possible that when caregivers selected goals for the COPM, they might be aware of the functional activities that are relevant to their children. This may have motivated goal practice despite that caregivers from both groups were not instructed to practice these goals at home and no information in how to progress performance of these goals was offered during the intervention period. On average, goal practice totaled ~3 hours, which is a small fraction of the amount of treatment time. Moreover, the children and interventionists from the USG were not aware of the goals. Furthermore, it is possible that there were indeed differences between groups that either was not measured (eg, ICF body structure and function level) or that the employed measures were not sensitive enough to detect. Finally, the study may have been underpowered to detect group differences. However, as indicated by the η^2 values (Table 2), the differences in changes were extremely small (favoring the UPG) and would require an extremely large sample size for this difference to be statistically significant, which would bring the clinical significance of such changes into question.

Clinical Implications

The present study shows that the intensive nature of bimanual practice seems to be the main contributor for improvements in manual dexterity. It is not known whether these findings would hold true at lower dosages. It may be possible to combine interventions that involve bimanual play with specific functional training. This may reduce the costs of the provision of high-intensive interventions without compromising the benefits. This possibility needs to be tested in a larger cohort of children.

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APPENDIX G

Research Articles

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Bimanual Training and Constraint-Induced Movement Therapy in Children With Hemiplegic Cerebral Palsy: A Randomized Trial

Andrew M. Gordon, PhD[1,2], Ya-Ching Hung, Ed.D.[3], Marina Brandao[4], Claudio L. Ferre, MA[1], Hsing-Ching Kuo, MS[1], Kathleen Friel, PhD[5], Electra Petra[1], Ashley Chinnan[1], and Jeanne R. Charles, PhD[6]

Abstract

Background. Constraint-induced movement therapy (CIMT) promotes hand function using intensive unimanual practice along with restraint of the less-affected hand. CIMT has not been compared with a treatment with equivalent dosing frequency and intensity in children with cerebral palsy (CP). *Objectives.* The authors report a randomized trial comparing CIMT and a bimanual intervention (hand-arm intensive bimanual therapy; HABIT) that maintains the intensity of practice associated with CIMT but where children are engaged in functional bimanual tasks. *Methods.* A total of 42 participants with hemiplegic CP between the ages of 3.5 and 10 years (matched for age and hand function) were randomized to receive 90 hours of CIMT or an equivalent dosage of functional bimanual training (HABIT) conducted in day-camp environments. A physical therapist blinded to treatment allocation tested hand function before and after treatment. The primary outcomes were changes in Jebsen-Taylor Test of Hand Function (JTTHF) and Assisting Hand Assessment (AHA) scores. Secondary measures included the Goal Attainment Scale (GAS). *Results.* Both the CIMT and HABIT groups demonstrated comparable improvement from the pretest to immediate posttest in the JTTHF and AHA ($P < .0001$), which were maintained at 6 months. GAS, however, revealed greater progress toward goals for the HABIT group ($P < .0001$), with continued improvement across test sessions for both groups ($P < .0001$). *Conclusions.* Both CIMT and bimanual training lead to similar improvements in hand function. A potential benefit of bimanual training is that participants may improve more on self-determined goals.

Keywords

hand, upper-extremity rehabilitation, hemiplegic cerebral palsy, bimanual training, grasp, constraint-induced movement therapy

Introduction

Cerebral palsy (CP) is the most common pediatric physical disability, with congenital hemiparesis comprising one of the most common forms.[1] Strong evidence supporting any upper-extremity treatment approach is lacking.[2,3] Yet there is increasing evidence that hand function in individuals with CP does improve during development[4-6] and with intensive practice.[7,8]

One means to achieve intensive practice is constraint-induced movement therapy (CIMT), involving concurrent physical restraint of the less-affected hand and unilateral training of the hemiplegic hand.[9-11] There is increasing evidence supporting the efficacy of pediatric CIMT,[2,12,13] and its clinical use is proliferating.

Nevertheless, CIMT is potentially invasive,[14] and it is uncertain whether improved hemiparetic hand use results in improved function. Children with hemiplegic CP have impaired bimanual coordination above and beyond their unimanual impairments,[15-17] which may underlie some functional limitations. Furthermore, studies of CIMT (including our own)[18,19] have not compared it with other, equally

[1]Teachers College, Columbia University, New York, NY, USA
[2]College of Physicians and Surgeons, Columbia University, New York, NY, USA
[3]Queens College, CUNY, Flushing, NY, USA
[4]Universidade Federal de Minas Gerais, Brazil
[5]Columbia University Medical Center, New York, NY, USA
[6]Emory University School of Medicine, Atlanta, GA, USA

Corresponding Author:
Andrew M. Gordon, Department of Biobehavioral Sciences, Box 199, Teachers College, Columbia University, 525 West 120th Street, New York, NY 10027, USA
Email: ag275@columbia.edu

intense treatments. Thus, it is unknown whether similar improvements in hand function can be achieved with an equally intensive bimanual treatment.

We report a randomized clinical trial comparing CIMT and a bimanual intervention (hand-arm intensive bimanual therapy, HABIT)[20,21] that maintains the same dosing frequency and intensity and the progressive task-specific practice associated with CIMT, but children are engaged in functional bimanual rather than unimanual tasks. HABIT has a proposed neuroscientific rationale[13,22] and is a formalized approach that has an equal amount of personal interaction and intensity as CIMT, encourages functional gains,[23] and has preliminary evidence suggesting efficacy.[15,21] Yet bimanual training is different from CIMT because of the type of activities practiced. Based on theories of motor learning (practice specificity),[24,25] we hypothesized that the unimanual training associated with CIMT would lead to greater improvements in unimanual dexterity, whereas bimanual training would lead to greater improvements in bimanual hand use and goal attainment.

Method

Participants

Participants were recruited from clinics in the NYC area, our Web site (http://www.tc.edu/centers/cit/), ClinicalTrials.gov (NCT00305006), and online support groups. Potential participants were first screened via e-mail and/or telephone. Those between the ages of 3.5 and 10 years with congenital hemiplegia were invited to receive an on-site physical examination or an examination videotaped by their physical or occupational therapist. The inclusion criteria were established based on prior CIMT and HABIT trials[19,20]: (1) ability to extend wrist >20° and fingers at the metacarpophalangeal joints >10° from full flexion, (2) the ability to lift the more affected arm 15 cm above a table surface and grasp light objects, (3) >50% difference in the Jebsen-Taylor Test of Hand Function (JTTHF) score between the 2 hands and a time below the maximum possible (1080 s) of the paretic hand, (4) mainstreamed in school and a Kaufman Brief Intelligence test score >70, and (5) demonstrated ability to follow instructions during screening and complete the testing. Exclusion criteria included the following: (1) health problems unassociated with CP, (2) current/untreated seizures, (3) visual problems interfering with treatment/testing, (4) severe muscle tone (Modified Ashworth score > 3.5), (5) orthopedic surgery on the paretic hand within 1 year, (6) botulinum toxin therapy in the upper extremity within the past 6 months or intended treatment within the study period, and (7) balance problems precluding wearing a sling. Informed consent was obtained from participants and caregivers. The study was approved by the University Institutional Review Board.

Procedures

General intervention procedures. CIMT and HABIT procedures share in common intensive progressive task practice based on motor learning approaches. They differ in that CIMT requires hand restraint and progression of unimanual tasks, whereas in HABIT, there is absence of restraint, and tasks are progressed bimanually. We first describe the common procedures followed by procedures specific to CIMT and HABIT.

Six HABIT and CIMT day camps were conducted at the University from July 2007 to 2009. The day camps were held in 2 separate rooms. Participants in 1 room received CIMT, and the other received HABIT; 2 to 5 children participated in each treatment at each camp. Participants were engaged in treatment 6 h/d for 15 consecutive weekdays (adjusted for holidays; 90 hours, making up any missed hours) during school recess by trained interventionists. Eight of the interventionists (out of 42 interventionist/child pairs) were physical or occupational therapists (PT/OT; 3 in the CIMT group and 5 in the HABIT group). The remaining interventionists were graduate students in Kinesiology, Neuroscience, Speech Pathology, or Psychology programs and undergraduates. Despite a higher level of knowledge and awareness of how to interact in a therapeutic manner, the PT/OT interventionists were required to provide only the specific procedures related to CIMT/HABIT and were restrained from using other treatment modalities. The expected outcomes were not discussed with interventionists. The pretreatment training, administered by the supervisors, was standardized based on the established manual of procedures for each treatment and reinforced by supervisors and during daily meetings. Each room was always supervised by additional experienced PTs/OTs, who modeled and ensured uniformity of treatment.

Participants worked individually with their interventionist or in groups (1:1 interventionist to participant ratio always maintained). Interventionists were paired with children prior to randomization using family-centered approaches considering caregiver and supervisors' best judgment based on the child's age and gender. Emphasis was placed on making participation enjoyable. With the exception of goals (see the following), tested task items were never trained. Children participated in whole and part task practice. Whole task practice involved sequencing successive movements within the context of tasks (eg, card games). The activities were performed continuously for at least 15 to 20 minutes. Targeted movements and spatial and temporal coordination were practiced within the context of completing the task. Part task practice (analogous to "shaping")[19,20] required breaking down motor skills into smaller components (eg, playing-card turning to promote forearm supination) while increasing repetitions and skill requirements. It also increased intensity of treatment by requiring as many repetitions as

possible over repeated 30-s intervals (typically a minimum of 5 intervals).

Task difficulty was graded by varying the spatial/temporal constraints or by providing tasks that required progressive skilled use as performance improved. Task difficulty was increased when the participant was successful on 7 of 10 repetitions. Task performance was recorded, and task- and age-specific knowledge of results was provided for encouragement.[26-28]

Supervisors instructed interventionists regarding the main focus of the treatment (eg, supination) for each child, based on his/her pronounced movement deficits, interests, and potential for improvement. Data activity logs were collected, which included activities performed, time spent in part/whole practice, and the number of repetitions.

Caregivers were instructed to engage participants in home practice (unimanual without restraint for CIMT and bimanual for HABIT) for 1 h/d during and for 6 months following the intervention and document practice using activity logs.

CIMT procedures. CIMT was modified to be child focused (for details see Gordon et al[19]). Participants' less-affected hands were restrained with slings, and unimanual activities were performed with the paretic hands. The sling was strapped to participants' trunks with the distal end sewn shut and was continuously worn except when toileting or during breaks (not >15 min/d).

Participants performed fine-motor and manipulative gross-motor activities that elicit general movements of interest and that included a range of age-appropriate, unimanual functional and play activities. The interventionist provided assistance where appropriate. For example, during scissor use, the child cut paper while the interventionist held and rotated the paper.

HABIT procedures. HABIT[20] did not use a physical restraint, but instead, participants were engaged in age-appropriate fine- and gross-motor bimanual activities using motor learning approaches.[28] Activities were selected by considering the role of the paretic hand, increasing in complexity from a non-dominant passive assist (eg, stabilizing paper while drawing) to active manipulator (eg, reorienting paper while cutting) using increasingly complex bimanual coordination and participants' interests. Task demands were graded, and participants were engaged in active problem solving.

Interventionists avoided verbal prodding to use the paretic hand and instead constrained the environment by providing tasks necessitating the use of both hands to elicit desired movements. Part practice included both bilateral symmetrical (eg, reaching toward object[s] with both hands) and asymmetrical (eg, pulling apart objects) movements.

Measures

Participants were evaluated directly prior to treatment (pre-test), within 2 days ("immediate"), and 1 and 6 months after treatment by a physical therapist blinded to group allocation (verified following testing). Two primary outcome measures were used to quantify unimanual capacity and bimanual performance under the International Classification of Functioning and Health (ICF) "activity and performance" domain.[29]

The Assisting Hand Assessment (AHA, version 4.3,[30,31]) quantifies the effectiveness with which a child with unilateral disability uses his/her affected (assisting) hand in bimanual activity. It has excellent validity and reliability (interrater = .97, intrarater = .99).[31] The test was videotaped and scored off-site by an experienced evaluator blinded to group allocation.

The JTTHF is a standardized test of simulated functional tasks quantifying the time to complete a battery of unimanual activities.[32] The activities performed with the paretic hand include flipping index cards, object placement, simulated eating, stacking checkers, and manipulating empty and full cans. Reliability for children with stable hand disabilities is high (.95-.99).[33] To determine whether the less-affected hand was affected by the restraint (CIMT) or use (HABIT), we also measured the JTTHF for this hand before and immediately after CIMT/HABIT.

Three secondary measures were also used. The dissociated movements (ICF "body function") and grasp (ICF "body function" and "activity and performance") subtests of the Quality of Upper Extremity Skills Test (QUEST)[34] were used to characterize dissociation of distal and proximal upper-extremity movements and attainment of specific grasp patterns of the 2 hands (ICF activity and performance and body function/structure). The sum score for both hands is converted to a standardized score (maximum = 100).

The Goal Attainment Scale (GAS; ICF "activity and performance" and "participation") was used to quantify progress on established goals before group assignment (1 functional and 1 play goal defined by caregivers and/or older participants). Goals were assessed for appropriateness based on age and current abilities and scaled off-site by a physical therapist. The goals were practiced up to 30 min/d. However, because emphasis was placed on embedding practiced movements into fun activities rather than providing "goal-training" as is often done in traditional occupational/physical therapy, interventionists were given latitude as to how much training was provided (if at all) within the 30-minute limit. The CIMT group was unable to practice bimanual goals and, instead, practiced unimanual movement components comprising the goal. Goal achievement[35] was rated by the caregiver and verified by a physical therapist. Scores were transformed to standardized T scores (mean = 50; standard deviation = 10)[35]:

$$T = 50 + \frac{10\sum x_i}{\sqrt{n - np + n^2 p}},$$

where x_j is the attainment score, n the number of scales, and P the expected correlation of scales ($P = .3$). A T score of 50 indicates that goals were attained.

Children wore activity monitors (Manufacturing Technology Inc, Fort Walton Beach, Florida, #7164; 5.1 cm × 2.6 cm × 1.5 cm, 42.9 g)[36] on their wrists during the AHA test sessions. The units sample (10 Hz) and store summed values in memory and data were downloaded to a PC. The number of accelerations is measured as activity counts (0.01664 g for an acceleration of 2.13 g directed parallel to the x-axis at 0.75 Hz), which were used to determine the percentage of time each hand was used while performing AHA activities.[21]

Statistical Design

Sample size calculations were performed based on AHA scores derived from an earlier HABIT trial.[21] A mean improvement of 0.94 ± 0.54 logits for the HABIT group was reported. With $\alpha = .05$, $1 - \beta = 0.80$, and an estimated 20% dropout, 21 participants in each group were required. Intention-to-treat principles were used.

A 2 (Group) × 4 (Test Sessions) ANOVA with repeated measures on test sessions was performed on raw and log-transformed AHA, JTTHF, QUEST, and accelerometry data using SPSS 15. Since the findings were qualitatively similar, only analyses of raw data are reported. The overall Group × Test Session interaction tested whether the time course differed between treatment groups. Planned comparisons were used between the pretest and each of the posttests and the immediate posttest with the 1- and 6-month posttest. Bonferroni corrections were used, resulting in a significance level of $P < .01$. A 2 (Group) × 3 (Posttests) ANOVA with repeated measures on posttests was performed on the GAS T scores, with the group factor indicating whether one group had greater goal attainment.

Results

Patient Flow

Patient flow is shown in the CONSORT flow diagram (Figure 1). During recruitment (June 2007-2009), 183 individuals were screened. Ultimately, 44 qualified individuals agreed to participate and were randomized into the HABIT or CIMT group (see Figure 1 legend for details). Participants (4-10 in each camp) were randomized offsite using concealed allocation stratified by age and JTTHF screening score. A total of 42 participants (21 in each group) completed the study. Table 1 describes participant characteristics. There were no significant group differences in baseline scores for any measure (Table 2).

Treatment Characteristics

Participants in the HABIT and CIMT groups completed all 90 hours of treatment and averaged 79% and 81% of the time in structured practice, respectively (remaining time was spent in transitioning between tasks, toileting, etc). Children spent 17% and 16% of the practice time, respectively, in part practice, with the remainder in whole practice. Direct observation by the supervisors and monitoring of daily logs confirmed that both treatment protocols were completely adhered to and only the intended treatments were received. Home logs indicated that children averaged 286 minutes of the requested 360 min/wk engaging in home practice during the 6 months following the intervention.

No adverse events were reported. Participants stopped usual and customary care (UCC) during the treatment but resumed afterward (proportion receiving therapy was similar between groups: $P > .05$, Table 1). There were no changes to preexisting therapy.

Quality and Amount of Movement

Table 2 shows the means for the HABIT and CIMT groups at each time point and the pretest to immediate posttest difference for all measures. For the JTTHF, there was a 141.7 s (37.8%) and a 131.2 s (34.5%) decrease for the CIMT and HABIT groups, respectively (Figure 2A). Similarly, the AHA scaled logit scores improved 2.24 and 3.0 points for the CIMT and HABIT groups, respectively (Figure 2B). Planned comparisons revealed that the changes were attributed to differences between the pretest and immediate posttest that were maintained over the 6 months. There were no Group × Test Session interactions for either primary measure.

There was no difference in the less-affected hand JTTHF for either group (CIMT change score = 6.1, 95% confidence interval [CI] = −3.5, 15.7; HABIT change score = 5.2, 95% CI = −0.8, 11.3; $P > .05$ in both cases).

To account for the asymmetric distribution of right- and left-side hemiparesis across the 2 groups, we repeated the analyses adding side as an additional factor. There was no effect of side or group interaction for either measure.

A main effect of testing session was also seen for secondary measures (QUEST dissociated movements and grasp subtests, and the accelerometry, Table 2) for both treatments. QUEST changes were largely a result of increased scores for the paretic hand ($P < .001$, not shown separately in Table 2). However, scores for the less-affected hand increased slightly (raw score pretest to immediate posttest across both groups, 23.3 to 23.9) for the grasp ($P < .001$) but not for the dissociated movements subtest. Accelerometry results indicated that the less-affected hand moved ~91% and the paretic hand ~64% of

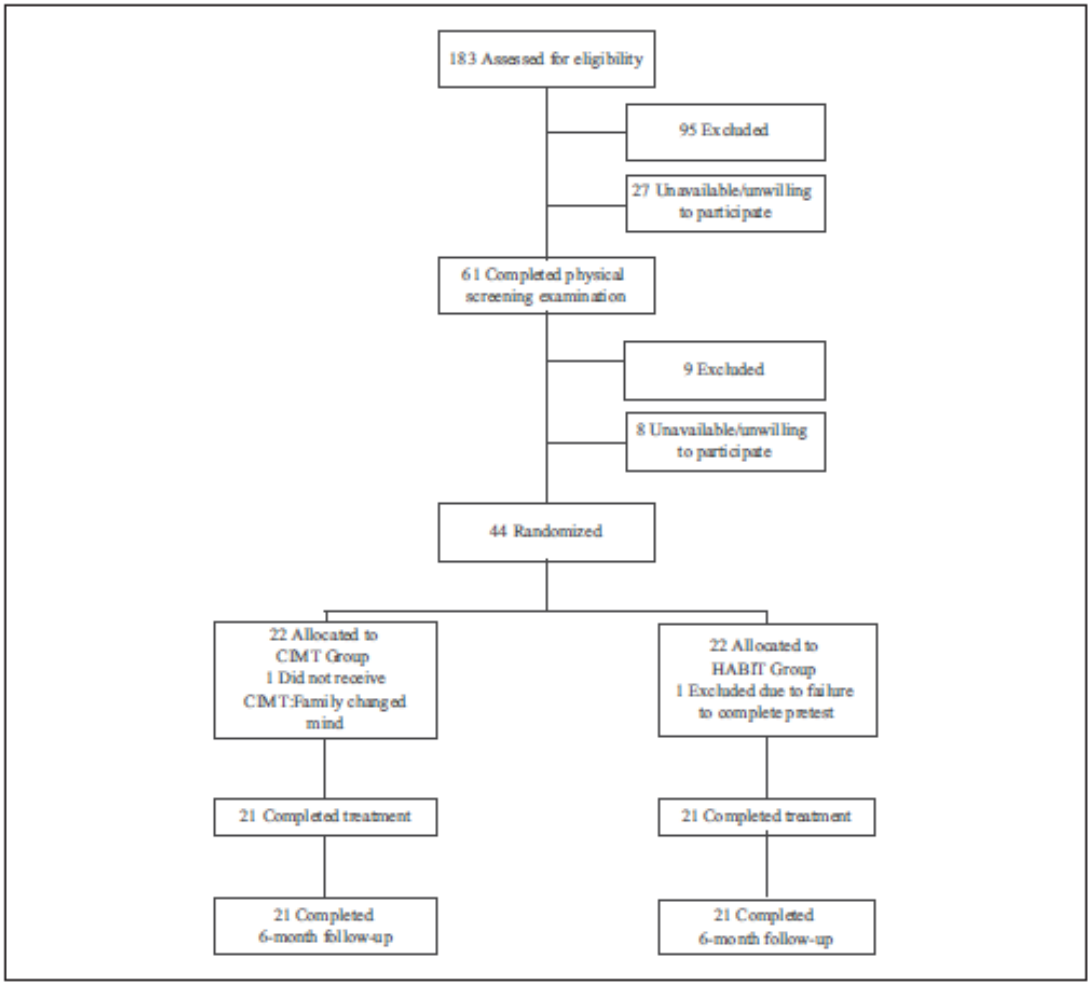

Figure 1. CONSORT flow diagram showing progress through the stages of the study, including flow of participants, withdrawals, and inclusion in analyses. A total of 183 individuals were screened via telephone/e-mail, and 95 of these were excluded for the following reasons: too old (n = 11), too young (n = 35), poor cognition (n = 11), wrong diagnosis (n = 22), botulinum toxin treatment within prior 6 months or planned within study period (n = 2), uncontrollable seizures (n = 7), prior CIMT within 6 months (n = 5), surgery (n = 1), and disease too severe (n = 1). A total of 88 individuals potentially met the study criteria and were invited to undergo physical screening; 27 individuals chose not to undergo physical screening. Of the remaining 61 individuals, 9 were excluded on the basis of the physical exam: too severe (n = 8) and too mild (n = 1). Of these individuals who qualified, 8 chose not to participate: not available during day-camp period (n = 5) and hardship associated with participating (n = 3). Ultimately, 44 of the remaining individuals agreed to participate and were randomized into the HABIT or CIMT group. One participant dropped out after randomization (unaware of group allocation), and another was excluded after the intervention for inability to comply with testing procedures. A total of 21 individuals from each group completed the intervention and subsequent posttests. Abbreviations: CIMT, constraint-induced movement therapy; HABIT, Hand-Arm Intensive Bimanual Therapy.

the time for each group at baseline. Hand use increased ~14% for both groups (ie, making up half of the baseline difference) for the hemiparetic hand, whereas it remained unchanged for the less-affected hand. The changes did not correlate with AHA scores changes ($r = -0.041$) but did correlate with JTTHF changes ($r = 0.48$; $P < .001$).

Table 1. Baseline Participant Characteristics

Characteristics	CIMT (n = 21)	HABIT (n = 21)
Mean age (SD), years, months	6, 3 (2, 2)	6, 4 (1, 11)
Gender		
Male	9 (43%)	11 (52%)
Female	12 (57%)	10 (48%)
Paretic hand		
Right	15 (71%)	9 (43%)
Left	6 (29%)	12 (57%)
Lesion location[a] (type)		
Right	5(1,[b] 2,[c] 2,[d] 0[e])	8 (2,[b] 2,[c] 4,[d] 0[e])
Left	8 (3,[b] 2,[c] 3,[d] 0[e])	7 (1,[b] 3,[c] 2,[d] 1[e])
Bilateral	1 (0,[b] 0,[c] 1,[d] 0[e])	1 (0,[b] 1,[c] 0,[d] 0[e])
Race		
White	15 (71%)	12 (57%)
African American	3 (14%)	4 (19%)
Hispanic	2 (10%)	4 (19%)
Asian	1 (5%)	1 (5%)
MACS		
I	2 (10%)	3 (14%)
II	18 (85%)	17 (81%)
III	1 (5%)	1 (5%)
Baseline JTTHF, mean (SD), s	375 (254)	381 (226)
KBIT, mean (SD)	96.7 (20.7)	99.3 (16.5)
2PD[f]	3.1 (0.83)	3.4 (1.5)
Controlled seizures	3 (14%)	7 (33%)
Therapy		
PT	20 (95%)	21(100%)
OT	20 (95%)	18 (85%)

Abbreviations: CIMT, constraint-induced movement therapy; HABIT, Hand-Arm Intensive Bimanual Therapy; SD, standard deviation; MACS, Manual Ability Classification System; JTTHF, Jebsen-Taylor Test of Hand Function; KBIT, Kaufman Brief Intelligence Test; 2PD, 2 point discrimination (average of thumb and index); PT, physical therapy received off-site (number of individuals); OT, occupational therapy received off-site (number of individuals).

[a]MRI from 12 children were not available.

[b]Brain malformation.

[c]Abnormality of periventricular white matter.

[d]Cortical/subcortical lesion.

[e]Nonprogressive postnatal brain injury (CIMT child, left precentral gyrus, right corona radiata; HABIT child, bilateral swelling, more pronounced on right, minimal hyperdensity along body of left ventricle).

[f]Out of 30 participants, 12 were undetermined because of age.

Goal Attainment

Goal characteristics and attainment are described in Table 3. The majority of goals were bimanual (remaining goals were unimanual with paretic hand). All goals were related to the ICF "activity and performance" domain. The HABIT group spent more intervention time practicing goals than the CIMT group, but home time practice of goals did not significantly differ between groups.

Both groups achieved (T score of 50) or exceeded their expected level of goal performance and continued to improve across test sessions. However, the HABIT group made greater progress than the CIMT group, as indicated by significant group differences in their T scores across all test sessions (Table 3). The combined amount of camp and home practice time did not correlate with GAS T scores ($r = 0.06$) at immediate posttest.

Approximately 25% of the identified goals were not practiced in either group during the interventions or at home. However, the T scores for these unpracticed goals were significantly higher for the HABIT group (Table 3). These scores appeared to improve across test session, but the improvement and interaction with group was not statistically significant.

Predictors of Improvement

Participants with higher initial JTTHF scores had greater absolute ($r = 0.69$), but not percentage change in, JTTHF scores for CIMT and HABIT. Neither age nor any other variable was related to primary measure improvement for either treatment.

Finally, in the present study, only 8 of the 42 participants were assigned a physical or occupational therapist for the intervention. There was little difference in AHA or JTTHF changes (as evidenced by small mean differences and overlapping distributions) regardless of whether the interventionist was a PT/OT or not (PT/OT AHA change score = 1.37, 95% CI = −1.11, 3.86; non-PT/OT AHA change score = 2.91, 95% CI = 1.47, 4.35; PT/OT JTTHF change score = 118.9, 95% CI = 48.8, 189.0; non-PT/OT JTTHF change score = 140.6, 95% CI = 98.4, 182.9). Similar absences of differences were observed for the secondary measures.

Discussion

Bimanual training and CIMT resulted in similar improvements in the primary measures, which did not support our hypothesis of specificity of training. This suggests that improvements in hand function associated with CIMT can be achieved with an equally intensive bimanual approach. However, there was specificity of training for goal attainment, whereby the HABIT group made better progress on established goals and transfer to unpracticed goals. These findings have important implications, given the increasing popularity and potential invasiveness of CIMT. It is important to note that our results suggest that intensive progressive task-specific training improves hand function.

Both CIMT and Bimanual Training Improve Hand Function

In agreement with our quasirandomized CIMT/HABIT trial,[15] there were significant changes in all measures following CIMT and HABIT. The partial η^2 (Tables 2 and 3) indicated that 26% and 48% of the variance in AHA and

Table 2. Results

	Pretest (95% CI)	Immediate Posttest (95% CI)	1-Month Posttest (95% CI)	6-Month Posttest (95% CI)	Change Score (Pretest to Immediate Posttest) (95% CI)	Test Session Effect *P* Value (Partial η^2)	Interaction *P* Value (Partial η^2)
AHA (Logits)							
CIMT	0.38 (–0.42, 1.17)	0.80 (0.01, 1.58)	0.90 (0.12, 1.67)	1.05 (0.35, 1.74)	0.42 (0.08, 0.76)	—	—
HABIT	0.38 (–0.41, 1.17)	0.94 (0.16, 1.72)	0.98 (0.21, 1.76)	0.99 (0.30, 1.69)	0.56 (0.23, 0.90)	—	—
Mean	0.38 (–0.20, 0.90)	0.87 (0.30, 1.40)	0.94 (0.40, 1.50)	1.02 (0.60, 1.50)	0.49 (0.25, 0.73)	*P* < .0001 (.260)	*P* = .806 (.008)
JTTHF (s)							
CIMT	374.8 (268.9, 480.6)	233.1 (154.0, 312.2)	207.1 (146.1, 268.1)	221.0 (150.1, 291.8)	–141.7 (–195.4, –88.0)	—	—
HABIT	380.8 (275.0, 486.7)	249.6 (170.5, 328.7)	236.9 (176.0, 297.9)	222.7 (151.9, 293.5)	–131.2 (–185.0, –77.5)	—	—
Mean	377.8 (306.1, 449.5)	241.4 (187.7, 295.0)	222.0 (180.5, 263.6)	221.8 (173.9, 269.8)	–136.5 (–174.0, –98.9)	*P* < .0001 (.480)	*P* = .857 (.006)
Accelerometry (%)							
CIMT	65.3 (62, 69)	77.6 (75, 81)	77.8 (75, 81)	79.0 (76, 82)	12.3 (9, 15)	—	—
HABIT	63.1 (60, 67)	78.3 (75, 81)	76.4 (74, 79)	77.8 (75, 81)	15.2 (13, 18)	—	—
Mean	64.2 (60, 70)	78.0 (76, 80)	77.1 (76, 79)	78.4 (77, 80)	13.8 (12, 16)	*P* < .0001 (.774)	*P* = .346 (.027)
QUEST—Dissociated Movement (standardized scores)							
CIMT	85.2 (81.6, 88.8)	90.3 (88.1, 92.6)	91.3 (89.0, 93.5)	89.1 (86.4, 91.8)	5.2 (1.7, 8.7)	—	—
HABIT	87.7 (84.1, 91.3)	91.2 (89.0, 93.4)	90.8 (88.5, 93.1)	90.9 (88.2, 93.6)	3.4 (0.2, 6.9)	—	—
Mean	86.5 (84.1, 89.0)	90.8 (89.2, 92.3)	91.0 (89.5, 92.5)	90.0 (88.2, 91.8)	4.3 (1.9, 6.8)	*P* < .0001 (.200)	*P* = .369 (.026)
QUEST—Grasp (standardized scores)							
CIMT	69.5 (63.6, 75.4)	80.6 (75.8, 85.4)	81.2 (76.8, 85.7)	78.8 (72.8, 85.0)	11.1 (6.1, 16.1)	—	—
HABIT	68.6 (62.7, 74.5)	79.4 (74.6, 84.2)	79.9 (75.5, 84.4)	76.2 (70.1, 82.3)	10.8 (5.8, 15.8)	—	—
Mean	69.0 (65.0, 73.0)	80.0 (76.8, 83.3)	80.6 (77.6, 83.6)	77.5 (73.3, 81.7)	11.0 (7.5, 14.5)	*P* < .0001 (.359)	*P* = .948 (.005)

Abbreviations: CIMT, constraint-induced movement therapy; HABIT, Hand-Arm Intensive Bimanual Therapy; JTTHF, Jebsen-Taylor Test of Hand Function; AHA, Assisting Hand Assessment; QUEST, Quality of Upper Extremity Skills Test; Mean, represents the average for the CIMT and HABIT groups.

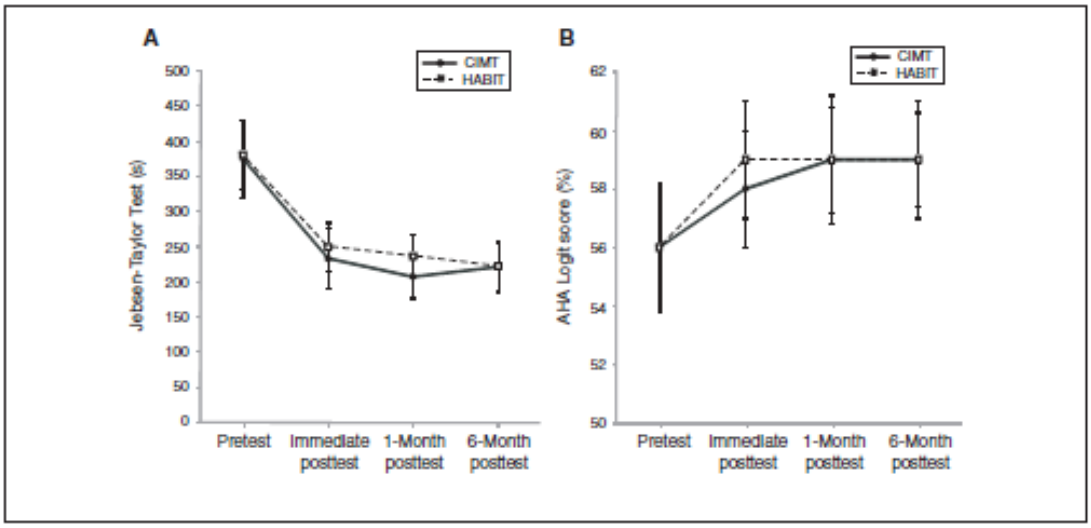

Figure 2. A. Mean ± standard error of the mean (SEM) time to complete the 6 timed items (writing excluded) of the Jebsen-Taylor Test of Hand Function. Faster times correspond to better performance. The maximum allowable time to complete each item was 180 s, resulting in a maximum score of 1080 s. B. Mean ± SEM scaled logit scores on the AHA; higher scores represent better performance. Abbreviations: CIMT, constraint-induced movement therapy; HABIT, Hand-Arm Intensive Bimanual Therapy; AHA, Assisting Hand Assessment.

JTTHF, respectively, and up to 77% for secondary measures, is attributable to the treatments. Similar findings have been reported following adult stroke cerebral vascular accident (CVA) interventions.[37,38] The robust JTTHF changes for both treatments (137 s) were nearly twice those reported in a 13-year follow-up study of hand function

Table 3. Goal Attainment Scale

Goal Category	Number of Goals					
Unimanual	6 (17.0%)					
Bimanual	36 (83.0%)					
Functional goal type						
Dressing	26 (62.0%)					
Eating	12 (28.6%)					
Improve object manipulation	3 (7.1%)					
Other	1 (2.3%)					
Play goal type						
Ball play	19 (45.2%)					
Computer/video game	6 (14.2%)					
Scissors	5 (11.9%)					
Other[a]	12 (28.7%)					
Goal Practice (minutes)	Intervention	Home				
CIMT	70.8 (78.7)	12.1 (22.1)				
HABIT	138.0 (98.2)	25.9 (32.3)				
t Test	$t = 2.45, P < .05$	$t = 1.6, P = .11$				
	Immediate Posttest (95% CI)	1-Month Posttest (95% CI)	6-Month Posttest (95% CI)	Group Effect *P* Value (Partial η^2)	Test Session Effect *P* Value (Partial η^2)	Interaction *P* Value (Partial η^2)
GAS (*T* score—all goals)[b]						
CIMT	51.0 (47.5, 54.4)	54.5 (51.5, 57.6)	59.0 (55.8, 62.3)	—	—	—
HABIT	59.1 (55.6, 62.7)	61.3 (58.1, 64.4)	63.8 (60.5, 67.0)	—	—	—
Mean	55.0 (52.6, 57.5)	57.9 (55.7, 60.1)	61.3 (58.9, 63.7)	$P < .001$ (.264)	$P < .001$ (.235)	$P = .412$ (.022)
GAS (*T* score—unpracticed)						
CIMT	47.5 (39.4, 55.6)	49.5 (42.9, 56.1)	59.5 (53.5, 65.5)	—	—	—
HABIT	60.0 (50.3, 69.7)	60.7 (52.8, 68.6)	61.4 (54.2, 68.7)	—	—	—
Mean	53.8 (47.4, 60.1)	55.1 (50.0, 60.3)	60.5 (55.8, 65.2)	$P < .05$ (.258)	$P = .076$ (.158)	$P = .174$ (.110)

Abbreviations: CIMT, constraint-induced movement therapy; HABIT, Hand-Arm Intensive Bimanual Therapy; CI, confidence interval; GAS, Goal Attainment Scale.
[a]Other play activities included braiding hair, holding a book or cards, playing with Legos, dressing a doll, and using a remote car.
[b]Average of functional and play *T* scores; Mean, refers to the mean of the CIMT and HABIT groups.

development in CP.[4] Yet these improvements were achieved in just 3 weeks and were maintained at the 6-month follow-up.

Since early development of corticospinal tract (CST) is activity dependent, prolonged movement restraint in the developing infant could conceivably affect the restrained hand.[15,39] Treatments that potentially promote rebalancing M1 excitability, such as bimanual practice or inhibiting contralesional M1 using transcranial magnetic stimulation,[40] may facilitate recovery. Our findings indicate that bimanual training is an effective alternative to CIMT.

Intensive approaches such as CIMT may be additive over repeated exposures.[41] Thus, emphasis should be placed on providing treatment in a child-friendly manner. There could also be an additive effect of combined (CIMT/bimanual) ingredients. Combined treatment showed improvement compared with a group of participants who received the same cumulative amount of an alternative treatment, although the dosing schedules were different.[42] Our findings of similar improvements in hand function for both treatments may indicate that both dosing schedule and intensity may be important.

Bimanual Training and Goal Attainment

Bimanual training affords learning strategies that have direct impact on daily routines. Although both groups achieved expected goal performance, the attainment was higher for the HABIT group at all test sessions. Goal attainment continued to improve for both groups across test sessions, with the CIMT group appearing to make greater continued gains at the 6-month posttest (but $P > .05$). Not surprisingly, most goals were bimanual because

participants had a well-functioning dominant hand. Performance of bimanual activities in hemiplegic CP is a complex process influenced by internal and external factors, with strategies chosen based on the least negative alternative.[43] Thus, improved bimanual performance is functionally important, and bimanual training allows direct practice of these goals.

It would not be surprising that goals that are practiced more improve more. However, despite the greater goal practice in the HABIT group, this was not the case because the amount of practice did not correlate with GAS improvements. It is particularly interesting that there was greater improvement even for unpracticed goals for the HABIT group. Thus, there was better transfer of practice from other bimanual skills to goal achievement. This transfer could be a result of improvements in the ability to coordinate movements between the 2 hands and/or increased problem solving (identified as important for plasticity)[44] required during bimanual performance, given the increased degrees of freedom. Accordingly, action planning in children with hemiplegia may improve following a combined CIMT/bimanual training.[45]

Therapeutic Considerations

One potential advantage of CIMT is that the restraint allows the interventionist to focus solely on the more affected hand. Our protocol relies on a 1-to-1 interventionist to child ratio. In the absence of this possibility, the restraint may result in greater intensity because participants would have no choice but to use their more-affected hand (ie, forced use). However, the restraint would preclude opportunity to practice functionally meaningful (bimanual) movements.

The similar improvements regardless of whether or not interventionists were clinicians suggest that the 1-to-1 ratio can be maintained in an economically feasible manner. The similarity is likely a result of the fact that preintervention training is standardized, and clinicians were required to closely adhere to standardized procedures and not administer therapeutic techniques incompatible with motor learning approaches used by HABIT and CIMT.[28] Both treatments were supervised by PT/OTs, who maintained the integrity of the standardized training, which may have further reduced differences.

Limitations

There may be individual differences that make 1 treatment more effective for a given individual or yield a response difference that depends on the hemiparetic side. There was an unequal distribution of side of hemiparesis in each group. However, when this was taken into account, the results did not change.

It is conceivable that the measures used are not sensitive enough to detect subtle differences between treatment outcomes. For example, there could be differential changes in the spatial–temporal coordination of the hands that can only be determined kinematically. Furthermore, although the sample size was fairly large for a physical rehabilitation study, differences may emerge with a larger and more diverse sample.

We did not randomize interventionist assignment or include a no-treatment/UCC group. However, for the latter, nearly all the improvement occurred at the immediate posttest. Although we only had 1 pretest, the JTTHF during screening (~1 month prior) and at pretest did not significantly differ. Furthermore, earlier studies of CIMT[4,18] and HABIT[21] indicate that these measures do not change over 6 months in a UCC group.

Finally, treatment efficacy may differ at lower dosages (ie, the 90 hours may wash out differences). In fact, an earlier 60-hour HABIT study found that hand function began to return toward baseline at 1-month posttest,[21] whereas we did not find this following 60 hours of CIMT[18] (albeit using different measures) or for either 90-hour treatment here.[46] Further research is required to establish both optimal ingredients, dosage responses, and feasibility in real-world settings.

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APPENDIX H

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Development/Plasticity/Repair

Using Motor Behavior during an Early Critical Period to Restore Skilled Limb Movement after Damage to the Corticospinal System during Development

Kathleen Friel,[1,3] Samit Chakrabarty,[3,5] Hsing-Ching Kuo,[1,3] and John Martin[3,4]

Departments of [1]Psychiatry and [4]Neuroscience, Columbia University, New York, New York 10032, [2]Department of Biobehavioral Sciences, Teachers College, Columbia University, New York, New York 10027, [3]Department of Physiology, Pharmacology, and Neuroscience, City College of the City University of New York, New York, New York 10031, and [5]Institute of Membrane and Systems Biology, University of Leeds, Leeds, LS2 9JT, United Kingdom

This study investigated the requirements for restoring motor function after corticospinal (CS) system damage during early postnatal development. Activity-dependent competition between the CS tracts (CSTs) of the two hemispheres is imperative for normal development. Blocking primary motor cortex (M1) activity unilaterally during a critical period [postnatal week 5 (PW5) to PW7] produces permanent contralateral motor skill impairments, loss of M1 motor map, aberrant CS terminations, and decreases in CST presynaptic sites and spinal cholinergic interneuron numbers. To repair these motor systems impairments and restore function, we manipulated motor experience in three groups of cats after this CST injury produced by inactivation. One group wore a jacket restraining the limb ipsilateral to inactivation, forcing use of the contralateral, impaired limb, for the month after M1 inactivation (PW8–PW13; "restraint alone"). A second group wore the restraint during PW8–PW13 and was also trained for 1 h/d in a reaching task with the contralateral forelimb ("early training"). To test the efficacy of intervention during adolescence, a third group wore the restraint and received reach training during PW20–PW24 ("delayed training"). Early training restored CST connections and the M1 motor map, increased cholinergic spinal interneurons numbers on the contralateral, relative to ipsilateral, side, and abrogated limb control impairments. Delayed training restored CST connectivity and the M1 motor map but not contralateral spinal cholinergic cell counts or motor performance. Restraint alone only restored CST connectivity. Our findings stress the need to reestablish the integrated functions of the CS system at multiple hierarchical levels in restoring skilled motor function after developmental injury.

Introduction

The corticospinal (CS) system integrates motor systems information to regulate spinal motor circuits for skilled limb control (Lemon, 2008). CS system damage typically produces debilitating weakness or paralysis and, especially during development, maladaptive control (Volpe, 2009). How can we leverage knowledge of normal CST development to restore motor function after early postnatal injury? Two key determinants for establishing CST connections between motor cortex (M1) and spinal cord are CS system activity (Martin and Lee, 1999; Friel and Martin, 2007) and early motor experiences (Martin et al., 2004). By manipulating CST activity, we demonstrated the importance of activity-dependent competition between the developing CSTs from each hemisphere. Asymmetric levels of activity on the two sides during an early critical period leads to aberrant bilateral development of CST spinal terminations (Martin et al., 2009). This results in reduced efficacy of M1-to-contralateral spinal motor circuitry (Chakrabarty et al., 2009a; Chakrabarty and Martin, 2010) and skilled motor impairments (Friel et al., 2007). These circuit impairments are similar to those in hemiplegic cerebral palsy, a developmental motor disorder affecting 1–3 in 1000 births (Himmelmann et al., 2005).

The circuit and skill impairments produced by perinatal activity imbalance are permanent if left untreated. However, manipulating CS system activity after CST developmental impairment, by M1 inactivation or CST electrical stimulation, repairs aberrant CST spinal circuitry and abrogates movement errors (Friel and Martin, 2007; Salimi et al., 2008). The repair mechanism is activity-dependent competition. Electrical stimulation of the impaired CST gives it a competitive advantage to secure more connections. Inactivation of the unimpaired CST removes its competitive advantage.

The goal of this study was to harness activity-dependent competition to repair CST circuitry and restore function by altering behavioral experiences. We manipulated competition behaviorally at two treatment levels. By using constraint of the ipsilateral limb, we intended to reduce the competitive advantage of that limb and its associated control circuitry and to provide a compet-

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Correspondence should be addressed to either of the following: Dr. Kathleen M. Friel, Department of Psychiatry, Columbia University, New York, NY 10032, E-mail: kf2105@columbia.edu; or Dr. John H. Martin, Department of Physiology, Pharmacology, and Neuroscience, City College of the City University of New York, 160 Convent Avenue, New York, NY 10031, E-mail: jmartin@ccny.cuny.edu.

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itive advantage to the impaired contralateral limb and its control circuits. By combined contralateral limb constraint plus impaired limb reach training, we augment the competitive advantage to the behaviorally impaired limb. We chose these two levels to help inform therapies for humans with developmental motor impairments, because many patients with developmental motor disorders can receive limb restraint but are too impaired to engage in skilled training. We applied these manipulations immediately after establishment of aberrant CST circuitry after M1 inactivation [8 postnatal weeks (PW) of age] and during adolescence (older than PW20). We examined performance of all animals in a visually guided locomotor task dependent on CST control and, in trained animals, reach accuracy. CST outcomes were spinal axon termination pattern, varicosities, and M1 representation. We also studied choline acetyltransferase (ChAT) expression in spinal interneurons, which we showed is under activity-dependent CST developmental regulation (Chakrabarty et al., 2009a).

Our findings stress the need to reestablish a normal CST spinal termination pattern and M1 motor map and to increase cholinergic spinal interneuron numbers on the contralateral, relative to ipsilateral, side to restore skilled motor function after developmental injury. This was only achieved by combined constraint of the unaffected limb and early training of the affected limb.

Materials and Methods

All experimental procedures were approved by and conducted in accordance with the Institutional Animal Care and Use Committees of Columbia University, the New York State Psychiatric Institute, and The City College of the City University of New York. Cats were purchased from an Association for Assessment and Accreditation of Laboratory Animal Care-accredited supplier. Kittens of either sex were delivered in litters of four to five, with a lactating mother, at PW4.

General surgical procedures. For all surgical procedures, animals were given atropine (0.04 mg/kg, i.m.) to reduce oral secretions. Animals were anesthetized with a mixture of acepromazine (0.03 mg/kg, i.m.) and ketamine hydrochloride (30 mg/kg, i.m.). For osmotic minipump insertion and tracer injection procedures, animals were maintained in an areflexive state with 1–2% isoflurane. For intracortical microstimulation, anesthesia was maintained with ketamine hydrochloride as needed, typically 10 $mg \cdot kg^{-1} \cdot h^{-1}$ intravenously.

Animals were given a broad-spectrum antibiotic (Cephazoline; 25 $mg \cdot kg^{-1} \cdot d^{-1}$, i.m.) before surgery, immediately after surgery, and twice daily for 2 d after surgery. Likewise, an analgesic (Burprenorphine; 0.03 $mg \cdot kg^{-1} \cdot d^{-1}$, i.m.) was given immediately after surgery and twice daily for 2 d after surgery. Animals were kept in a warming incubator until they recovered from anesthesia. Kittens were then returned to their home cage, with their mother and littermates. Cats typically resumed nursing within 4–6 h after surgery and were given supplemental milk (KMR feline milk replacement) as needed to ensure adequate weight gain.

M1 activity blockade. To block M1 neural activity, the $GABA_A$ agonist muscimol (10 mM in sterile saline; Sigma) was continuously infused using an osmotic minipump (Alzet model 2002) (0.5 μl/h) into the center of the left M1 forelimb representation, located primarily in the lateral sigmoid gyrus (Chakrabarty and Martin, 2000), as in our previous studies (Martin et al., 1999; Friel and Martin, 2005; Friel et al., 2007). A 28 gauge hypodermic needle cannula (Alzet), beveled at the tip, was connected with vinyl tubing (size 4; Scientific Commodities) to the pump. The cannula was inserted 1.5 mm below the pial surface, to the approximate location of pyramidal cell bodies. The cannula was fixed to the skull with screws and dental acrylic cement. Neuronal activity was blocked in left M1 from PW5 to PW7. The osmotic pump delivered muscimol for 2 weeks.

We have shown using the metabolic marker cytochrome oxidase that this infusion maximally inhibits a 2.5–3 mm patch of cortex at the infusion site and less inhibition for an additional 4–5 mm (Martin et al., 1999). This inactivation also produces a reduction in the level of neuropil immunostaining of the calcium binding protein parvalbumin over approximately the same distance as observed for the cytochrome oxidase reduction (Friel et al., 2007) (for the location of maximal activity marker reductions in relation to the M1 motor representations, see Fig. 7*A*). In previous studies, we verified that the infusion did not produce a lesion by comparing, within the infused and non-infused cortex, cell body density, the distribution of neurofilament-F (SMI-32) immunoreactivity, and parvalbumin cell body staining (Martin et al., 1999; Friel et al., 2007).

Post-inactivation treatments. The osmotic minipumps that delivered muscimol were removed at the end of PW7. The goal of this study was to determine the effects of different post-inactivation treatments on CS system anatomy, physiology, and function. After M1 inactivation, animals were assigned to one of three treatment groups: (1) restraint of unimpaired forelimb limb and training of impaired forelimb in the month immediately after the M1 inactivation (PW8–PW13) ("early training"); (2) restraint of unimpaired forelimb limb in the month immediately after the M1 inactivation (PW8–PW13) ("restraint only"); and (3) restraint of unimpaired forelimb limb and training of impaired forelimb 3 months after the M1 inactivation (PW20–PW24) ("delayed training").

Jacket restraint. Animals wore a soft mesh jacket to prevent use of the limb ipsilateral to inactivation (left). The jacket fit over the animal's head and fit snugly around the torso of the animal. The jacket had a mesh sleeve that was fitted over the animal's left forelimb (ipsilateral to the M1 inactivation). The distal end of the sleeve was secured to the torso of the jacket, thereby preventing any purposeful movements. The jacket had a hole near the animal's right shoulder through which the animal's right forelimb was placed. Thus, when the animal was wearing the jacket, it had unrestrained use of the forelimb contralateral to M1 inactivation and was not able to use the arm ipsilateral to M1 inactivation. Animals walked on three limbs. Within 1 d, animals exhibited acclimation to the jacket, ably mobilizing to eat, drink, and move about the cage. All animals wore a jacket for the prescribed period, which was 23 h/d for 4 weeks. The jacket was removed for 1 h each day. For animals in the trained groups, they performed a reach task during most of this period; the rest of the time was spent in the cage. For the restraint-only group, they were free to walk about in their cage during this hour. Performance on the horizontal ladder-walking task was tested two times each week during the treatment period, during the 1 h when the cats were not wearing the restraint.

Forelimb reaching task. The animals in the early (PW8–PW13) and delayed (PW20–PW24) training groups were trained in a forelimb reaching task. Animals were not provided food in their home cage on the days of training. For the reaching task, animals were placed in a 36 × 36 × 50 cm box. Five sides of the box were made of mesh plastic; one side was open. A metal grid was affixed to the box so that the grid blocked the open side of the box from 10 cm above the box floor to the top of the box. This left a 10 cm opening between the metal grid and the box floor. A flat surface ("reaching surface") was placed on a 5-cm-high platform outside the box and placed up against the opening in the box under the metal grid.

During training, cats were placed in the box. Before cats were placed in the box, the jacket was removed so the animal could use all four limbs for postural support. The reaching surface was placed up against the opening in the box under the metal grid. A 5 mm cube of food (beef or tuna) was placed on the reaching surface ~13 cm from the metal grid. The clearance between the reaching platform and the bottom edge of the metal grid was sufficient for the cats to reach to the platform and retrieve the meat. Cats would retrieve the food cubes, one by one. After the cat retrieved one cube, the experimenter would place another cube on the reaching platform.

Cats were trained to reach with the contralateral, impaired forelimb (right; contralateral to the M1 inactivation). They were discouraged from reaching with their ipsilateral unimpaired limb by quickly removing the food as they began their reach, so they did not receive positive reinforcement. Cats were not exposed to the reaching task before or during the cortical inactivation, because even normally developing cats younger than 7 weeks of age are not capable of performing the task. Typically,

early in training, cats would initiate each reach with their unimpaired (left) forelimb. If so, the experimenter would remove the food cube or block the path between the unimpaired forelimb and the food. Early in training, all attempted reaches with the impaired forelimb were rewarded by the experimenter placing the meat close to the cat's mouth. Within one to three sessions, cats learned that reaching with the impaired forelimb was necessary for reward and would successfully retrieve the meat with its impaired forelimb. Cats were trained for 30 min, two times per day, 5 d/week, for 4 weeks. All cats made ~4000 total reaches during training (average ± SE, 3866 ± 105).

Horizontal ladder-walking task. We examined animals' performance while walking on a horizontal ladder (88 cm long × 18.4 cm wide; 0.9 cm square rungs; 6 cm rung interval) (Friel et al., 2007). All animals in all treatment groups were tested on the ladder-walking task 2 d/week during the period of intervention (PW8–PW13 or PW20–PW24). Animals were not tested before or during the period of cortical inactivation because cats younger than 7 weeks of age are not capable of performing the task. Ladder walking was tested twice a week, on 2 consecutive days. Rung spacing was 6 cm. Cats were placed on a platform at one end of the ladder, and meat cubes were placed at the other end. Testing was videotaped. During testing, the cat walked across the ladder from the start platform to the food reward. After the cat traversed the ladder in one direction, the food reward for the next trial was placed on the other end of the ladder, requiring the cat to traverse the ladder in both directions. This ensured that both sides of the cat were captured on film. To prevent cats from memorizing rung position, we placed them at different positions on the platform for each trial, while keeping the distance between rungs constant. This resulted in their starting to step on the rungs with either forelimb. Moreover, the first ladder rung that was stepped on differed from trial-to-trial. We showed previously that control animals adjusted their step distance in accurate proportion to surprise changes in rung distance (Friel et al., 2007).

In each testing session, cats completed 20 passes across the ladder. In each pass, cats made an average of four steps, providing ~80 steps per session for analysis. Testing took ~10 min per session. For the restraint-only group, ladder testing was performed during the 1 h when the cat's jacket had been removed. For the reach training groups, ladder testing was done immediately after reach training, before the jacket was placed back on the animal. We showed previously that this testing frequency provided sufficient trials for analysis and did not result in a training effect. When animals were tested on the ladder-walking task for up to 4 months using this schedule, in the absence of any intervention, stepping accuracy did not improve; stepping errors were not significantly different at the end of testing compared with initial testing (Friel et al., 2007).

Analysis of reaching. Videotapes of training sessions were imported into a video editing program (iMovie; for the Apple Macintosh computer). A 1 cm^2 grid was superimposed on the video, using the software program Afloat. This made both the video and the grid simultaneously visible. The grid was aligned to the video so that the meat cube was in the center of the grid. For each reach, the video was paused each time the cat's paw touched the reaching platform. The grid square in which the paw tip (tip of D3) had landed was tallied. Two reaching sessions per week were analyzed.

After reach endpoint positions were tallied, percentage reaches landing in each grid square were calculated for each session. Percentages were imported into MATLAB (MathWorks) and converted to density maps, with blue representing lowest values and red representing highest values.

Analysis of ladder step movements. Videotapes of testing sessions were imported into a video editing program (iMovie; for the Apple Macintosh computer). Images from the video files were analyzed at 30 Hz, pausing at the frame in which the paw made contact with the rung. We measured the distance that the tip of the cat's forepaw extended in front of the rung of the ladder (termed forward distance). The forward distance was measured on a flat computer screen. Distance measures from the computer screen were converted to centimeters by scaling according to a calibrated distance on each video file. We compiled a database of mean forward distance of animals after alternate inactivation. These data were compared with control data from a previous study (Friel et al., 2007): (1) age-matched non-inactivated animals; (2) M1 saline infusion; and (3) unilateral inactivation.

Tracer injections. Biotinylated dextran amine (BDA; 5% in PBS; Invitrogen) and Lucifer yellow dextran (LY; 1% in PBS; Invitrogen) were pressure injected into M1 4 weeks before killing the animal. These anatomical tracers were used to examine the CS projection from neurons in the forelimb areas of each hemisphere. Injections were made ≥2 d after cessation of muscimol infusion. Previous experiments indicated that tracer injected at the time of an intracortical infusion failed to label CS terminals.

All injections were made under visual guidance within a band of cortex just lateral to the tip of the cruciate sulcus, as in our previous studies (Li and Martin, 2001; Friel and Martin, 2005). These injections were all within M1. Three injections of BDA, 300 nl each, were made in the left M1 of each cat, whereas three 300 nl injections of LY were injected into the right M1 of each cat. Injections were made 1.5 mm below the pial surface. Injections were separated by 1.5 mm and placed rostral, lateral, and posterior to the former cannula implantation site, which was just lateral to the tip of the cruciate sulcus. We ensured that differences in the distribution of CS axons in the spinal cord were not attributable to differences in the locations of injections sites. In all animal groups, the locations of injection sites, relative to the cruciate sulcus, were identical.

Histology and tracer histochemistry. Four weeks after tracer injection, cats were deeply anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and perfused transcardially with warm saline, followed by a solution of 4% paraformaldehyde, pH 7.4. Heparin was injected (200–500 U, i.v.) at the onset of perfusion. For perfusion, a peristaltic pump was used at a predetermined flow rate that depended on the animal's weight. The total perfusion time was 20–30 min. The brain and spinal cord were removed, postfixed in the same fixative at 4°C for 2–3 h, and then transferred to 20% sucrose in 0.1 M phosphate buffer overnight. Frozen transverse sections (40 μm) through the cervical spinal cord (C7–C8) were cut and processed for BDA histochemistry and LY immunohistochemistry to visualize the distribution of labeled CS terminals. Parasagittal sections through the cortex were cut, and alternate sections were processed for BDA or LY to determine the location of tracer injection sites. Parasagittal cortical sections were also Nissl stained for assessing cortical cytoarchitecture. In our extensive previous experience with BDA after M1 inactivation, we have found that 4 weeks is sufficient transport time in mature animals (Li and Martin, 2001; Friel et al., 2007).

For visualization of BDA, sections were incubated in PBS containing 1% avidin–biotin complex reagent (ABC kit; Vector Laboratories) and 0.2% Triton X-100 for 2 h at room temperature. After rinsing, sections were incubated with the chromogen diaminobenzidine (DAB; Sigma) for 6–30 min. After rinsing, sections were mounted on gelatin-coated slides, air dried overnight, dehydrated, and coverslipped. For visualization of LY, sections were incubated at 4°C overnight in PBS containing 0.01% rabbit anti-LY antibody (Invitrogen) in blocking buffer (3% goat serum in 1× PBS with 0.2% Tween 20, pH 7.4). After rinsing, sections were incubated for 2 h at room temperature in blocking buffer containing 0.2% anti-rabbit antibody conjugated to peroxidase, pH 7.4. After rinsing, sections were incubated with the chromogen DAB for 5–30 min. Sections were mounted on gelatin-coated slides, air dried overnight, dehydrated, and coverslipped.

To mark putative synapses between CST axons and spinal neurons, we triple labeled spinal sections for BDA, the presynaptic marker synaptophysin (SYN), and the postsynaptic marker postsynaptic density-95 (PSD-95). Nonspecific binding was blocked in sliced tissue with 3% donkey serum for 1 h at room temperature. To visualize BDA, sections were incubated with conjugated ExtrAvidin Cy3 (1:500 to 1:800; Sigma). For visualization of PSD-95, tissue was incubated with the primary antibody (1:500 polyclonal rabbit anti-PSD-95; Invitrogen) for 4 h at room temperature, washed with PBS, and incubated in the secondary antibody for 1 h at room temperature (1:500 goat anti-mouse IgG conjugated to FITC; Jackson ImmunoResearch). For visualization of SYN, tissue was then incubated with the primary antibody (1:1000 monoclonal mouse anti-SYN; Millipore Bioscience Research Reagents) overnight at 4°C. Sections were washed and incubated with the secondary antibody and incubated for 1 h at room temperature (1:500 ExtrAvidin Cy5; Sigma). Sections

were washed, mounted on gelatin-coated slides, air dried, and coverslipped with Vectashield (Vector Laboratories).

For visualization of ChAT, we used a goat polyclonal anti-ChAT primary antibody (AB144P; Millipore; SwissProt number P28329; 748 aa; single band on Western blot, 68–70 kDa), as in our previous study (Chakrabarty et al., 2009a). Sections (40 μm) were incubated overnight at a 1:100 concentration at 4°C. After rinsing, sections were incubated with the chromogen DAB for 5–30 min. Sections were mounted on gelatin-coated slides, air dried overnight, dehydrated, and coverslipped.

Quantitative analysis of the topography of CS terminations. We previously developed a quantitative method for determining the topographic distribution of label within the gray matter in the cervical enlargement (Friel and Martin, 2007; Salimi et al., 2008). Briefly, contralateral BDA-labeled axons within the gray matter were traced from transverse spinal sections at 200× magnification using Neurolucida (MicroBrightField), by moving the cursor along the length of the labeled axon. Boutons were defined as punctate axonal swellings (i.e., varicosities) with a diameter of ≥3 than the diameter of the adjoining nonvaricose axon. Bouton sites along axons were marked.

Tracings of axon label and boutons were separately exported and quantified using a suite of programs written in MATLAB (MathWorks). The gray matter was divided into 40 × 40 μm square regions of interest (ROIs). For each ROI, the mean density of traced axons or marked boutons was determined. A matrix of mean axon or bouton density was generated in MATLAB that preserved the mediolateral and dorsoventral dimensions of the distribution of label in the gray matter.

We quantified the regional axon and bouton density to generate maps of regional distribution of axonal label distribution and to quantify the amounts of label in different spinal laminae. Density is represented according to a color scale, from the lowest density (blue) to the highest (red). Red represents an axon density of 135 μm/mm^2 and a bouton density of 3.5 boutons/mm^2. Regional distribution maps were generated for individual animals and averaged for all animals within a particular treatment group. For group averages, we aligned the data from different animals according to the point of intersection between the gray matter above the central canal and the dorsal median septum.

To compare the locations of label across animals, we transformed the regional distribution maps (i.e., dorsoventral and mediolateral) to dorsoventral distributions only. This was done by summing all label present at a single depth. Because the size of the gray matter differs from animal to animal, we normalized dorsoventral distributions of labeling. For this analysis, we marked the dorsal and ventral gray matter borders and interpolated 100 rows between.

Quantitative analysis of ChAT distribution. We used a stereologic method to assess the amount and distribution of ChAT-positive cells. ChAT-positive cells were marked in five randomly selected section image files of DAB-labeled ChAT cells using Stereoinvestigator (MicroBrightField) at 200×. A grid size of 150 μm and frame size of 200 μm were used. The gray matter borders and borders between dorsal, intermediate, and ventral laminae were drawn at 100×. Counting windows were randomly distributed among all laminae. Markers were placed in the center of the nucleus of the cell. Files containing markers were exported and quantified using a suite of programs in MATLAB.

We quantified the regional density of ChAT-positive cells to generate maps that show the regional distribution of label in different spinal laminae. Density is represented according to a color scale, from the lowest density (blue) to the highest (red). Regional distribution maps were generated for individual animals and averaged for all animals within a particular treatment group.

Statistical analyses. Statistical analyses were done in Microsoft Excel and MATLAB (MathWorks). For comparisons of group means, the Student's t test or ANOVA was performed. ANOVAs were followed by Bonferroni's-corrected *post hoc* comparisons. For comparisons of distributions, χ^2 tests were performed. Error bars in all figures represent SEM.

For the analysis of reaching endpoint, principal components analysis was done. x–y coordinates in space of endpoints for each animal by week were transformed into absolute coordinates in one coordinate system. Transformed coordinates were plotted, and best-fit ellipses were determined using MATLAB. Ellipse areas were calculated using MATLAB.

Results

All cats in this study were subjected to inactivation of the forelimb representation of left M1 between PW5 and PW7. The region inactivated encloses the forelimb motor representations (Friel et al., 2007). Within this region, transient reversible inactivation in the mature cat produces contralateral forelimb impairments (Martin and Ghez, 1993), demonstrating its importance in moment-to-moment limb control. Furthermore, the CST projection to the cervical spinal cord originates from this area in immature and mature cats (Martin, 1996; Li and Martin, 2001). Thus, we reversibly blocked activity of the M1 area responsible for CST control of the contralateral forelimb. Left untreated, this produces permanent forelimb control impairments (Martin et al., 2000), permanent M1 motor map defects (Chakrabarty et al., 2009b), and permanent reductions in neurotransmitter marker expression on the affected side of the spinal cord (Chakrabarty et al., 2009a). Beginning 1 week after cessation of inactivation, animals were assigned to one of three groups: (1) restraint only; (2) early training; or (3) delayed training. We present, in sequence, the effects of treatment on motor control (horizontal ladder walking; reaching), regional distribution of CST spinal terminations, ChAT interneurons, and M1 motor map.

Horizontal ladder walking

Starting at PW8, 1 week after cessation of M1 inactivation, cats were tested on a horizontal ladder-walking task. The task is quickly learned by the cats, typically requiring one to two sessions before the cats readily perform the task (Friel et al., 2007). Cats were tested two times per week during the intervention period. As we reported previously (Friel et al., 2007), this M1 inactivation produces impairments paw placements in this task. Animals overstep the ladder rungs with the contralateral limb; the ipsilateral limb performs as control cats. Overstepping resulted in limb instability, with the limb occasionally slipping off the ladder rung. In these circumstances, animals were able to correct and position their paw on the rung. Despite overstepping and occasional slips, animals walked across the ladder using a quadrupedal gait. Early during intervention, the distance the cat's impaired paw extended beyond the target ladder rung (termed forward distance) was significantly higher than the forward distance of historical age-matched controls (Friel et al., 2007) ($t = 7.34$, df = 11, $p < 0.0001$). There was no difference in the forward distance of the three treatment groups in week 1 of intervention; each was similarly impaired ($F_{(2,10)} = 0.21$, $p = 0.82$).

Figure 1 compares scores between week 1 and week 4 of intervention, showing that forward distance decreased to normal for early training only. There was an overall effect of group on forward distance ($F_{(2,10)} = 4.33$, $p = 0.045$). Forward distance of the early training group decreased significantly ($t = 2.62$, df = 3, $p = 0.04$) from week 1 to week 4, reaching normal levels by week 4. There was a significant difference in forward distance at week 4 among all groups ($F_{(2,10)} = 17.19$, $p < 0.0001$). In contrast to the reduction in overstepping with early training, there was no change in forward distance from week 1 to week 4 for the restraint-alone ($F_{(3,11)} = 0.054$, $p = 0.97$) or delayed training ($F_{(3,7)} = 0.08$, $p = 0.97$) groups. These findings indicate that reach training combined with restraint is necessary to improve forepaw placement accuracy during visually guided stepping but only during an early period.

Reaching

Cats were trained to reach for cubes of food with their affected forelimb for 60 min/d, 5 d/week, for 4 weeks. All cats in the early

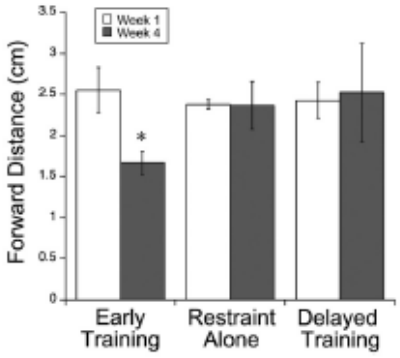

Figure 1. Stepping on a horizontal ladder. The distance from the forward edge of the ladder rung and the tip of the cat's paw was measured (forward distance). All cats showed an overstep during the first week of intervention. By week 4, forward distance in the early training group had recovered to normal levels ($p = 0.04$). No improvement was found in the restraint-alone or delayed training groups.

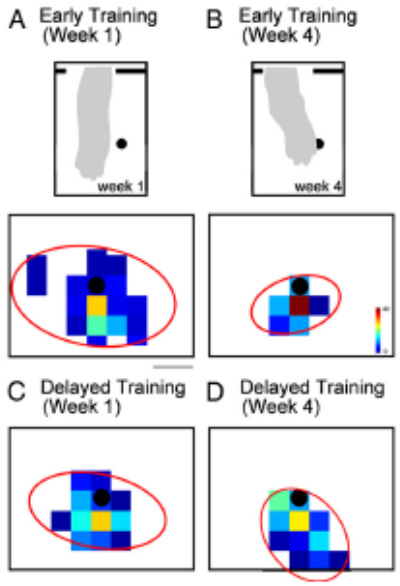

Figure 2. Reaching to a target. Cats in the early and delayed training groups were trained five times per week, 1 h/d for 4 weeks. ***A–D***, Density maps of reach endpoint positions. The black dot in the center of each density map indicates the position of the food target. Scale bar, 2 cm. Both the early (***A***) and delayed (***C***) training groups showed a dispersion of reaching endpoints in the first week of training. By week 4, reaching accuracy of the early training (***B***) group had significantly improved, indicated by a narrower distribution of reaching endpoints near the target. Insets in ***A*** and ***B*** show a drawing from a still-frame video image of the position of the arm at point of first contact. Note greater over-reaching in ***A*** than ***B***. Accuracy in the delayed training group (***D***) did not improve by week 4. In ***A–D***, the red ellipses indicate the distribution of reaches, as determined by principal components analysis.

training and delayed training groups performed ~4000 reaches over the period of intervention (mean ± SD, 3878 ± 273). Figure 2*A* shows drawings of the video frame in which the cat first contacted the surface for a typical reach during week 1 (left) and week 4 (right) from the early training group. Cats reached for the cube of food in the center of the field (black dot). Early in training (week 1), reaching accuracy was poor, with cats reaching beyond the cube of meat. As we described previously (Martin et al., 2000), when the animal's paw contacted the meat, grasping was not performed; rather, the arm was swept around the food and the meat was raked toward their mouth. This strategy was effective in retrieving the food. By the end of training (week 4), reaching accuracy was substantially improved in the early training group, with cats directing their affected forelimb to the meat without overreaching.

Figure 2, *C* and *D*, shows color-coded distributions of reach endpoints during week 1 and week 4, from all cats in the early training group. To further describe this dispersion, we used principle components analysis to compute the size of the ellipse that enclosed 90% of endpoints. The *x–y* coordinates of reaching endpoints were transformed to a normalized coordinate frame. An ellipse best fitting the dispersion of the endpoints for all cats in each group was fitted to the data (red ellipses). During week 1, endpoints showed a wide distribution, most often overshooting the target. During week 4, endpoints were more focused around the target. After early training (week 4), there were significantly more reaches within 1 cm of the target than early (week 1) ($t = 8.64$, df = 3, $p = 0.003$). In contract, delayed training did not improve reaching accuracy. Figure 2, *C* and *D*, shows reach endpoint distributions during week 1 and week 4 from all cats in the delayed training group. During week 1, endpoints showed a wide distribution, and the distribution did not change by week 4 of intervention. The percentage of reaches within 1 cm of the target did not change ($t = 0.3$, df = 2, $p = 0.78$). The apparent tighter distribution of endpoints in the delayed training group at week 1 compared with the early training group could reflect better skills in older kittens. However, there was no significant difference in the percentage of reaches within 1 cm of the target during week 1 between the early and delayed training groups ($t = 2.62$, df = 2, $p = 0.12$). These findings show that reach training combined with restraint only during an early postnatal period improves reach endpoint accuracy. This finding is similar to visually guided stepping.

Redistribution of CST connections

To help inform the repair mechanism underlying abrogation of stepping and reaching impairments, we examined four features of motor systems organization that animals studies, including our own using the cat inactivation cat model, have shown to be essential for normal forelimb motor skill: (1) CST axon terminal distribution in the contralateral cervical enlargement; (2) distribution and density of CST presynaptic sites; (3) segmental motor circuits, assayed using ChAT immunostaining; and (4) the M1 motor map. In addition, we examined the effects of the different treatments on the density of ipsilateral CST terminations, which we have shown previously is permanently increased after unilateral M1 inactivate between PW5 and PW7 (Martin et al., 1999).

Figure 3*A–C* shows color-coded density maps of the distribution of terminations (top row) of axons originating in the M1 that had been inactivated during PW5–PW7 and that terminate in the contralateral spinal cord. Density plots are shown for the three animal groups, and *D* shows control and inactivation-only distributions for comparison (replotted from Friel and Martin, 2007). Beginning with *D*, the normal distribution of CST terminations (*D1*) in the cervical enlargement are shown as the green region. These correspond to the areas enclosing 60% of the labeling (Friel and Martin, 2007). Labeling is mostly within the intermediate zone (i.e., Rexed's laminae VI–VII). In contrast, after unilateral M1 inactivation between PW5 and PW7, there is a

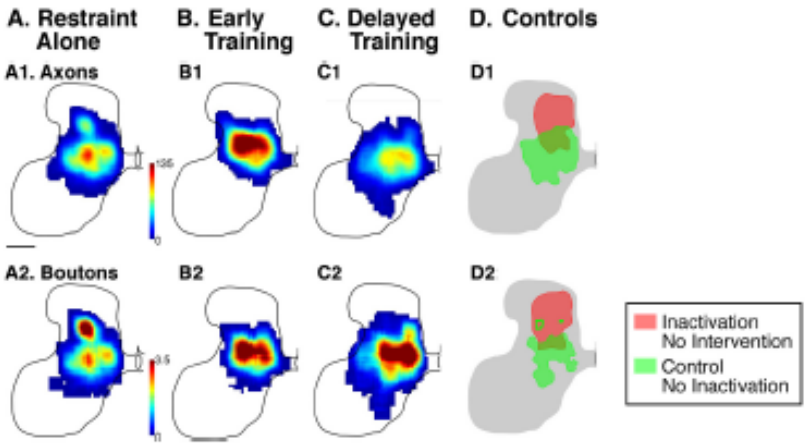

Figure 3. *A–C*, Density maps of the distribution of axon terminations and synaptic boutons in the lower cervical spinal cord contralateral to the inactivated M1. Axons were traced from the inactivated M1. In all treatment groups, axon terminals and boutons were located in the lower part of the dorsal horn and the intermediate zone contralateral to the inactivated M1, their normal targets. *D*, Overlays of the distribution areas for untreated animals: inactivation alone, without intervention; control, no inactivation (from Friel and Martin, 2007). Although inactivation alone shifted axons and varicosities to the upper dorsal horn, all three interventions redirected axons and varicosities to the same region as controls. The upper row (*A1–D1*) presents data for CST axons; the lower row (*A2–D2*), for CST axon varicosities.

dorsal shift, which is permanent unless treated further (Friel and Martin, 2007). Thus, without intervention, CST terminations are restricted to the upper part of the dorsal horn (red distribution). The goal of treatment is to return labeling to the green region. The color density maps (*A–C*, top rows) use red to indicate a higher axon density, whereas blue indicates lower axon density. Although there are subtle differences in local CST axon density, remarkably there are no systematic difference between groups. All label is centered within the deep dorsal horn and intermediate zone. Importantly, the densest labeling is within the normal distribution, as defined by the green field in *D*.

Figure 4*A* (left) plots the mean axon density value from the dorsal to ventral gray matter surfaces for controls (green) and inactivated-only (red) animals, replotted from Friel and Martin (2007). These plots quantify the distribution of axon terminations from the inactivated M1 to the contralateral spinal cord. The dorsal shift after inactivation can be seen. Data from the three treatment groups are plotted on the right. The three are overlapping, and each shows maximal labeling within the deep dorsal horn and intermediate zone, just like the normal pattern (Fig. 4*A*, green).

To assay putative CST synaptic density, we examined axon varicosities. As in our previous studies, we quantified the distribution and density of axon varicosities, defined as three times the diameter of adjoining nonvaricose segments (based on DAB labeling). These CST axon varicosities colabel the presynaptic vesicle protein SYN (Meng et al., 2004). Importantly, they appose sites of PSD-95. Figure 5 shows two representative examples that CST axon varicosities mark putative synapses, based on presynaptic and postsynaptic immunostaining of SYN and PSD-95, respectively. The top two rows show a varicosity in a control animal. *A* is a low-magnification projection image (i.e., *z*-stack; 10 optical slices) of BDA (green), PSD-95 (red), and SYN (blue) labeling in the intermediate zone. Note that the red–green colocalization is colored yellow, blue–green is cyan, and red–blue is magenta. *A1–A3* and *A4–A6* are two adjacent optical slices that are part of the total *z*-stack. The slice in *A1*, which shows the varicosity morphology well, also colocalizes a large puncta of PSD-95 (yellow arrow). Although PSD is ubiquitous, there is

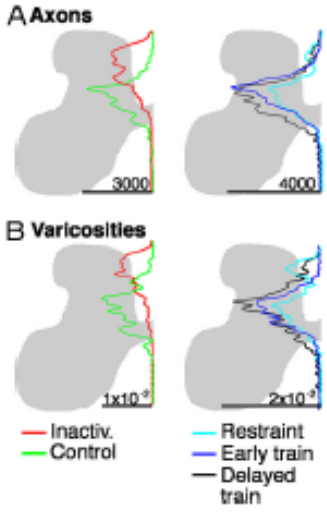

Figure 4. Dorsoventral distribution of CST axon within the gray matter of the lower cervical spinal cord contralateral to the inactivated M1 (*A*) and CST axon varicosities (*B*). Line graphs plot density on a left to right scale.

colocalization within part of the varicosity. At this depth in the tissue, there is minimal SYN label (gray arrow). The slice in *A2* grazes the varicosity and better shows a puncta of SYN colocalized to the varicosity than PSD-95. The insets in *A1* and *A4* are triple-label images of these single optical slices. Note the yellow (BDA–PSD) colocalization in the varicosity in *A1* and cyan (BDA–PSD) colocalization in *A4*. *B* shows CST axons within five adjacent sections in the cervical enlargement of an early trained animal. The small red box is the location of axon in *C* (projection stacked image; 10 optical slices); this is at an axon branch point. The small yellow box in *C* is the focus of the single 1 μm optical slice in *C1–C4*. *C1* shows colabeling of the CST axon, SYN, and PSD-95. Corresponding single-label images are shown (CST

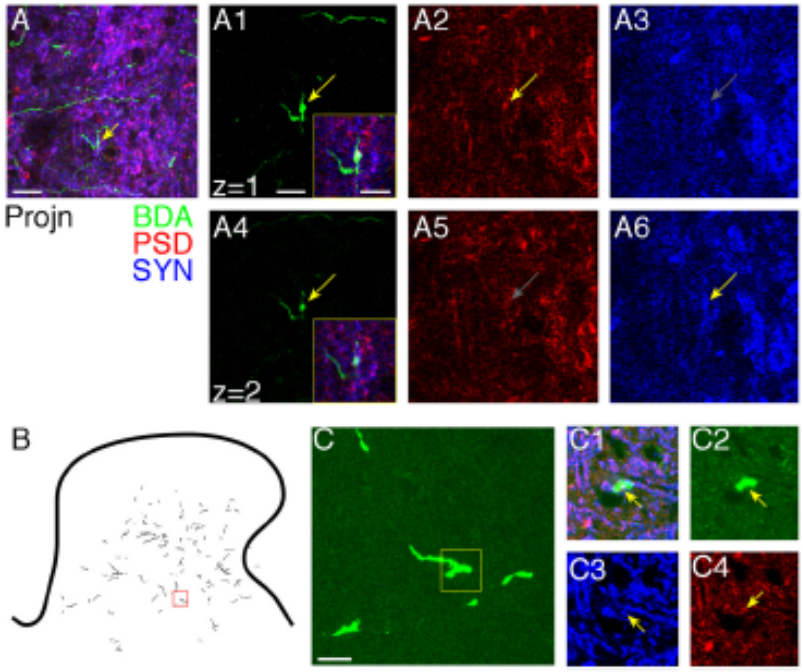

Figure 5. Confocal images of CST axon varicosity showing colocalization of BDA, PSD-95, and SYN. ***A***, Axon terminal in the spinal intermediate zone from a control animal. ***A***, Projection image (10 1-μm optical slices), triple labeled. ***A1–A3***, One micrometer optical slice showing a BDA staining only (***A1***), PSD staining (***A2***), and SYN staining (***A3***). Yellow arrows point to labeling present; gray arrow point to the location in which optimal labeling for SYN (***A3***) and PSD (***A5***) is not present. ***A4–A6*** are similar to ***A1–A3*** but for the next optical slice. Insets in ***A1*** and ***A4*** show triple label for the single optical slices. ***B***, Low-magnification view of location of CST label in the contralateral cervical spinal cord. Red box contains the axon used for analysis in ***C***. ***C***, Axon terminal in the spinal intermediate zone in a projection image; BDA staining only. The region outlined by the yellow square in ***C*** is shown in ***C1–C4***, which are the same 1 μm optical slice but with different staining. The yellow arrow points to the same location in ***C1–C4***. ***C1***, Overlay of BDA, PSD-95, and SYN labels. ***C2***, BDA only. ***C3***, SYN only. The arrow points to a puncta of SYN. ***C4***, PSD-95 only. The arrow points to a puncta of PSD-95. Scale bars, 10 μm.

bouton alone, *C2*; SYN-labeled puncta, *C3*; PSD-95-labeled puncta, *C4*). As in *A*, there is colocalization of the presynaptic and postsynaptic proteins within the labeled CST axon. Thus, axon varicosities can be used to mark putative synapses between CST axons and spinal cord neurons.

Figure 3*B* shows the density and distribution of CST axon varicosities, marking putative synaptic sites (boutons). As with axon density, the normal distribution (green) is within the intermediate zone and, after M1 inactivation only, the upper dorsal horn. The three treatment groups all show return of CST varicosities within the intermediate zone. Figure 4*B* shows the dorsoventral distributions of CST varicosities. Again, the varicosities from each of the three treatment groups overlap and are located within the normal CST territory, the intermediate zone.

We confirmed these findings by quantifying the amounts of axonal label and boutons in the dorsal, intermediate, and ventral regions of the cervical enlargement for the three treatment groups. We plotted regional axon density by summing the amount of label in dorsal, intermediate, and ventral spinal laminae and calculating the percentage of the total label in each region (Fig. 6*A*). As in Figure 3, we compare these data from the different treatment groups with an inactivation/no-treatment and control/no-inactivation groups from a previous study (Friel and Martin, 2007). We compared the amounts of label in each region across the treatment groups. There was a significant effect of group on the distribution of axons (ANOVA, $F_{(4,14)} > 5.59$, $p < 0.007$) and varicosities ($F_{(4,11)} > 6.59$, $p < 0.006$) for dorsal and intermediate spinal regions. We next compared labeling in the treatment groups with that of control (no inactivation) and inactivation only (no treatment). The control and inactivation data were from a previous study (Friel and Martin, 2007). Mean percentage CST axons in control animals (no intervention) are represented by the dotted lines in Figure 6*A*; means for inactivation only (no intervention) are the dashed lines. Control and inactivation-only data are from a previous study (Friel and Martin, 2007). There was significantly more axon and varicosity label in the intermediate zone in all three treatment groups than inactivation/no-intervention animals ($t > 3.36$, $p < 0.02$) and significantly less label in the upper dorsal horn in all three treatment groups than inactivation/no-intervention animals ($t > 3.81$, $p < 0.017$). Importantly, the distribution of axon and varicosity label in the dorsal region ($t < 0.80$, $p > 0.45$) and intermediate regions ($t < 0.60$, $p > 0.57$) were not significantly different from control/no-inactivation animals. Sparse ventral horn labeling was also the same as controls. We then examined the density of putative synaptic boutons (i.e., CST axon varicosities; Fig. 6*B*). We measured varicosity density in the medial intermediate zone, which is the location of the highest density of CST terminations (box in Fig. 6, inset). Bouton density was not significantly different among the groups ($F_{(2,8)} = 0.98$, $p = 0.42$). Thus, for both CST axons and putative CST synapses, each treatment group restored CST connections to the proper gray matter regions. Our findings indicate restitution of CST axon

terminations within the normal intermediate zone territory for all three treatment groups.

Our previous studies in which CS system activity was directly manipulated with neural inactivation or electrical stimulation demonstrated yoked, reciprocal, bilateral changes: as contralateral CST connections prospered, ipsilateral connections receded. In the present study, we also identified the presence of aberrant ipsilateral CST terminations. As previous reports, they were located predominantly within the intermediate zone. For this analysis, we traced CST axons labeled with LY (restraint only, $n = 3$; early training, $n = 4$; delayed training, $n = 3$). Axons from the M1 contralateral to the inactivated M1 were traced. Axons typically terminated bilaterally in the lower cervical spinal cord. Here, we traced the ipsilateral terminations, that is, the axons that originate in the right M1 and terminate in the right side of the spinal cord. Figure 7 shows the distribution of ipsilateral CST labeling for the three animal groups. Whereas electrical stimulation and inactivation therapies result in consistent and significant reductions in aberrant ipsilateral CST terminations (Friel and Martin, 2007; Salimi et al., 2008), surprisingly we did not observe any consistent changes to ipsilateral CST terminations after the behavioral approaches in this study. Statistical analysis revealed no differences in the amount of ipsilateral axonal label (Kruskal–Wallis $K = 0.64$, $p = 0.73$) or varicosities (Kruskal–Wallis $K = 1.80$, $p = 0.41$) among the three groups.

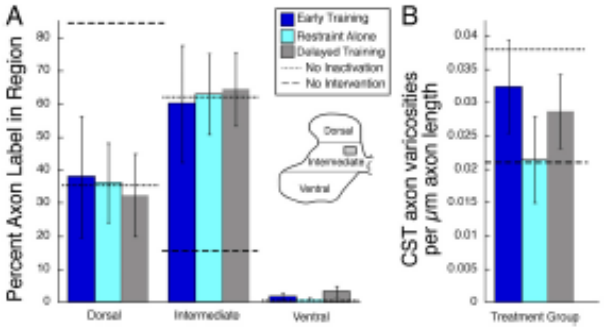

Figure 6. Quantification of axon label and synaptic bouton density. *A*, Percentage axon label in dorsal, intermediate, and ventral spinal regions. Dashed lines represent values from inactivation/no-intervention and control/no-inactivation groups. All three treatment groups resulted in axons distributions similar to control/no-inactivation animals, with the greatest amount of label residing in the intermediate spinal laminae. *B*, Density of synaptic boutons in axon terminals in the intermediate zone. There were no statistically significant differences among treatment groups.

Distribution of ChAT

Previously we showed that development of ChAT in spinal segmental interneurons was under activity-dependent regulation by the CST (Chakrabarty et al., 2009a). Here, we examined changes in the distribution of ChAT-positive interneurons produced by the three treatments. Figure 8*A–C* shows color density plots of ChAT-positive interneurons and motoneurons for the three groups. We quantified changes in the numbers of cells using stereological methods. We distinguished between putative motoneurons (i.e., large multipolar neurons in the motor pools) and ChAT-positive interneurons located elsewhere. Because we did not expect the treatments to influence motoneuron counts (Chakrabarty et al., 2009a), we used this measure to control for inter-animal/treatment immunohistochemical differences. We determined the ratio of the number of ChAT-positive neurons on the side contralateral to inactivation versus ipsilateral to inactivation (Fig. 8*D*). Previously, we found that inactivation of M1 on one side during PW5–PW7 resulted in a decrease in the ratio of ChAT-positive cells on the affected/contralateral sides (Chakrabarty et al., 2009a). In that study, the affected/contralateral ratio was 0.45. In the present study, we found that, within the dorsal and intermediate regions, which is the territory in which the CST terminates, early training not only increased the number of ChAT-positive cells to the level of the contralateral side but remarkably increased these numbers to 5.4 times the level of the contralateral side (Fig. 6). In the restraint-alone and delayed training groups, the levels of ChAT-positive cells also were elevated in the affected side relative to the contralateral side but to a much lesser extent (1.9 times). There were no differences in motoneurons on the two sides (Fig. 8*D*). The ratio of ChAT-positive cells on the affected to the contralateral sides was significantly higher in the early training group than the restraint-alone and delayed training groups ($\chi^2 = 5.9$, df = 2, $p = 0.05$). Although there were differences in the ratios of ChAT-positive cells among treatment groups, there were no significant differences in the number of ChAT-positive cells in dorsal/intermediate or ventral laminae (Kruskal–Wallis $K < 1.83$, $p > 0.40$). These findings show an unsuspected strong effect of early reach training on neurotransmitter phenotype.

Changes in motor maps

To determine physiological changes in M1 in response to training, we used intracortical microstimulation to map motor cortex in the three animal groups. Representative examples of motor maps from individual animals are shown in Figure 9*A*. The dashed circles correspond to the region of maximal inhibition, as reported from other studies (see Materials and Methods). The more extended regions of reduced activity suppression is not shown. There was a striking difference between the two trained animals and the untrained (restraint-alone) animal; the untrained animals had few effective sites (average number of effective sites per animal − early training = 17.7, delayed training = 17.3, restraint alone = 3). Furthermore, and quantified below, the maps of early and delayed training animals look much like controls, and the map of the restraint-only animal looked like inactivation-only animals (Chakrabarty et al., 2009).

We quantified the difference in the motor maps by examining the proportion of difference response types. Motor responses were categorized as distal (digit, wrist), proximal (elbow, shoulder), or multijoint (combined movement of two different joints within 1.1× the threshold of the lowest-threshold response). Importantly, both early and late training restored the M1 map to its normal proportions of distal, proximal, and multijoint sites. In contrast, restraint alone was no different from untreated animals (i.e., inactivation alone). Specifically, there was a significantly greater percentage of distal sites in the early training group than in the no-intervention or restraint-alone groups ($\chi^2 = 42.9$, df = 4, $p < 0.0001$). The percentage distal sites was not statistically different among the early training, delayed training, and no-inactivation groups ($\chi^2 = 1.69$, df = 2, $p = 0.43$). There was a significantly lower percentage of proximal sites in the early training group than in the no-intervention or restraint-alone groups ($\chi^2 =$

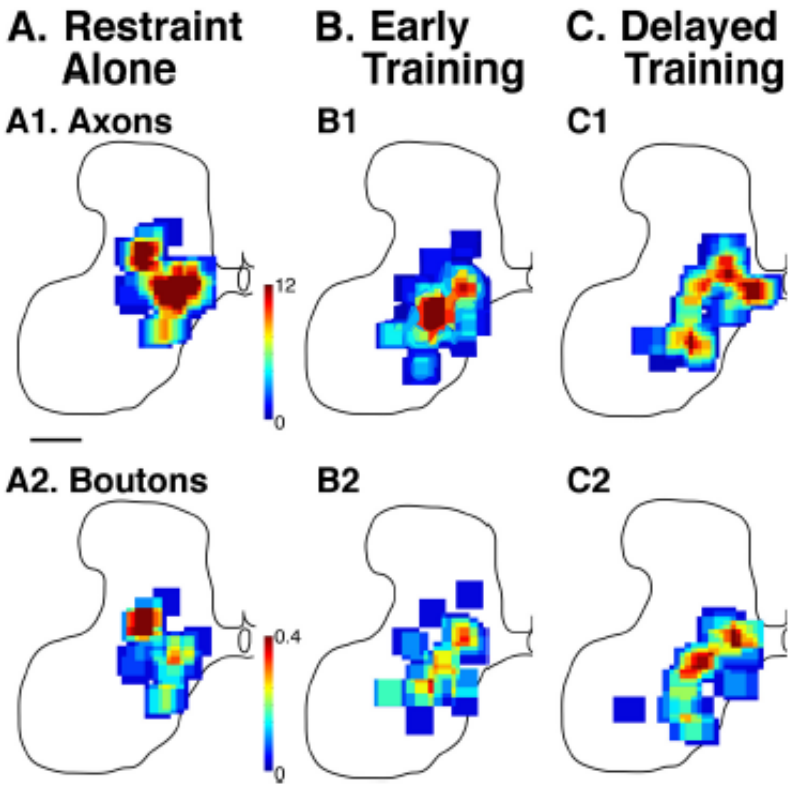

Figure 7. CST labeling of axons that originate in the right (contralateral to inactivation) M1 and terminate ipsilaterally, in the right side of the spinal cord. ***A–C***, Density maps of the distribution of axon terminations (top) and CST axon varicosities (bottom) in the lower cervical spinal cord. There were no group differences in the amount of ipsilateral terminations or the density of synaptic boutons on these terminations. The upper row (***A1-C1***) presents data for CST axons; the lower row (***A2-C2***), for CST axon varicosities.

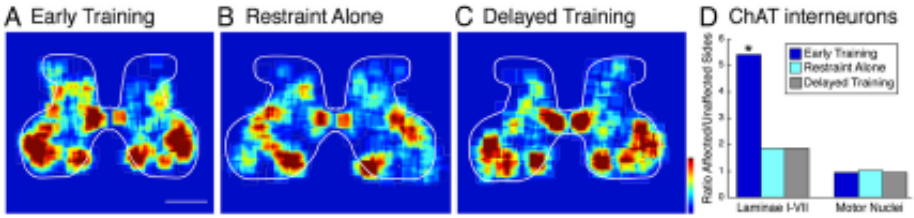

Figure 8. Distribution of ChAT-positive cells in the lower cervical spinal cord. ***A–C***, Density maps of ChAT-positive cells in the cervical enlargement. The large dense clusters of cells in the ventral horn correspond predominantly to motoneurons. Note, the color scale is the same for all panels (red = 1.25×10^{-5} cells/square μm). Scale bar, 1 mm. ***D***, Ratios of ChAT label on the affected (left side of spinal cord in figures) to the contralateral (right) sides of the spinal cord. Note that in the early training group, there was a much greater ratio of ChAT-positive cells in the intermediate zone (laminae I-VII) on the affected/trained side than the untrained side. The ratio of ChAT label in motor nuclei in all groups was symmetrical on the two sides.

64.69, df = 4, $p < 0.0001$). The percentage of proximal sites was not statistically different among the early training, delayed training, and no-inactivation groups ($\chi^2 = 0.83$, df = 2, $p = 0.66$). There was a significantly higher percentage of multijoint sites in the early training group than in the no-intervention or restraint-alone groups ($\chi^2 = 197.2$, df = 4, $p < 0.0001$). The percentage of multijoint sites was not statistically different among the early training, delayed training, and no-inactivation groups ($\chi^2 = 0.11$, df = 2, $p = 0.66$).

The threshold at which a visually discernible response could be evoked was compared among the three treatment groups (Fig. 9*C*). There was a significant effect of group on threshold (ANOVA, $F_{(2,7)} = 24.33$, $p < 0.001$). The threshold for the restraint-alone group (79 μA) was significantly higher than the early training (59.4 μA, $t = 5.2$, $p = 0.035$) and the delayed training groups (52.1 μA, $t = 6.7$, $p = 0.022$). The thresholds for the early training and delayed training groups were not statistically different from one another ($t = 1.961$, $p = 0.3$) and not

significantly different from controls (i.e., no inactivation; 37.7 μA, $t = 1.48$, $p = 0.2$). Our findings are in agreement with others that the M1 motor map retains its representational plasticity into maturity. Furthermore, we show that it was necessary for the animal to engage in reach training to restore the M1 motor map; it was not sufficient simply to increase limb use.

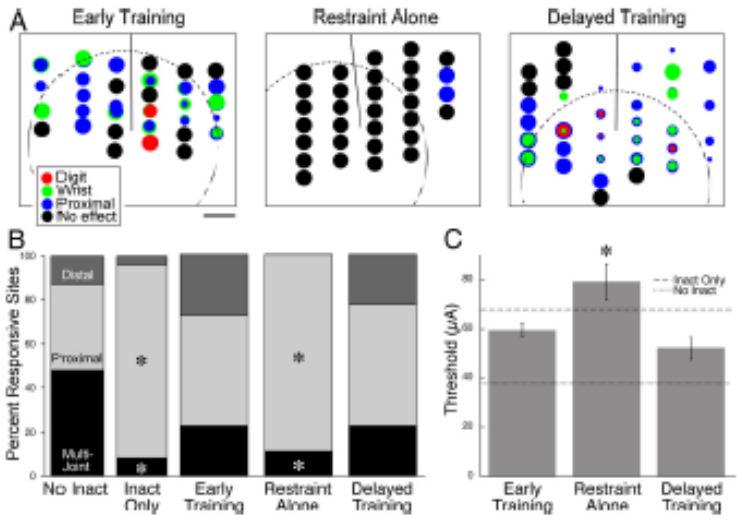

Figure 9. Intracortical microstimulation. ***A***, Representative motor maps from one animal in each treatment group. The dotted circle represents the approximate extent of the muscimol inactivation (Martin et al., 1999; Friel et al., 2007). The line midway between the columns of stimulation sites in each animal represents the cruciate sulcus. Site markers of multiple colors represent multijoint movements. Elbow and shoulder sites were combined and labeled proximal. No effect sites were tested up to 100 μA. The size of each site marker is proportional to the stimulus intensity needed to evoke a movement at that site. Scale bar, 1 mm. ***B***, Percentage responsive sites in all groups. The percentage distal and multijoint sites were decreased in the inactivation, no-intervention, and restraint-alone groups, whereas the early and delayed training groups had similar amounts of distal and multijoint sites as control/no-inactivation animals. The inactivation, no-intervention, and restraint-alone groups had significantly more proximal sites than the no-inactivation, early, and delayed training groups. ***C***, The thresholds at which movements could be evoked were statistically higher in the restraint-alone group than the early training, delayed training, and no-inactivation groups (*$p < 0.05$).

Discussion

We show that a behavioral approach during an early critical period repairs a hierarchically ordered set of CS system circuit elements, and this is associated with restoring restore-effective targeting of skilled limb movement after CS system developmental injury. The normal organization of the M1 motor map and the distribution of CST terminations in the spinal cord, rather than a new organization, was necessary but not sufficient for motor recovery. It was the capacity of combined restraint, training, and early intervention to substantially increase the numbers of ChAT-expressing interneurons on the affected side of the spinal cord, relative to the opposite side, that was associated with recovery. We cannot say whether reestablishment of the motor map, CST spinal connectivity, and strong segmental ChAT expression reinstates normal limb control or, now with a more effective CS system, the animals are better able to implement new strategies for normal performance. Nevertheless, using combined limb restraint and training to achieve normally directed limb movement is significant. Our findings stress the need to reestablish the integrated functions of the CS system in bringing back skilled motor function, a consideration that is often lacking in repair studies in which emphasis is on promoting new connections.

Repairing the regional distribution of CST spinal terminations

Constraint of the unimpaired limb alone, which creates an asymmetrical pattern of limb use and presumably CST activity, is sufficient to repair CST terminations after inactivation of M1 during a critical period. Remarkably, constraint alone produced contralateral CST rewiring comparable with direct manipulation of the activity of the CST: either stimulation of the affected contralateral tract (Salimi et al., 2008) or inactivation of the ipsilateral M1 (Friel and Martin, 2007). On the basis of similarity between the effects of constraint of the unimpaired limb, inactivation of contralateral CST, and stimulation of the impaired CST, we conclude that each approach recruits a similar competition-based mechanism for restoring CST connectivity. However, we noted that restraint, alone or with training, failed to abrogate consistently the density of aberrant ipsilateral CST terminations from ipsilateral M1, whereas activity manipulations resulted in consistent reductions (Friel and Martin, 2007; Salimi et al., 2008). That behavioral manipulations failed to minimize consistently the aberrant ipsilateral connections from the unaffected side suggests a dose–response effect (i.e., behavioral intervention is more modest than activity manipulations). This further raises the question of whether aberrant dense ipsilateral terminations from the unimpaired side are adaptive and if functional recovery uses a bilateral CST repair strategy. Previously, we presented evidence favoring an adaptive role for ipsilateral CST terminations (Martin et al., 2000). Similarly, adult rat and monkey CST lesion studies show consistent association between recovery and ipsilateral CST outgrowth (Maier et al., 2008; Carmel et al., 2010; Rosenzweig et al., 2010). However, in normal monkey, there is no effective ipsilateral monosynaptic CST connection with motoneurons, again calling into question the functional role of the ipsilateral CST (Soteropoulos et al., 2011). Until it is possible to selectively inactivate ipsilateral CST axons, this question will not be resolved.

That restraint in adolescent cats was as effective as in younger cats suggests that either there is no critical period for local CST outgrowth or that it is highly protracted, more like a sensitive than critical period. Constraint of the unimpaired limb after pyramidal lesion in adult rats promoted outgrowth of the spared ipsilateral CST (Maier et al., 2008). Also in adult rats, augmentation of spared CST axons occurs with electrical stimulation (Brus-Ramer et al., 2007; Carmel et al., 2010). Mature CST neurons are incapable of axon regeneration after axotomy unless their growth state is manipulated (Liu et al., 2010) or the environment is made conducive for growth (Schnell and Schwab, 1990; Grimpe and Silver, 2004). Importantly, extensive CST sprouting within the gray matter after injury appears to be robust at all ages but only when their activity is augmented or the activity of the opposite side is reduced.

Repairing the M1 motor representation

With training at either age came restoration of the topography and efficacy of the M1 forelimb motor map. These findings parallel work in the primate stroke model, showing that forced use of the affected limb is not sufficient to recover the motor map; skilled training is required (Friel et al., 2000). Thus, there may little or no critical period for M1 motor map plasticity or CST local outgrowth. This is consistent with the large literature showing M1 motor map plasticity in mature animals (Monfils et al., 2005). Although the M1 motor map is often used as a proxy for CS system motor function, we find that restoring normal map parameters is not sufficient to restore function. The M1 motor map may encode motor recovery potential.

The M1 represents contralateral muscles and joints and, as the principal origin of the CST, provides functional somatotopic access for diverse M1 inputs to spinal motor circuits. With developmental injury/impairment there is never a period when the M1 motor representation is present. A period of map development may be necessary during recovery, such as the period of map formation that normally occurs between PW7/8 and PW13 (Chakrabarty and Martin, 2000). We propose that, during training, the motor systems learn to take advantage of the newly repaired spinal circuitry in much the same way as occurs during normal M1 motor map development.

Response of spinal cholinergic circuits to CST injury

Although there may by other neurotransmitter phenotypic changes after CST injury and treatment, we focused on cholinergic mechanisms because they are apt to be important for limb control and cholinergic interneuron development is under CST regulation. Cholinergic interneurons are an abundant class in the spinal cord (Barber et al., 1984; Huang et al., 2000). Many are movement related because they are active during fictive locomotion (Huang et al., 2000), and the Pitx2 group (Zagoraiou et al., 2009; Enjin et al., 2010) makes the M2-type muscarinic C-bouton on motoneurons (Miles et al., 2007) that may provide task-dependent regulation of motoneuronal excitability (Zagoraiou et al., 2009). Cholinergic agonists are potent modulators of spinal locomotor circuits (Dai et al., 2009; Dai and Jordan, 2010). Development of a cholinergic phenotype in most spinal interneurons occurs postnatally (Phelps et al., 1984; Chakrabarty et al., 2009a). We have shown that postnatal maturation of ChAT phenotype is CST dependent: blocking CST activity between PW5 and PW7 prevented normal ChAT expression throughout the deep laminae of the dorsal horn and intermediate zone (Chakrabarty et al., 2009a), key termination regions of the CST.

A critical period effect could explain why early but not late training differentially increased ChAT expression. Why is training required to upregulate ChAT? A cholinergic phenotype appears to be dependent on the constitutive level of neural activity, similar to neurotransmitter switching in other systems (Borodinsky et al., 2004). Whereas it may depend on particular spatiotemporal firing patterns associated with skilled task performance, we suspect that it is a dose–response effect. Electrical stimulation of the affected CST leads to behavioral improvements (Salimi et al., 2008). Plausibly, this direct activity manipulation powerfully drives spinal circuits more than asymmetric limb use associated with restraint, and, in consequence, might also lead to upregulation of contralateral segmental ChAT expression. Intriguingly, inactivation of the unaffected ipsilateral CS system also leads to behavioral recovery (Friel and Martin, 2007), but this would not be expected to drive the affected contralateral segmental motor circuits. If motor recovery after reducing the unaffected CS system also is accompanied by increased ChAT expression, it implies a complex bilateral regulation and would help inform why it is that upregulation of ChAT expression in the affected, relative to opposite, side is associated with motor recovery. One should keep in mind the dominant reciprocal bilateral organization of spinal circuits, whereby there is a phasic locomotor drive to one side of the spinal cord, there is reciprocal inhibition on the other, and this inhibition is mediated by segmental commissural circuits.

Combinatorial hierarchical recovery code

Constraint alone creates asymmetric limb use. We propose that segmental circuitry can amplify this imbalance through commissural inhibition, normally recruited to inhibit contralateral central pattern generator circuitry during locomotion. The more active side of the cord could effectively inhibit contralateral circuits, and this, in turn, would lead to disinhibition of the more active side. These spinal excitability changes could create an environment permissive for greater CST outgrowth. Perhaps because there is expression of guidance cues, because the animals are not fully mature, targeting of outgrowth is appropriate.

Reach training could amplify this process through greater activation of the affected side, greater inhibition of the opposite side, and more disinhibition. Importantly, given CS control of reaching and visually guided locomotion, this activity boost would more selectively affect the spinal follower circuits of CST. Thus, we propose that functional recovery of visually guided control can be achieved by either direct activity manipulations of the CST or preferentially recruiting the CST during particular tasks. In the context of this hypothesis, combined training and direct activity manipulations are apt to yield the strongest effect. The normal topography of the M1 motor map likely reflects the efferent organization of the CST, which even after restraint alone is well directed. What is lacking in the late-trained group is a critical function of segmental circuitry.

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APPENDIX I

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RESEARCH ARTICLE

Improvements in hand function after intensive bimanual training are not associated with corticospinal tract dysgenesis in children with unilateral cerebral palsy

Kathleen M. Friel · Hsing-Ching Kuo · Jason B. Carmel · Stefan B. Rowny · Andrew M. Gordon

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Abstract Unilateral cerebral palsy (CP) results from damage to the developing brain that occurs within the first 2 years of life. Previous studies found associations between asymmetry in the size of the corticospinal tract (CST) from the two hemispheres and severity of hand impairments in children with unilateral CP. The extent to which CST damage affects the capacity for hand function improvement is unknown. This study examines the association between an estimate of CST dysgenesis and (1) hand function and (2) the efficacy of intensive bimanual training in improving hand function. Children with unilateral CP, age 3.6–14.9 years, $n = 35$, received intensive bimanual training. Children engaged in bimanual functional/play activities (6 h/day, 15 days). Peduncle asymmetry, an estimate of CST dysgenesis, was measured on T1-weighted magnetic resonance imaging scans. Hand function was measured pre- and post-treatment using the assisting hand assessment (AHA) and Jebsen–Taylor test of hand function (JTTHF). AHA and JTTHF improved post-treatment ($p < 0.001$). Peduncle asymmetry was correlated with baseline AHA and JTTHF ($p < 0.001$) but not with AHA or JTTHF improvement post-training ($R^2 < 0.1$, $p > 0.2$). An estimate of CST dysgenesis is correlated with baseline hand function but is a poor predictor of training efficacy, possibly indicating a flexibility of developing motor systems to mediate recovery.

Keywords Bimanual training · Rehabilitation · Corticospinal · Motor development

Abbreviations

AHA	Assisting hand assessment
CM	Cortical malformation
CP	Cerebral palsy
C/SC	Cortical/subcortical lesion
CST	Corticospinal tract
DTI	Diffusion tensor imaging
HABIT	Hand–arm bimanual intensive therapy
JTTHF	Jebsen–Taylor test of hand function
M1	Primary motor cortex
MRI	Magnetic resonance imaging
PV	Periventricular injury

K. M. Friel (✉) · J. B. Carmel
Burke-Cornell Medical Research Institute, 785 Mamaroneck Avenue, White Plains, NY 10605, USA
e-mail: kfriel@burke.org

K. M. Friel · J. B. Carmel
Department of Neurology, Brain Mind Research Institute, Weill Cornell Medical College, New York, NY 10021, USA

K. M. Friel · H.-C. Kuo · A. M. Gordon
Department of Biobehavioral Sciences, Teachers College of Columbia University, New York, NY 10027, USA

K. M. Friel · H.-C. Kuo · S. B. Rowny
Division of Experimental Therapeutics, Department of Psychiatry, Columbia University Medical Center, New York, NY 10032, USA

J. B. Carmel
Department of Pediatrics, Weill Cornell Medical College, New York, NY 10021, USA

A. M. Gordon
Department of Rehabilitation Medicine, Columbia University Medical Center, New York, NY 10032, USA

Introduction

Poor function in the affected hand is among the greatest functional impairments for children with unilateral cerebral

palsy (CP) (Gordon and Friel 2009). Whereas multiple neural systems help control hand function, the corticospinal system predominates for skilled voluntary movement in humans. Previous studies have demonstrated a strong association between asymmetry in the size of the corticospinal tract (CST) or cerebral peduncles and various features of hand function in children with unilateral CP (Bleyenheuft et al. 2007; Duque et al. 2003) and adult stroke patients (Barnes et al. 2008; Pineiro et al. 2000). Peduncle asymmetry is an estimate of CST dysgenesis, since the CST passes through the peduncles. Peduncle asymmetry is a consequence of the extent of initial damage to the motor system during development.

Asymmetry in the size of the CST, measured using diffusion tensor imaging (DTI) (Bleyenheuft et al. 2007; Yoshida et al. 2010) or by cross-sectional area of the cerebral peduncles (Bouza et al. 1994; Duque et al. 2003), is strongly correlated with stereognosis, and a measure of hand function in activities of daily living in children with unilateral CP. The timing of grip forces during a lifting task was also associated with peduncle asymmetry in children with unilateral CP (Duque et al. 2003). Another study found a correlation between peduncle asymmetry and stereognosis in children with unilateral CP (Bleyenheuft et al. 2007).

Intensive bimanual training has shown efficacy in improving hand function in children with unilateral CP (Facchin et al. 2011; Gordon et al. 2007, 2008, 2011; Sakzewski 2012; Sakzewski et al. 2011). However, the neuroanatomical substrate required for this improvement has not been examined. These therapies require great time and effort and are resource-consuming for patients, families, and clinicians. It is important to identify predictors of efficacy, so that therapy can be targeted to children who are most likely to benefit from it.

Two recent studies have examined associations between magnetic resonance imaging (MRI) features and efficacy of constraint-induced movement therapy (CIMT) in children with unilateral CP and adults with chronic stroke. Rocca et al. (2013) reported that fiber integrity of the lesion area was predictive of improvement in quality of upper extremity movement (Rocca et al. 2013). In a similar study, associations were found between baseline arm function and integrity of the CST on the affected side, as measured by DTI (Rickards et al. 2013). However, integrity of the CST was not predictive of improvement in arm function after CIMT in children or adults with unilateral CP or stroke.

There were two goals to this study. We first aimed to examine the association between peduncle asymmetry and bimanual and unimanual hand function. We next sought to examine the relationship between peduncle asymmetry and efficacy of bimanual training for improving hand function. We hypothesized that peduncle asymmetry would be associated with amount of improvement in hand function shown by children after bimanual therapy—those with large asymmetry were predicted to have less functional recovery. If an estimate of dysgenesis of the CST is associated with efficacy of therapy, MRI could inform decisions about which treatment is most appropriate for individual children with unilateral CP (Z'Graggen et al. 1998; Carmel et al. 2013).

Methods

Participants

Thirty-five children participated in this study. All participants had been diagnosed with congenital unilateral CP before the age of 1 year. Demographics of study participants are summarized in Table 1. Participants were included in the bimanual training protocol in accordance with previously established criteria (Gordon et al. 2011). Briefly, participants were required to have the ability to extend the wrist at least 20° and the fingers at the metacarpophalangeal joint at least 10° from full flexion in the more-affected hand. Participants were also required to have the ability to lift the more-affected arm 15 cm from a table and to grasp objects, to have a greater than 50 % asymmetry in Jebsen–Taylor test of hand function (JTTHF) scores between the two hands, and be mainstreamed in school with a Kaufman brief intelligence score >70. Potential participants with contraindications to MRI (e.g., metallic implants) were also excluded.

Table 1 Demographic and clinical measures (±SD)

Measure	
n	35
Age	7.9 ± 2.9
Gender	12 F, 23 M
Pre-training JTTHF (s)	294.2 ± 220.0
Post-training JTTHF (s)	199.3 ± 163.2
% Improvement JTTHF	31.1 ± 21.8
Pre-training AHA (AHA unit)	61.0 ± 11.3
Post-training AHA (AHA unit)	64.0 ± 12.1
% Improvement AHA	58.4 ± 62.3
Side of lesion	15 R, 20 L
Peduncle asymmetry	73.8 ± 14.6
Lesion type	6 CM
	20 PV
	9 C/SC

CM cortical malformation, *PV* periventricular injury, *C/SC* cortical/subcortical lesion

Participants were recruited for the bimanual training study from clinics in the NYC area, our center website (http://www.tc.edu/centers/cit/), ClinicalTrials.gov (NCT00305006), and online parent forums. All research was approved by the Institutional Review Boards of Teachers College and Columbia University Medical Center. Informed assent/consent was obtained from all participants and their parents. Some participants (18 out of 35) were recruited for a randomized clinical trial testing the efficacy CIMT and HABIT, and their motor outcomes have been published (Gordon et al. 2011). In these participants, we received permission to acquire an MRI or analyze a previously obtained MRI after the children had participated in the intervention. An additional 17 participants were recruited after the published RCT had been completed and received HABIT training as described below (Gordon et al. 2011).

MRI acquisition and measurement

Magnetic resonance imagings were obtained in one of two ways. For 10 participants, parents were provided a copy of an MRI that the child had received as part of their clinical care. For these children, the average age when the MRI was performed was 24.7 ± 27.6 months (range 0–90 months, median 13.8 months). Eight of the ten children were 2 years of age or younger at the time of the MRI). T1-weighted scans were used in this study.

For an additional 25 participants, T1-weighted MRI scans were obtained at the Program for Imaging and Cognitive Sciences facility at Columbia University Medical Center (CUMC), using a 3T Phillips magnetic resonance scanner with a six-channel head coil. Before the MRI was performed, a physician administered a safety screening evaluation. Images with a 2 mm^3 voxel size were acquired in the axial plane with scan duration of 297 s. For these children, the average age at the time of the scan was 9.1 ± 3.0 years.

The size of the cerebral peduncles was measured at the level of the rostral midbrain, as indicated by the presence of the mammillary bodies. Analysis was performed on a Macintosh computer using the public domain NIH image program (http://rsb.info.nih.gov/nih-image/). NIH image is a versatile program that has been shown to be accurate and efficient in analyzing structural features of MRI scans[19]. Since it was not possible to dissociate the substantia nigra from the peduncles, the substantia nigra was included in the measure, which encompassed the area from the lateral sulcus to the interpeduncular fossa. The borders of the outlined area could be finely adjusted in the software program to maximize accuracy of tracing. The software provided an areal measure of the outlined territory, which was converted to mm^2 using scale bars on the image. An example of the measurement of the peduncle (outlined) is shown in the inset of Fig. 2. Asymmetry in peduncle size was calculated as a ratio of the area of the more-affected peduncle divided by the area of the less-affected peduncle × 100 %. A lower number indicates a greater difference in the sizes of the peduncles.

Table 2 Peduncle asymmetry remains stable over time

Subject	Age at MRI (years months)	Peduncle asymmetry	Percent difference
1	1.11	1.10	−3.7
	6.11	1.06	
2	1.11	1.38	−8.3
	7.11	1.27	
3	0.6	1.34	+3.6
	0.9	1.39	
4	0.9	1.44	−7.1
	1.2	1.43	
	1.10	1.34	

For part of our analysis, we used a normalized peduncle asymmetry measure. We normalized peduncle size to the circumference of the brain in an axial slice, taken at the level of largest brain circumference. An MRI viewing program (OsiriX, Pixmeo Sarl) was used to scroll through each child's MRI from dorsal to ventral. At the level of maximum brain circumference, the image was saved and exported to NIH image. Brain circumference was measured by tracing the outline of the brain on the image. The software provided the area of the outlined brain cross section. If it was visually unclear which section contained the brain at maximum circumference, several sections were exported and measured in NIH image. The largest circumference from the series was used in the calculation of normalized peduncle asymmetry. The raw peduncle asymmetry was divided by the cross-sectional area of the brain at the point of maximum circumference.

Lesion type was determined for all cases. For statistical comparisons, lesion type was categorized as cortical malformation (CM), periventricular injury (PV), or cortical/subcortical lesion (C/SC) and is described in detail in Table 2. For participants who received MRIs at CUMC, a pediatric neurologist categorized the lesion type. For MRIs that were obtained as part of the child's clinical care, lesion type was obtained from the MRI report and verified by a pediatric neurologist. For subjects who had more than one scan, the most recent scan was used for the measurements and analysis.

Intensive bimanual training

Intensive bimanual training [hand–arm intensive bimanual training (HABIT)] was performed to improve hand and arm

function. HABIT employs principles of motor learning, including repetitive practice and skill progression to engage the more-affected hand in dexterous activities. In HABIT, participants were actively engaged in bimanual tasks during the training period. HABIT was conducted in a day-camp setting using child-friendly activities. Seven HABIT camps were performed from July 2007 to July 2012. Every participant received 90 h of treatment over 3 weeks (6 h/day, 15 days). HABIT was conducted one-to-one with a trained interventionist and supervised by a physical or occupational therapist. Details of HABIT procedures are presented elsewhere (Gordon et al. 2011).

Assisting hand assessment (AHA)

The AHA is a standardized test that quantifies the effectiveness of assisting hand use in performing bimanual activities in children with unilateral upper limb disabilities. The AHA has excellent validity and reliability (0.97–0.99) and responsiveness to change. The test was videotaped and scored off-site by an experienced blinded evaluator. Transformed logit data (AHA unit) were analyzed.

Jebsen–Taylor test of hand function (JTTHF)

Jebsen–Taylor test of hand function for the affected hand was the primary outcome measure (Jebsen et al. 1969). JTTHF is a standardized test that quantifies the time to complete skilled tasks. It consists of functional subtests including card flipping, small objects manipulation and placement, simulated eating, checker stacking, and empty and full can manipulation. Nonetheless, the JTTHF is considered a measure of hand dexterity, as movement speed is a correlate of how well a child can use the hand. Each child was evaluated prior to and within 2 days of completing treatment.

Statistical analyses

A paired t test was used to evaluate changes in JTTHF and AHA before and after treatment using SPSS (IBM, version 21). Associations between peduncle asymmetry and JTTHF measures were evaluated using linear regression analyses. Regression models were generated in SPSS. First, we generated models between one dependent and one independent variable. Then, the following covariates were added to regression models as noted in the results: initial severity, age, gender, lesion type, side most affected by the lesion, and the lag time between when the MRI was taken and when the child participated in the hand training. These covariates were chosen because it was suspected that these variables could affect the relationship between the dependent and independent variables. Correlation coefficients and R^2 values are reported. p values less than 0.05 were considered statistically significant.

Results

Study participants

Table 1 summarizes the demographic and clinical features of participants. Lesion type was categorized as periventricular, cortical/subcortical, and CM. Of the children with cortical and subcortical damage, three had a lesion restricted to the sensorimotor cortex. The other children all had involvement of the basal ganglia and parietal lobe. Although all children had a diagnosis of unilateral CP, bilateral damage was found on the MRI of five children. Due to the heterogeneity of lesion type among participants in the study, we are unable to draw conclusions on the impact of lesion location on motor function and capacity for recovery.

For some children, the MRI was done several years before the child participated in the hand training protocol. Lag time between MRI time and training (average 42.5 months, SD = 33.0, range 0–111.7 months) was added to the regression models that are presented below.

Bimanual training improves hand function

Some participants ($n = 18$) in this study were included in a randomized clinical trial that demonstrated efficacy of HABIT in improving unimanual and bimanual hand use, and their motor outcomes have been published (Gordon et al. 2011). Figure 1 shows change in bimanual use (AHA) and unimanual capacity (JTTHF) after HABIT in all participants of the current study. There was a significant improvement in AHA score (Fig. 1a, $df = 33$, $t = 4.41$, $p < 0.0001$) and JTTHF score (Fig. 1b, $df = 33$, $t = 4.47$, $p < 0.0001$) after HABIT.

Stability in peduncle asymmetry measures between raters and across time

Peduncle measurements were performed by analysts (KF, HK) blinded to behavioral data at the time of performing analysis. Inter-rater reliability was tested by comparing measures done independently by two different analysts, both blinded to behavioral data, on a subset of the MRIs ($n = 15$). Pearson's correlation between the measures of the two raters was 0.95 (95 % CI 0.85–0.98). Among these images measured by two analysts were five repeated MRI scans done on two different children at different ages. The Pearson's correlation between measures of the two analysts for the repeated MRIs was 0.96 (95 % CI 0.84–0.99).

Four children had MRI scans at more than one time point. Table 2 summarizes peduncle asymmetry measured from

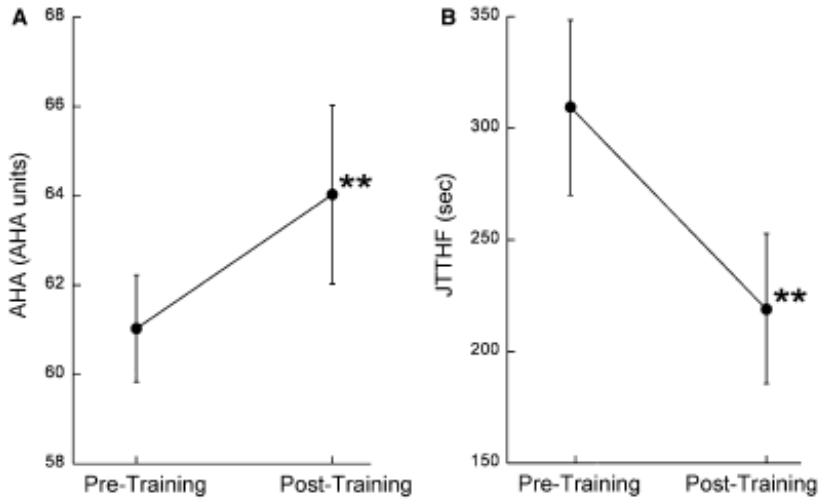

Fig. 1 Intensive bimanual therapy improves **a** bimanual and **b** unimanual hand function (*$p < 0.001$). *Error bars* represent SEM

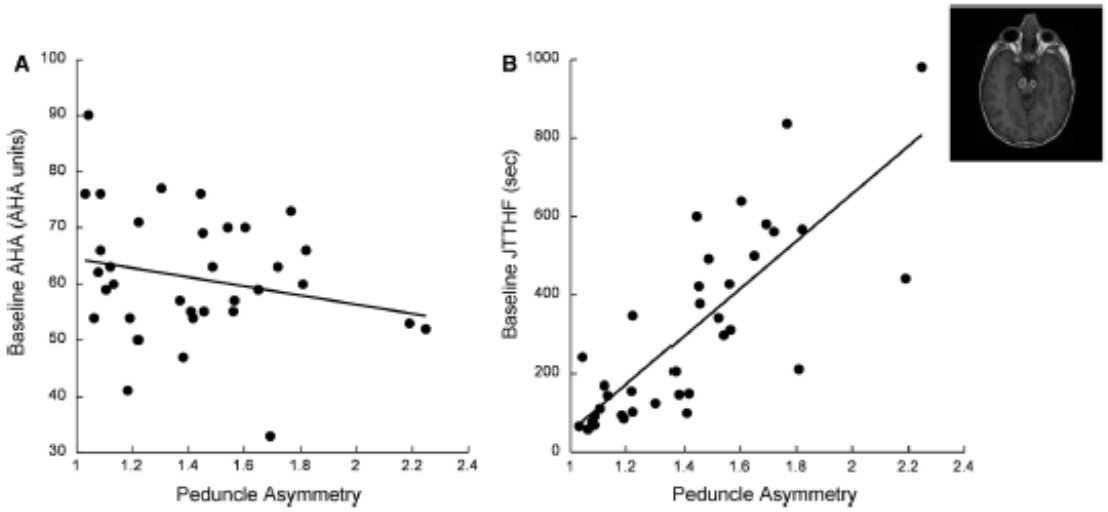

Fig. 2 Peduncle asymmetry predicts baseline **a** bimanual and **b** unimanual hand function. Pre-treatment AHA and peduncle asymmetry were positively linearly associated [$F(1,33) = 12.56.93$, $p = 0.01$, $R^2 = 0.28$]. Pre-treatment JTTHF and peduncle asymmetry were strongly and positively linearly associated [$F(1,33) = 50.93$, $p < 0.0001$, $R^2 = 0.6$]. *Inset* T1-weighted MRI showing cerebral peduncles, outlined

each of the scans. In all four children, the first MRI scan was performed when the child was less than 2 years of age. The percent difference in peduncle asymmetry measured from the different scans was 3.6–8.3 %. Although repeat scans were obtained on only a small number of children, these findings indicate that peduncle asymmetry shows low variability between scans, even when scan times are separated by many years. The stability in asymmetry suggests that there is little change in the CST between these developmental time points.

Peduncle asymmetry is associated with baseline hand function

We examined the linear association between baseline hand function and peduncle asymmetry. There was a significant linear correlation between baseline AHA score and peduncle asymmetry [Fig. 2a, $F(1,33) = 12.56$, $p = 0.01$, $r = 0.53$, $R^2 = 0.28$]. Greater asymmetry was associated with poorer bimanual hand use before the intervention. There was a

significant linear correlation between baseline JTTHF and peduncle asymmetry [Fig. 2b, $F(1,33) = 50.33$, $p < 0.0001$, $r = 0.78$, $R^2 = 0.60$]; greater asymmetry was associated with poorer unimanual capacity before the intervention.

We examined the contribution of the following covariates to the linear relationship between peduncle asymmetry and baseline AHA and JTTHF: age, gender, side of lesion, lesion type, and the lag time from when the MRI was done to the time of training. None of these covariates were independently a significant contributor to the association between bimanual hand use and peduncle asymmetry. For unimanual capacity, age was a significant contributor to this relationship [model $F(6,28) = 12.35$, $p < 0.0001$, $r = 0.85$, $R^2 = 0.73$, $t(\text{age}) = 2.7$, $p(\text{age}) = 0.017$]. The time to complete the JTTHF was lower in older children than younger children, indicating better unimanual capacity.

The interaction between age and peduncle asymmetry is also highly correlated with baseline JTTHF [$F(1,33) = 20.51$, $p < 0.0001$, $r = 0.62$, $R^2 = 0.38$] as well as baseline AHA [$F(1,33) = 8.41$, $p = 0.007$, $r = 0.45$, $R^2 = 0.20$]. This means that the relationship between peduncle asymmetry and baseline hand function differs with age.

Peduncle asymmetry does not predict improvement in hand function

We examined the linear association between the percent improvement in AHA and JTTHF and peduncle asymmetry. The linear association between peduncle asymmetry and percent improvement in AHA was not significant [Fig. 3a, $F(1,33) = 1.29$, $p = 0.26$, $R^2 = 0.038$]. Similarly, there was not a significant linear association between peduncle asymmetry and percent improvement in JTTHF [Fig. 3b, $F(1,33) = 2.72$, $p = 0.11$, $R^2 = 0.074$].

To further examine any possible relationships between peduncle asymmetry and improvement in hand function, we used multivariable statistics to determine the effects of the following covariates: initial severity, age, gender, side of lesion, lesion type, and the lag time from when the MRI was done to the time of training. The inclusion of covariates did not significantly strengthen the association between percent improvement in AHA and peduncle asymmetry [$F(7,27) = 1.16$, $p = 0.36$, $r = 0.48$, adjusted $R^2 = 0.032$, $t < 1.0$, $p > 0.1$ for all covariates]. Likewise, including these covariates in a regression model did not reveal a significant association between percent improvement in JTTHF and peduncle asymmetry [$F(7,27) = 0.86$, $p = 0.55$, $r = 0.43$, adjusted $R^2 = 0.03$, $t < 1.4$, $p > 0.1$ for all covariates].

We also normalized the peduncle size relative to the whole brain cross-sectional area and tested the association between normalized peduncle asymmetry and hand function improvements. There were no significant associations between normalized peduncle asymmetry and AHA improvement [$F(1,33) = 0.15$, $p = 0.90$] or JTTHF improvement [$F(1,33) = 1.69$, $p = 0.20$].

To further test the robustness of this result, we compared the relationship between recovery and peduncle asymmetry in children who were within the top 75 % ("strong responders") in terms of improvement in AHA ($n = 23$) or JTTHF ($n = 24$) after training, or those in the lower 25 % of recovery ("weak responders"). The association between peduncle asymmetry and percent improvement

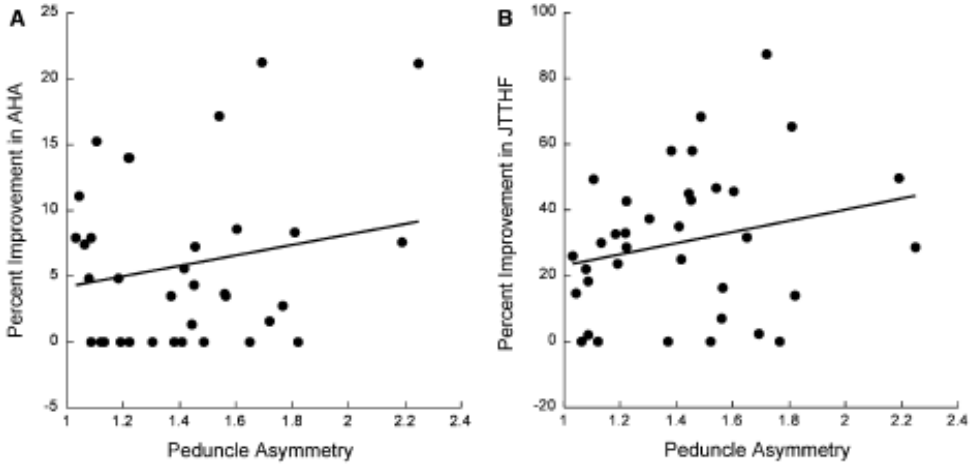

Fig. 3 Response to therapy is not linearly associated with peduncle asymmetry. **a** Percent improvement in AHA was not significantly linearly correlated with peduncle asymmetry [$F(1,33) = 1.29$, $p > 0.2$, $R^2 = 0.038$]. **b** Percent improvement in JTTHF was not significantly linearly correlated with peduncle asymmetry [$F(1,33) = 2.72$, $p > 0.11$, $R^2 = 0.074$]

was not statistically significant in the strong responders [AHA: $F(1,21) = 0.23$, $p = 0.63$, JTTHF: $F(1,22) = 2.96$, $p = 0.10$] or the weak responders [AHA: $F(1,9) = 2.5$, $p = 0.15$, JTTHF: $F(1,8) = 0.13$, $p = 0.9$]. Thus, response to intensive hand upper extremity training is not linearly associated with peduncle asymmetry.

Discussion

We examined the association between an estimate of CST dysgenesis, peduncle asymmetry, and unimanual and bimanual skill in children with congenital unilateral CP. Since previous studies described a linear association between CST damage and impairment in manual skill, in children with CP and adults with stroke (Bleyenheuft et al. 2007; Bouza et al. 1994; Duque et al. 2003; Feys et al. 2010; Mark et al. 2008), we hypothesized that peduncle asymmetry would be associated with baseline hand function and the capacity for recovery with intensive training.

Consistent with prior studies, we found that baseline unimanual capacity, measured by the JTTHF, was strongly linearly associated with peduncle asymmetry (Bleyenheuft et al. 2007; Duque et al. 2003). The magnitude and strength of the association found in this study were similar to previous studies. We also showed that bimanual hand use, measured by the AHA, is associated with peduncle asymmetry. Even though prior studies used different measures of hand function than the JTTHF and AHA, the linear association between CST dysgenesis and manual skill deficits was a consistent finding. It is important to note that neither the JTTHF nor the AHA measure how the task is performed (e.g., kinematics).

Contrary to our hypothesis, we found that there was no linear association between peduncle asymmetry and the capacity of children to improve unimanual or bimanual hand function with HABIT. The independence of functional improvement and peduncle asymmetry held despite correction of a large number of covariates, including lesion type, age at time of HABIT, age at MRI, and brain size. This represents an exciting, novel finding, indicating that children with a range of severity in peduncle asymmetry can benefit from HABIT.

Two studies have examined the relationship between MRI measures and improvement in hand abilities after CIMT in children with unilateral CP and adults with chronic stroke. Rocca et al. (2013) found a significant association between integrity of fibers in the lesion area and improvement in hand function after CIMT. In contrast, Rickards et al. (2013) did not find a significant association between improvement after CIMT and CST integrity in children or adults. Both studies enrolled a low number of participants ($n < 30$). Further research regarding the predictive power of MRI features and recovery after intensive hand training is needed.

Our results suggest that improvements in hand function after training are not exclusively mediated by the contralaterally projecting CST. Other motor pathways, including ipsilateral connections from the less-affected M1, are possibly important in recovery. Developmental studies have suggested that lesions occurring earlier in life are more likely to result in increased ipsilateral connections from the less-affected M1 (Fowler et al. 2010; Kuhnke et al. 2008; Tian et al. 2010; Holmstrom et al. 2010; Yoshida et al. 2010; Eyre 2003), likely by preserving the bilateral CST projections that occur during development (Staudt 2010). Lesions resulting in strong ipsilateral connections also can produce more severe deficits in hand function, inviting speculation that these ipsilateral connections are maladaptive (Kuhnke et al. 2008). Laboratory models of injury have also shown that other pathways can be involved in motor recovery (Z'Graggen et al. 1998; Carmel et al. 2013). Moreover, Rose et al. (2011) examined the relationship between hand function and asymmetry of corticospinal and corticothalamic pathways in children with unilateral CP. They did not find a significant association between CST asymmetry and hand function, but instead found that asymmetry of the corticothalamic pathway was strongly correlated with hand function. Thus, it is possible that integrity of corticothalamic fibers may be related to improvement after intensive hand therapy.

Lesion type was not a significant predictor of baseline hand function or amount of recovery. Some studies indicate a relationship between lesion type and hand function, suggesting that children with periventricular lesions have better hand function than children with cortical or subcortical lesions (Cioni et al. 1999; Feys et al. 2010). It is possible that we failed to find an effect of lesion type on hand function since children in this study had a variety of lesion types. Therefore, we potentially lacked the power to discern differences based on lesion type.

A limitation of our study is that structural images were used. Some studies have used structural images to examine motor pathway dysgenesis in children with CP (Duque et al. 2003) and adult stroke patients (Pineiro et al. 2000). Our regression model of baseline hand function shows highly similar results as the Duque et al. study (2003), suggesting that these findings are reproducible. An important study compared peduncle asymmetry analysis from structural scans to analysis using DTI to identify the CST (Bleyenheuft et al. 2007). In this study, DTI was more effective at finding associations between motor symptoms and neuroanatomy than peduncle asymmetry on structural scans. When the peduncle size is measured, the size includes not only the CST but also other tracts and the substantia nigra. Thus, peduncle measure is an estimate of CST dysgenesis

but is not as precise as a DTI-based analysis. Bleyenheuft et al. suggested that peduncle measures systematically underestimate CST dysgenesis.

Moreover, structural images provide no information about the integrity of neural pathways. Indeed, the integrity of motor pathways may be more predictive of the potential for recovery than the estimated size of motor pathways. DTI provides information about the integrity of fibers by determining the anisotropy of fibers, a measure of directional diffusivity of water along a fiber bundle. Anisotropy of motor pathways is lower than normal in children with CP (Bleyenheuft et al. 2007; Thomas et al. 2005; Yoshida et al. 2010). Future studies should examine the relationship between DTI-determined motor fiber integrity and potential for improvement in hand function.

Another limitation of our study is that for many children, there was a lag time between the time at which the MRI was taken and the time of intervention (average 42.5 months). Although lag time between MRI and training was not a significant variable in the regression models, it is possible that children's peduncle asymmetry had changed between the time of MRI acquisition and training. Two pieces of evidence suggest this is not the case. First, there was a strong relationship between peduncle asymmetry at the time of MRI and baseline hand function. Second, the asymmetry of the peduncles remained remarkably stable over time in the children who had repeated MRI scans. Further study of CST development, particularly in early development, will help to explore how CST anatomy changes in early development, as has been done with physiological measures of CST function (Eyre et al. 2007; Staudt 2010). Additionally, we only had sufficient power to test linear relationships between variables. It is possible that a more complex nonlinear relationship might exist between peduncle asymmetry and recovery.

This study is limited to children with unilateral CP who have mild to moderate impairments of the affected hand. Our finding that the independence of recovery from peduncle asymmetry invites the possibility that children with a great amount of peduncle dysgenesis, and more severe hand deficits than children studied here, might respond to intensive treatment. It is likely that other factors are stronger predictors of treatment efficacy, such as intensity of training, treatment schedule, and attentiveness of the child during therapy. Moreover, other neurological factors that were not assessed in this study could be predictors of recovery. For example, neurological damage that impairs vision, attention, or cognitive potential may impact recovery, as children with these impairments may be limited in their ability to participate in intensive training protocols. Future studies that can identify predictors of recovery will be extremely valuable, so that treatment can be tailored to children based on these parameters.

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APPENDIX J

Research in Developmental Disabilities 49–50 (2016) 129–139

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The effects of intensive bimanual training with and without tactile training on tactile function in children with unilateral spastic cerebral palsy: A pilot study

Hsing-Ching Kuo [a], Andrew M. Gordon [a], Aline Henrionnet [b], Sylvie Hautfenne [b], Kathleen M. Friel [c,d], Yannick Bleyenheuft [b,*]

[a] Department of Biobehavioral Sciences, Teachers College, Columbia University, 525 West 120th Street, Box 93, New York, NY 10027, USA
[b] Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium
[c] Burke-Cornell Medical Research Institute, 785 Mamaroneck Avenue, White Plains, NY 10605, USA
[d] Brain-Mind Research Institute, Weill Cornell Medical College, 1300 York Ave, New York, NY 10021, USA

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ABSTRACT

Children with unilateral spastic cerebral palsy (USCP) often have tactile impairments. Intensive bimanual training improves the motor abilities, but the effects on the sensory system have not been studied. Here we compare the effects of bimanual training with and without tactile training on tactile impairments. Twenty children with USCP (6–15.5 years; MACS: I–III) were randomized to receive either bimanual therapy (HABIT) or HABIT + tactile training (HABIT + T). All participants received 82 h of standardized HABIT. In addition 8 sessions of 1 h were provided to both groups. The HABIT + T group received tactile training (without vision) using materials of varied shapes and textures. The HABIT group received training with the same materials without tactile directed training (full vision). Primary outcomes included grating orientation task/GOT and stereognosis. Secondary outcomes included two-point discrimination/TPD, Semmes-Weinstein monofilaments/SWM. The GOT improved in both groups after training, while stereognosis of the more-affected hand tended to improve (but $p = 0.063$). No changes were found in the TPD and the SWM. There were no group × test interactions for any measure. We conclude tactile spatial resolution can improve after bimanual training. Either intensive bimanual training alone or incorporation of materials with a diversity of shapes/textures may drive these changes.

What this paper adds

- This is the first study in USCP to test the effect of systematic tactile training.
- Tactile spatial discrimination was improved following intensive bimanual training.
- HABIT with and without tactile training improved tactile function similarly.

* Corresponding author. Tel.: +32 27649349.
E-mail addresses: hk2455@tc.columbia.edu (H.-C. Kuo), ag275@tc.columbia.edu (A.M. Gordon), aline.henrionnet@gmail.com (A. Henrionnet), sylvie.hautfenne@outlook.com (S. Hautfenne), kaf3001@med.cornell.edu (K.M. Friel), yannick.bleyenheuft@uclouvain.be (Y. Bleyenheuft).

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1. Introduction

Impaired function in children with unilateral spastic cerebral palsy (USCP) does not purely result from motor impairments, but is also affected by concomitant sensory impairments. Tactile registration, tactile perception, and sensorimotor integration are essential for grasping and releasing objects (Gordon & Duff, 1999a, 1999b), dexterous manipulation (Bleyenheuft & Gordon, 2013), and activities of daily living (Auld, Boyd, Moseley, Ware, & Johnston, 2012). There have been studies investigating sensory contribution to motor control in children with USCP (Auld et al., 2012; Auld, Russo, Moseley, & Johnston, 2014; Gordon & Duff, 1999a). However, whether intensive bimanual training or tactile training is effective in modifying tactile impairments in children with USCP has never been investigated.

Early animal studies directly tested how sensory deprivation affected the motor system. Mott and Sherrington (1895) showed that deafferentation, an abolition of sensory input, impaired the performance of skilled movements in monkeys. Although the underlying pathophysiology is different in deafferented monkeys and in USCP, these studies highlighted the contribution of sensory input to fine motor control. Recently, neuroplastic changes were demonstrated in rats' sensory cortex (S1) after enhanced environmental or motor training. Joo et al. (2012) showed increased somatosensory evoked potentials that paralleled motor recovery. Alwis and Rajan (2013) showed that 8 weeks of environment enrichment increased electrophysiological responses to tactile stimuli. These studies highlight the interaction between sensory and motor systems.

Studies investigating effects of somatosensory training programs on modifying sensorimotor functions in adult stroke are emerging (Carey, Macdonell, & Matyas, 2011). While it has been acknowledged that the sensory impairment is a major contribution to motor impairments in children with USCP (Bleyenheuft & Gordon, 2013), effective therapy in improving tactile impairment is lacking (Auld et al., 2014). The study by Charles, Lavinder, and Gordon (2001) is the only study using intensive hand therapy which reported an improvement in tactile discrimination in 3 children after constraint-induced movement therapy (CIMT). They attributed the improvement in tactile discrimination to an increase in tactile input and its subsequent change in cortical receptor fields for the fingers. More recently, studies investigating neuroplastic changes associated with intensive hand therapy using functional Magnetic Resonance Imaging (fMRI) or magnetoencephalography (MEG) demonstrated increased activation associated with CIMT (Juenger et al., 2013) or HABIT (Bleyenheuft et al., 2015) in S1 or M1 in USCP and in adult stroke (Laible et al., 2012). In summary, tactile function could be improved after intensive hand therapy in children with USCP, and neurophysiological changes associated with intensive therapy may be found in S1 or M1. These studies prompted us to probe deeper into the relationship among intensive hand training, tactile training, and their impact on tactile function in children with USCP.

Both unimanual and bimanual intensive therapy have been shown to improve hand motor function in children with USCP (Gordon et al., 2011, 2008; Sakzewski, 2012; Sakzewski et al., 2011). Bimanual intensive therapy improved self-determined goals more than unimanual therapy as bimanual training allows use of both hands (e.g., tying shoes) (Brandao, Gordon, & Mancini, 2012; Gordon et al., 2011). In addition, bimanual training improved bimanual coordination more than unimanual training (Hung, Casertano, Hillman, & Gordon, 2011). Consequently, we adopted bimanual training as a common ingredient in our study. We further aimed to compare the efficacy of two interventions in this pilot study: intensive bimanual training (hand–arm bimanual intensive therapy, HABIT) vs. intensive bimanual training that includes tactile training (HABIT + T) on modifying tactile function in children with USCP. We hypothesized that tactile function could be enhanced after HABIT due to the enriched environment created by exposure to objects of varied textures, and tactile function could be further enhanced with additional tactile training.

2. Methods

2.1. Participants

Participants included a sample of convenience that was recruited from a subset of two ongoing trials (Bleyenheuft, Arnould, Brandao, Bleyenheuft, & Gordon, 2015; Brandao et al., 2013). The inclusion criteria were established based on prior HABIT trials (Brandao et al., 2013; Gordon et al., 2011; Gordon, Schneider, Chinnan, & Charles, 2007): (1) age 6 to 18, diagnosed with congenital USCP, (2) the ability to lift the more-affected arm 15 cm above a table surface and grasp light objects, (3) cognition level defined as mainstreamed in school (Kaufman Brief Intelligence test score >70), (4) demonstrated ability to follow instructions and complete testing. Exclusion criteria included: (1) health problems unrelated to USCP, (2) uncontrolled seizures, (3) visual problems interfering with intervention/testing, (4) severe muscle tone at any joint (Modified Ashworth score >3.5), (5) orthopedic surgery on the more-affected hand within one year, and 6) botulinum toxin therapy in the upper limb within the last 6 months or intended treatment within the study period. Informed assent/consent forms were obtained from participants and caregivers. This study was approved by the respective Universities' Institutional Review Boards.

2.2. Procedures

2.2.1. General intervention procedures

One bimanual training camp was conducted at Teachers College in New York City in early July 2012. Another bimanual training camp was conducted at Université Catholique de Louvain in Brussels, Belgium in late July 2012. In each site,

participants were randomized offsite using concealed allocation stratified by their baseline tactile discrimination threshold (measured by Grating Orientation Task) and baseline unilateral dexterity (measured by Jebsen–Taylor Test of Hand Function) of the more-affected hand. Twenty participants were randomly assigned to a (1) HABIT including tactile training group (HABIT + T, $n = 4$ in New York, $n = 6$ in Brussels) or (2) HABIT group (HABIT, $n = 4$ in New York, $n = 6$ in Brussels).

HABIT is a form of intensive bimanual training for children with USCP using motor learning principles (Charles & Gordon, 2006). Children are engaged in using both hands in bimanual play and functional activities. The more-affected hand is treated as the assisting hand (active assist or stabilizer) in the context of task practice. Motor learning principles of whole-task and part-task practice are applied. Clinical trials of HABIT have shown efficacy in improving children's manual dexterity, bimanual hand use, and performance of functional goals (Brandao et al., 2013, 2012; Gordon et al., 2011, 2007).

2.2.2. Intervention details

All participants received 82 h of standardized intensive bimanual training within 3 weeks by trained interventionists. In both sites, an additional 8 h of treatment was provided in a separate room with a different interventionist (specifically trained). Children's regular interventionists were not allowed in this training room. During that time, the HABIT + T group received tactile training using tactile stimulating materials (without vision, described in Section 2.2.3). The HABIT group received the same dosage/schedule of controlled training with the same material but without specific tactile-directed training (standardized HABIT-full vision, see Section 2.2.3). Apart from the specially trained interventionist for those 8 h of tactile/control training, regular interventionists (for the 82 h of standardized HABIT) were trained at a pre-intervention session on procedures common to the 2 groups, such as strategies to engage children actively involving the use of both hands and safety. Daily team meetings reinforced individual treatment plans. The 2 camps had the same supervisor to ensure the uniformity of intervention.

2.2.3. Tactile and control training

The 8 h of specific tactile intervention or control training was conducted systematically by the same interventionists at both sites. During those 8 h, children received either tactile training or control training (1 h/session × 8 camp days, 8 sessions in total).

Children in the HABIT + T group received 8 sessions of 1 h tactile training. Specific training components encompassed (a) tactile discrimination and matching: training modalities included texture (e.g., fur and plastic), shape (e.g., circle and square), and size, (b) finger resistance discrimination and matching (cylinder blocks with different resistance for fingers to push in, see Fig. 1A and B). Training was primarily administered with the child blindfolded or exploring objects in bags (i.e., not visible). Yet, instructions and knowledge of results were given with vision. Both hands were required to engage in the tasks. Skill progression and engagement of both hands for bimanual manipulation were ensured. An example for a texture-matching task is that a child would first touch/feel an object with one hand (e.g., a sand-paper-top cylinder, see Fig. 1A). The child would be asked to identify the same object among various objects (e.g., fur-top cylinder, spiky-plastic-top cylinder, plastic alphabets) by exploring with the other hand. If children were not able to explore objects due to their motor impairments, interventionists supported the objects in children's hand to allow exploration. Children received knowledge of results after each trial by visual/verbal feedback from the interventionists. Positive reinforcement was always provided.

Children in the HABIT group did not receive tactile training. During the control training, they received standardized HABIT by playing with the same materials (full vision) in the same environment (same room/interventionist) as those provided to the HABIT + T group. Participants in this group received control training with the same schedule and frequency as those in the HABIT + T group (1hr/session on 8 camp days). Intervention materials were applied in the context of play and functional activities in this group. This design controlled for confounds of the differential effects by exposing children to different materials and different interventionists.

2.3. Measurements

Participants were evaluated directly prior to treatment (pre-test) and within 2 days after treatment (post-test) by one physical therapist blinded to group allocation. Primary outcomes included grating orientation task/GOT and stereognosis. Secondary outcomes included two-point discrimination/TPD, Semmes-Weinstein monofilaments/SWM, the Jebsen–Taylor Test of Hand Function/JTTHF and the Assisting Hand Assessment/AHA. The details of each assessment are described below.

The tactile spatial resolution was measured by the GOT using the JVP domes (Stoelting Co., Wood Dale, IL, USA). It was validated in children with USCP (Bleyenheuft & Thonnard, 2011). During testing, children had their palmer side of the index finger tip exposed and resting on the table. They were first given 4 practice trials with vision/verbal feedback, followed by 4 practice trials without vision and with verbal feedback. We used 11 domes presenting gratings with equal distances of bar & groove widths (0.35, 0.5, 0.75, 1.0, 1.2, 1.5, 2.0, 3.0, 3.5, 4.0, 4.5 mm). The domes were perpendicularly applied to the index finger pad for 1–2 s, which resulted in ~2 mm of deformation on the skin (a procedure validated in children, see Bleyenheuft, Cols, Arnould, & Thonnard, 2006; Bleyenheuft & Thonnard, 2007). Children determined the grating orientation (along or across the finger). Two-alternative orientations (forced-choice) were used for each trial. Each dome was tested in 10 trials using a pseudo-random presentation order of 5 trials with the gratings along horizontal axis and 5 trials with the gratings across the longitudinal axis of the finger. We started with the 3.0 mm dome. Children were tested with the next smaller width when the correct response rate of the current dome was ≥60%. They were tested with the next larger width when the

Fig. 1. (A) An example of an object-matching task during tactile training in the HABIT + T group. A child was first exposed to a sand-paper-top cylinder with the less-affected hand (on the right side of the picture), and was required to search for the same object with the more-affected hand (on the left side of the picture). The child's vision was occluded by a screen during the discrimination training. (B) An example of the materials used during tactile training.

correct response rate of the current dome was ≤50%. Threshold search stopped when the child consistently failed to discriminate (i.e., ≥2 domes showing the correct response rate ≤50%). A final threshold was calculated based on an estimate of 75% correct width (Van Boven & Johnson, 1994). A default threshold of 4.5 mm was assigned when they were unable to reach a correct response rate of 70% even when tested with the widest dome (4.5 mm).

Stereognosis was measured with the Manual Form Perception Test (Cooper, Majnemer, Rosenblatt, & Birnbaum, 1995). Children were asked to identify 10 objects by touching/feeling them. Five objects of daily use (toothbrush, tennis ball, comb, large cup, and candy-in-wrapper) and five shapes (circle, triangle, square, diamond, and octagon) were randomly presented to children. The total number of correctly identified items was the final score.

Static TPD was performed by using Disk-criminator® (Mackinnon & Dellon, 1985). Children were first instructed with the testing procedure of differentiating between 1-point and 2-points on the less-affected hand with vision. They were tested on thumb, index, and middle finger pads of both hands. The evaluator randomly assigned 1- or 2-point stimuli. Each finger was tested with 10 random trials at each distance. When a child achieved 7 correct responses at the distance tested, evaluator tested with the next smaller distance. When a child failed to achieve 7 correct responses at the distance tested, evaluator tested with the next larger distance. The minimal distance children were able to distinguish two discrete points, ranging from 2–15 mm, was recorded for each finger as the final score. If a child could not discriminate 1- or 2-point stimuli with a distance of 15 mm, a default threshold of 15 mm was assigned.

Tactile perception was tested with Semmes-Weinstein monofilaments (SWM) (Smith & Nepher Roylan Inc. Germantown, WI, USA) (Weinstein, 1993). We used a 20 monofilaments kit. Monofilaments were applied to the index finger pad. Children said "touch" or "yes" when they felt the filament. A few practice trials were given on the less-affected hand until they understood the procedure. We started with the 4.31 filament and searched for the threshold.

The Jebsen–Taylor Test of Hand Function (JTTHF) (Jebsen, Taylor, Trieschmann, Trotter, & Howard, 1969) is a standardized test quantifying unilateral dexterity as the movement time (in seconds) to complete motor tasks. It consists of subtests including card flipping, small objects manipulation and placement, simulated eating, checker stacking, and empty and full can manipulation. The maximum completion time of each subtest was 180 s, and if it was clear a child could not complete the items, the test was stopped to prevent frustration and fatigue and the maximum time was recorded. The total score (in seconds) is an addition of the 6 subtests time.

The Assisting Hand Assessment (AHA, version 4.3) (Krumlinde-Sundholm & Eliasson, 2003) quantifies the effectiveness of the more-affected hand use in bimanual activities (in 0–100 AHA-unit). The test was videotaped and scored off-site by an evaluator blinded to group allocation.

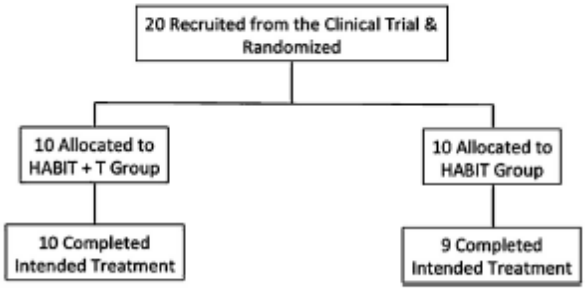

Fig. 2. Patient flow chart.

2.4. Statistical design

Statistical analyses were performed using SPSS (Version 21). A 2 (group) × 2 (test session) ANOVA with repeated measures on test sessions was performed on each measure for the more- and the less-affected hand. This design was to test efficacy of training on tactile and motor function and to examine if treatment efficacy differed depending on group assignment. As many of the measures violated assumptions of normal distributions, we logarithm-transformed the raw data using log base 10. Given that the ANOVA results on raw data and logarithm-transformed data were qualitatively similar, we thus reported the statistical results on the log-transformed data below. Figures show the raw values however. *t*-Tests were performed to test group differences at baseline. Pearson coefficient correlations were performed to examine the predictors of changes in function. *p*-Values under 0.05 were set as statistical significance.

3. Results

Patient flow is shown in the flow diagram (Fig. 2). Twenty children with USCP (ages 6–15.5 years; MACS level: I–III) met the inclusion criteria and were randomized to receive either HABIT or HABIT + T. One participant in the HABIT group in Brussels dropped out of the study. A total of 19 participants (ages 6–15.5 years; MACS level: I–III) completed 90 h of training. Clinical characteristics of participants are described in Table 1. There were no significant differences in the primary measures or secondary measures between the two groups at the baseline (all $p > 0.15$).

Table 1
Baseline participant characteristics.

Characteristics	HABIT + T (n = 10)	HABIT (n = 9)	Control group
Mean age (SD), years, months	8.9 (2.6)	8 (1.1)	8.2 (2.3)
Gender			
Male	4	6	4
Female	6	3	6
Paretic hand			
Right	6	5	4
Left	4	4	6
MACS			
I	2	0	3
II	6	8	6
III	2	1	1
Baseline tactile discrimination threshold, mean (SD), mm	4.23 (0.58)	4.35 (0.42)	4.11 (0.70)
Baseline stereognosis, mean (SD), n	6.5 (3.63)	5.22 (3.08)	4.4 (3.75)
Baseline TPD-thumb, mean (SD), mm	8.9 (5.36)	9.22 (5.41)	NA
Baseline SWM, mean (SD)	6.3 (2.5)	5.78 (2.74)	NA
Baseline JTHF, mean (SD),s	368.06 (280.42)	389.52 (308.25)	364.69 (305.60)
Lesion type			
CM	1	0	0
PV	4	4	2
C/SC	2	4	7
Unavailable	3	1	1

Abbreviations: HABIT, hand–arm bimanual intensive therapy; HABIT + T, HABIT with additional tactile training, SD, standard deviation; MACS, manual ability classification system; TPD, two-point discrimination, SWM, Semmes-Weinstein monofilament; JTHF, Jabsen-Taylor test of hand function, CM, cortical malformation; PV, periventricular injury; C/SC, cortical/subcortical lesion.

3.1. Changes in tactile spatial resolution & stereognosis after training

Fig. 3 shows the means of the more-affected hand for the HABIT + T and HABIT groups at pre-test and post-test for the GOT, stereognosis, the JTTHF, and the AHA. For the GOT, there was a 0.36 mm and a 0.82 mm improvement in the discrimination threshold in the more-affected hand for the HABIT + T and HABIT groups, respectively (Fig. 3A). There was a 1 mm and a 1.05 mm improvement in the discrimination threshold in the less-affected hand for the HABIT + T and HABIT groups, respectively (Fig. 3B). A test session effect showed that the improvement in the GOT was significant (Table 2, more-affected hand, $p = 0.028$; less-affected hand, $p = 0.002$). For the stereognosis, there was a 0.5 object and >1 object (1.7) improvement in the more-affected hand for the HABIT + T and HABIT groups, respectively (Fig. 3C). There was a trend of improvement in the stereognosis in the more-affected hand ($p = 0.063$). There was no significant Group × Test session interaction effect in the stereognosis in the more-affected hand. Finally, there were no significant changes in the stereognosis in the less-affected hand after training, (0.1 and 0.2 object more for the HABIT + T & HABIT groups, Fig. 3D). Nor did we find a significant Group × Test session interaction effect.

3.2. Changes in TPD and SWM after training

There were no significant changes after training in either hand in the TPD and SWM in either group (TPD all fingers, more-affected hand, $p > 0.16$; TPD all fingers, less-affected hand, $p > 0.57$; SWM more-affected hand, $p = 0.23$; SWM less-affected hand, $p = 0.74$). There were no Group × Test session interaction effects for either measure.

3.3. Changes in hand function after training

For the JTTHF, there was a 42 s (19.7%) and a 148 s (39.1%) decrease for the HABIT + T and the HABIT group, respectively (Fig. 3E, test session, $p < 0.001$). There was no significant Group × Test session interaction effect, indicating both groups did not improved in a significantly different way ($p = 0.053$). For the AHA, there was a 6.7 and a 4.9 AHA-unit improvement for the HABIT + T and the HABIT group, respectively (Fig. 3F, test session, $p = 0.002$). There was no Group × Test session interaction effect for the AHA, indicating both groups improved similarly ($p = 0.56$). These improvements were clinically meaningful (defined as a 5 AHA units improvement) for the HABIT + T group, and borderline clinically meaningful for the HABIT group.

3.4. Stability of measures in a control group without training

Since there were no differences between the two groups in the primary measures, we considered whether there was a learning effect in the participants simply by being tested twice across a 3-week period of time without training. We thereafter recruited an age-matched control group of 10 participants with USCP (see Table 3). They were tested two times with three-weeks in between on the primary and one secondary measure without any treatment. Paired t-tests confirmed stability of these measures across the two testing sessions in the control group (Table 3, no significant differences for any measure).

3.5. Predictors of functional outcome

Due to the heterogeneity of the lesion and functions in USCP, we tested whether individual differences, including initial severity in tactile & motor function and age, would impact changes in function. Given no group differences were found in previous analyses, we used all participants ($n = 19$) to explore correlations between individual differences and changes in function. We found that participants with worse unilateral dexterity (baseline JTTHF) had a trend to improve more in stereognosis after training in the more-affected hand ($R = 0.45$, $p = 0.052$). Baseline unilateral dexterity was not correlated with improvements in threshold of discrimination in the more-affected hand ($R = 0.3$, $p = 0.22$). Baseline tactile function was not correlated with percentage improvements in the AHA (GOT & AHA % change, $p = 0.88$; stereognosis & AHA % change, $p = 0.25$). No relation was found between age and improvements in threshold of discrimination ($p = 0.65$), or between age and improvements in stereognosis ($p = 0.95$).

4. Discussion

This study aimed to investigate (1) changes in tactile function associated with intensive bimanual training in USCP, (2) the effects of additional tactile training to HABIT in enhancing tactile function. We hypothesized that tactile function would be improved after HABIT, and be further improved with added tactile training. Our results demonstrated that tactile function, specifically tactile spatial discrimination was modifiable immediately after intensive bimanual training. These improvements were not an effect of repeated testing across a 3-week period of time. Contrary to our hypothesis, both groups improved similarly. Although the improvements in the stereognosis did not reach statistical significance across the two groups ($p = 0.063$), it is worthwhile noticing that 31.6% (4 in the HABIT + T group, 2 in the HABIT group) of participants achieved the highest value (10 objects) at baseline in the stereognosis in the more-affected hand. This ceiling effect in the stereognosis at baseline may not leave sufficient room for change.

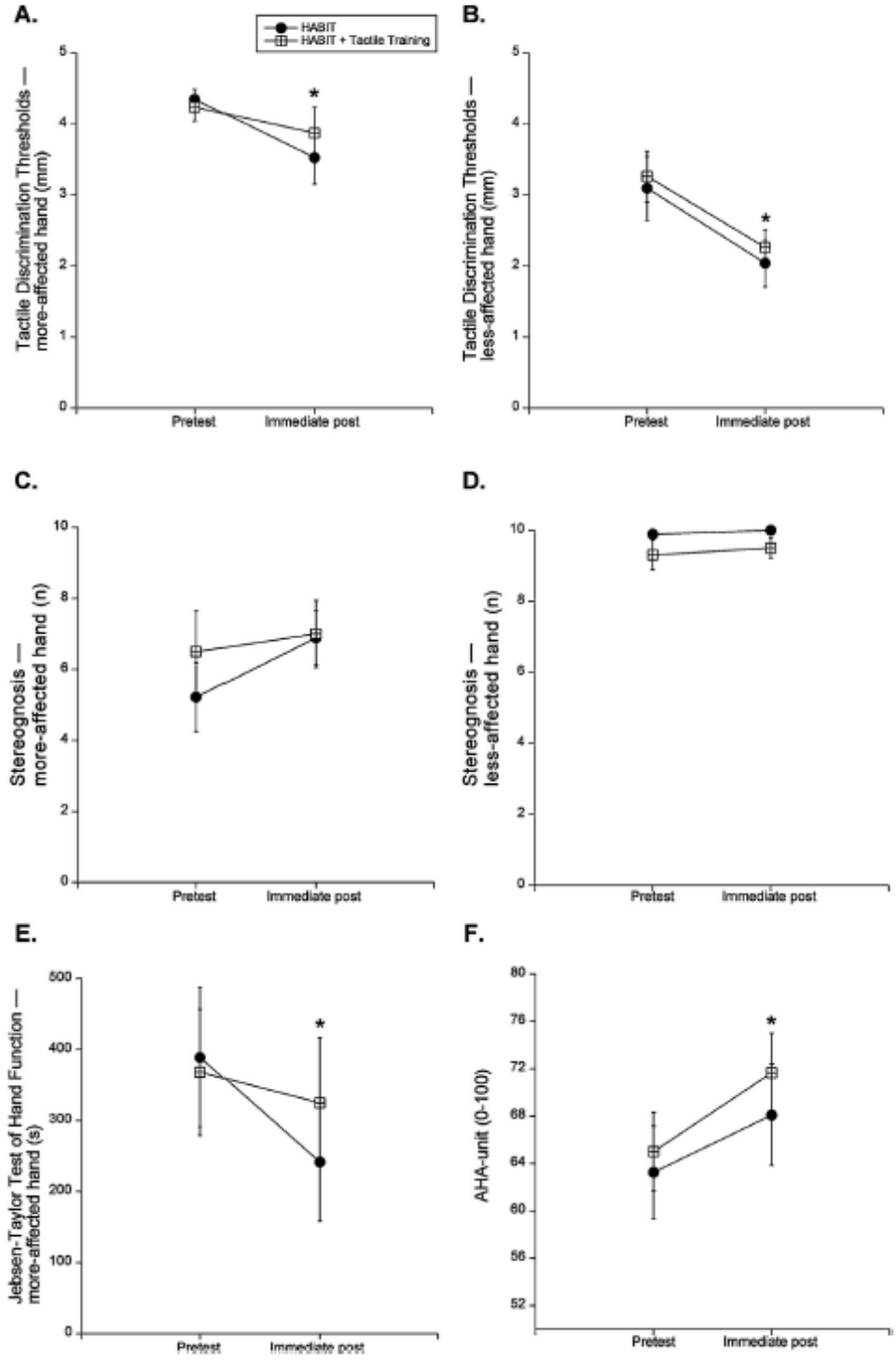

Fig. 3. (A) Mean ± standard error of the mean (SEM) threshold of grating orientation task (GOT) of the more-affected hand. Solid circle represents HABIT group. Open rectangle represents HABIT + T group. (* $p < 0.05$ for the main effect of test session). (B) Mean ± SEM threshold of grating orientation task (GOT) of the less-affected hand. (C) Mean ± SEM numbers of identified objects of stereognosis of the more-affected hand. (D) Mean ± SEM numbers of identified objects of stereognosis of the less-affected hand. (E) Mean ± SEM time to complete JTTHF of the more-affected hand. (F) Mean ± SEM AHA-unit measured by the AHA.

Table 2
Results for the more-affected hand.

	Pretest (95% CI)	Immediate posttest (95% CI)	Change score (pretest to immediate posttest) (95% CI)	Test session effect p value (partial η^2)[a]	Interaction p value (partial η^2)[a]	Power $(1-\beta)$[b]
Threshold of GOT (mm)						
HABIT+T	4.23 (3.89, 4.58)	3.87 (3.06, 4.68)	−0.36 (−0.99, 0.27)	–	–	–
HABIT	4.35 (3.99, 4.72)	3.53 (2.68, 4.38)	−0.82 (−1.48, −0.16)	–	–	–
Mean	4.29 (4.04, 4.54)	3.70 (3.11, 4.29)	−0.59 (−1.05, −0.14)	0.028 (0.253)	0.501 (0.027)	1.00
Stereognosis (n)						
HABIT+T	6.5 (4.19, 8.81)	7.00 (5.14, 8.87)	0.50 (−0.88, 1.88)	–	–	–
HABIT	5.22 (2.79, 7.66)	6.89 (4.92, 8.86)	1.67 (0.21, 3.12)	–	–	–
Mean	5.86 (4.18, 7.54)	6.94 (5.59, 8.30)	1.08 (0.08, 2.08)	0.063 (0.188)	0.522 (0.025)	0.99
TPD thumb (mm)						
HABIT+T	8.9 (5.2, 12.60)	8.6 (5.02, 12.18)	−0.30 (−1.40, 0.80)	–	–	–
HABIT	9.22 (5.32, 13.12)	8.89 (5.12, 12.66)	−0.33 (−0.80, 0.13)	–	–	–
Mean	9.06 (6.37, 11.75)	8.74 (6.14, 11.35)	−0.32 (−0.98, 0.35)	0.413 (0.04)	0.479 (0.03)	0.99
SWM						
HABIT+T	6.30 (4.50, 8.10)	5.40 (3.50, 7.30)	−0.90 (−2.16, 0.36)	–	–	–
HABIT	5.78 (3.88, 7.68)	6.00 (3.99, 8.00)	0.22 (−0.85, 1.29)	–	–	–
Mean	6.04 (4.73, 7.35)	5.70 (4.32, 7.08)	−0.34 (−1.24, 0.56)	0.228 (0.084)	0.106 (0.146)	0.97

Abbreviations: GOT, grating orientation task; HABIT, hand–arm intensive bimanual therapy; HABIT+T, HABIT with additional tactile training; TPD, two-point discrimination; SWM, Semmes-Weinstein monofilaments; Mean, represents the average for the HABIT+T and HABIT groups.
[a] Statistical results obtained from using ANOVA with repeated measure.
[b] Power was calculated for the test session effect.

4.1. Enriched environment may improve tactile function in both groups

The comparable results in the improvements associated with either training group in the GOT could primarily be explained by an enriched environment in both groups. Animal studies (Alwis & Rajan, 2013) showed that enriched environment induced neural responses to discriminative whisker behaviors. Studies in healthy adults demonstrated that perceptual learning occurred after one to several sessions of tactile training (Harris, Harris, & Diamond, 2001) and was transferred to the contralateral and adjacent fingers (Kaas, van de Ven, Reithler, & Goebel, 2013).

Two possible types of enriched environments have been applied to both groups in our study. First, both groups received 82 h of standardized HABIT. The increased amount of tactile stimulation during those 82 h of intensive bimanual manipulation may have created an enriched environment and may explain comparable improvements in the GOT. Second, the novel training materials with different textures, shapes, and sizes used during the 8 h of tactile and controlled training could also have enriched the environment. Thus 8 h of specific tactile training may not be needed to enhance tactile function in children with USCP on top of the intensive bimanual therapy they already received. Instead, either intensive tactile input during standardized HABIT or an introduction of new materials during tactile/controlled training might drive tactile improvement.

4.2. Insufficient dose of tactile training may cause similar findings

The similar findings in the two groups might also be explained by insufficient tactile training dose in the HABIT+T group. Carey et al. (2011) showed that 10 h (60 mins/session, 3 sessions/week) of somatosensory discrimination training effectively improved tactile discrimination capacity in adult stroke patients. It is possible that 8 h of tactile training was insufficient to drive differential effects of perceptual learning between the two groups in children with USCP, especially when cognitive capacity was required in perceptual learning. It is important to note that children with USCP were learning

Table 3
Outcomes remain stable over time in the control group.

Outcome	Test 1	Test 2[a]	t-Test p value[b]
Tactile discrimination threshold (mm)	4.11 (0.7)	3.95 (0.73)	0.42
Stereognosis (n)	4.4 (3.75)	4.4 (3.57)	0.95
JTTHF (s)	364.69 (305.6)	352.56 (232.59)	0.31

Values for Test 1 and Test 2 represents mean (SD).
[a] Test 2 was performed 3 weeks after Test 1.
[b] Statistical results obtained from using paired t-test.

new skills whereas adult stroke patients were re-learning the function they lost. Hence a more intensive training might be needed in children with USCP. Future studies should test an intensive dose of tactile training in a randomized controlled trial (RCT) or a cross-over study.

4.3. Improvements in spatial tactile discrimination in the both hands after intensive bimanual therapy

It is important to note that children with USCP had tactile impairments in both hands. In fact, the threshold of tactile discrimination of the less-affected hand is higher (worse acuity) than the dominant hand of typically developing (TD) children. The mean threshold of less-affected hand in USCP was 3.2 mm at baseline, while mean threshold in typically developing children varies from 1.37 (interquartile range = 1.12–1.83) at 6 years old to 1.10 (interquartile range = 0.71–1.28) at 16 years old (Bleyenheuft et al., 2006). Improvements were observed in both hands after training in our study. To our knowledge, this is the first study that reports tactile function can be improved in both hands after intensive bimanual training in children with USCP. This finding suggests that, when taking both hands into consideration, HABIT not only improves spatial-temporal control of the two hands (Hung et al., 2011), but also strengthens the tactile function in both hands. This may be an important consideration in choosing whether to do unimanual (CIMT) versus bimanual training.

4.4. HABIT and/or tactile training may improve tactile function involving reconstruction mental images of the stimuli

Despite the changes observed in the tactile spatial discrimination and the stereognosis, we did not find any significant changes in tactile pressure detection (SWM) and two-point discrimination. It has been previously shown that these sensory modalities are less affected than the others in children with CP (Arnould, Penta, & Thonnard, 2007; Cooper et al., 1995; Krumlinde-Sundholm & Eliasson, 2002; Van Heest, House, & Putnam, 1993). This may be related to the differential mechanisms associated with tactile spatial discrimination/stereognosis and pressure discrimination/TPD. Specifically, skills associated with spatial discrimination and stereognosis require a reconstruction of the mental image of the stimulus in the CNS, whereas skills associated with tactile pressure detection and two-point discrimination mainly require tactile input detection using non-spatial cues in the receptor population (Van Boven & Johnson, 1994). Thus neuroplastic changes after tactile training are more likely to be associated with changes in the mental representations of the tactile stimuli in the somatosensory cortex.

4.5. Correlation between baseline dexterity (JTTHF) and stereognosis

A moderate, albeit non-significant correlation between baseline JTTHF and stereognosis was found, whereby children with worse dexterity improved more in stereognosis in the more-affected hand after training. In two studies, researchers highlighted that stereognosis was correlated with manual ability in children with USCP (Arnould, Bleyenheuft, & Thonnard, 2014; Klingels et al., 2012). The association was possibly due to the requirement of active manipulation for allowing successful object identification. Children with the worst baseline dexterity are also more likely to change from a passive to an active exploration mode after training, due to their improvements in motor function. This probably accounts for improvement in stereognosis, whereas this association was not found between dexterity and tactile spatial discrimination in the more-affected hand (no active manipulation).

5. Limitations

Our study has a small sample size, which may render insufficient power to determine differences between groups, and the results may not be generalizable. However, our study is the first study to examine the effect of HABIT with or without tactile training in modifying tactile function in children with USCP, and no comparable studies using same measures were conducted in the literature. Therefore it is difficult to estimate an appropriate sample size a priori. We acknowledge that replication with a larger study is needed.

In this study, improvements in tactile function were measured following intensive bimanual training. Enriched environments may play a major role in the changes observed in both groups. Our study does not allow us to directly discriminate the effect of the amount of manipulative tasks (i.e. standardized HABIT) versus the manipulation of specific materials during the 8 h of specific tactile/control training as we collected our subjects from a sample of convenience. Future randomized controlled trials or cross-over studies should test the efficacy of these components in isolation and collect retention data several months after training.

We used a JVP dome-width of 4.5 mm as the widest bar width for testing based on a previous study (Bleyenheuft & Thonnard, 2011). We conservatively assigned 4.5 mm as the cap threshold for those who did not reach 70% of the correct rate when tested with 4.5 mm. In fact, 8 children in either group (16 out of 19 children, 84.2%) failed to reach 70% of the correct rate at baseline. Three out of 8 children in the HABIT + T group and 4 out of 8 children in the HABIT group improved from 4.5 mm to a measurable threshold after training. Conceivably, we could have underestimated improvements in these 7 children by capping their baseline threshold at 4.5 mm. Future studies using the GOT as an outcome measure may consider including a wider range of domes for allowing a more accurate threshold measure.

6. Conclusions

Our study is the first to report that tactile function can be enhanced in both hands after intensive bimanual training. Importantly, the improvements in outcome did not differ between the HABIT+T and the HABIT groups, suggesting the environment is driving the changes rather than specific tactile training. Our study suggests a window of opportunity for modifying tactile function by providing an enriched environment. The take-home message of our study is that tactile impairments can be improved when the tactile input is structured in the environment.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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APPENDIX K

Research in Developmental Disabilities 43–44 (2015) 136–149

Contents lists available at ScienceDirect

Research in Developmental Disabilities

Capturing neuroplastic changes after bimanual intensive rehabilitation in children with unilateral spastic cerebral palsy: A combined DTI, TMS and fMRI pilot study

Yannick Bleyenheuft [a,*], Laurence Dricot [a], Nathalie Gilis [a], Hsing-Ching Kuo [b], Cécile Grandin [a,c], Corinne Bleyenheuft [a,d], Andrew M. Gordon [b], Kathleen M. Friel [b,e]

[a] Institute of Neuroscience, Université catholique de Louvain, Brussels, Belgium
[b] Department of Biobehavioral Sciences, Teachers College, Columbia University, New York, USA
[c] Radiology Service, Cliniques Universitaires Saint-Luc, Brussels, Belgium
[d] Physical Medicine and Rehabilitation Service, CHU Mont-Godinne, Yvoir, Belgium
[e] Burke-Cornell Medical Research Institute, White Plains, NY, USA

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DTI

ABSTRACT

Intensive rehabilitation interventions have been shown to be efficacious in improving upper extremity function in children with unilateral spastic cerebral palsy (USCP). These interventions are based on motor learning principles and engage children in skillful movements. Improvements in upper extremity function are believed to be associated with neuroplastic changes. However, these neuroplastic changes have not been well-described in children with cerebral palsy, likely due to challenges in defining and implementing the optimal tools and tests in children. Here we documented the implementation of three different neurological assessments (diffusion tensor imaging-DTI, transcranial magnetic stimulation-TMS and functional magnetic resonance imaging-fMRI) before and after a bimanual intensive treatment (HABIT-ILE) in two children with USCP presenting differential corticospinal developmental reorganization (ipsilateral and contralateral). The aim of the study was to capture neurophysiological changes and to document the complementary relationship between these measures, the potential measurable changes and the feasibility of applying these techniques in children with USCP.

Independent of cortical reorganization, both children showed increases in activation and size of the motor areas controlling the affected hand, quantified with different techniques. In addition, fMRI provided additional unexpected changes in the reward circuit while using the affected hand.

Abbreviations: 3T, 3 tesla; 6MWT, Six-Minute Walk Test; ACPC, anterior commissure posterior commissure; AHA, Assisting Hand Assessment; BA, Brodmann area; CED, Cambridge Electronic Design; CIMT, constraint-induced movement therapy; COPM, Canadian Occupational Performance Measure; CP, cerebral palsy; CST, corticospinal tract; CVA, cerebral vascular accident; DTI, diffusion tensor imaging; EMG, electromyography; FDI, first dorsal interosseus; fMRI, functional magnetic resonance imaging; GMFCS, gross motor function classification system; HABIT, Hand and arm bimanual intensive therapy; HABIT-ILE, hand and arm bimanual intensive therapy including lower extremity; JTTHF, Jebsen–Taylor test of hand function; MACS, Manual Ability Classification System; MEP, motor evoked potential; MT, motor threshold; PEDI, Pediatric Evaluation of Disability Inventory; TE, echo time; TFE, Turbo Field Echo; TMS, transcranial magnetic stimulation; TR, repetition time; UE, upper extremity; USCP, unilateral spastic cerebral palsy; WF, wrist flexors.

* Corresponding author at: Institute of Neuroscience, Université catholique de Louvain, Avenue Mounier 53, 1200 Brussels, Belgium.
E-mail addresses: Yannick.bleyenheuft@uclouvain.be, yannick.bleyenheuft@gmail.com (Y. Bleyenheuft).

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1. Introduction

Over the last decade, intensive rehabilitation interventions have been successfully developed for addressing upper extremity function of children with unilateral spastic cerebral palsy (USCP; e.g., Charles, Lavinder, & Gordon, 2001; Charles, Wolf, Schneider, & Gordon, 2006; de Brito Brandão, Gordon, & Mancini, 2012; DeLuca, Case-Smith, Stevenson, & Ramey, 2012; Deppe et al., 2013; Eliasson, Krumlinde-Sundholm, Shaw, & Wang, 2005; Fedrizzi et al., 2013; Gordon, Charles, & Steenbergen, 2006; Gordon, Schneider, Chinnan, & Charles, 2007; Gordon et al., 2008, 2011; Hoare et al., 2013; Sakzewski et al., 2011). These interventions are based on motor learning principles applied to intensive unimanual (constrained-induced movement therapy – CIMT) or bimanual (e.g., Hand and arm bimanual intensive therapy – HABIT) training. More recently, a combined bimanual and lower extremity stimulation has been proposed (Hand and arm bimanual intensive therapy including lower extremities: HABIT-ILE; Bleyenheuft & Gordon, 2014; Bleyenheuft, Arnould, Brandao, Bleyenheuft, & Gordon, 2014).

While principles of motor learning indicate that changes in motor skill are associated with neuroplastic changes in animal models (Kleim et al., 2004; Nudo, 2003), there is a lack of evidence for these neuroplastic changes in children with cerebral palsy. This lack of evidence is likely due to challenges in defining and implementing the optimal tools for measuring these changes.

Neuroplastic changes might be influenced by the basic corticospinal (CST) organization of the children with USCP. It is now well-established that children with USCP may present an atypical development of the descending motor pathways (Eyre et al., 2007; Staudt et al., 2004, for a review see Gordon, Bleyenheuft, & Steenbergen, 2013). From the original bilateral projections developed during embryogenesis, CST may either pursue a "classical" crossed (contralateral) development (with a natural pruning of the ipsilateral projections), or maintain and strengthen the ipsilateral projections from the unaffected hemisphere to the affected hand. The maintenance of such projections results in the affected hand being controlled either with projections from both hemispheres (bilateral organization), or with projections from the unaffected hemisphere exclusively (ipsilateral projections). It is generally accepted that children with ipsilateral reorganization present a more severely affected hand function, accompanied by mirror movements in upper extremities (Holmström et al., 2010; Staudt et al., 2004).

Since motor pathways can be reorganized in very different ways in children with USCP (Carr, Harrison, Evans, & Stephens, 1993; Guzzetta et al., 2007; Staudt et al., 2002, 2004), tracking of CST circuits with diffusion tensor imaging (DTI) is a key imaging approach to understand the connectivity of the motor system in children with USCP. DTI measurements of CST dysgenesis are strongly related to the manual dexterity of a child with USCP (Bleyenheuft, Grandin, Cosnard, Olivier, & Thonnard, 2007), but does not appear to be predictive of the potential for improvement in these children (Friel, Kuo, Carmel, Rowny, & Gordon, 2014). DTI is also hypothesized to be sensitive to changes after motor learning in children with USCP since changes in DTI have been detected after learning in different contexts (Imfeld, Oechslin, Meyer, Loenneker, & Jancke, 2009; Lazaridou et al., 2013; Li, Wang, Hu, Liang, & Chen, 2013).

Cortical changes that are linked to motor changes elicited by intensive therapy sessions can be captured by cortical mapping using single-pulse transcranial magnetic stimulation (TMS). TMS can assess the topography of the motor map (Vandermeeren, Davare, Duque, & Olivier, 2009) as well as the strength of the connections. Single-pulse TMS is also a safe procedure that is well-tolerated by children (Krishnan, Santos, Peterson, & Ehinger, 2015). Disadvantages of TMS include not being safe for use in people with seizure disorders and not being readily able to assess cortical areas that are not on the brain surface, such as the leg motor map. Functional magnetic resonance imaging (fMRI) is a key instrument to investigate plastic changes in the brain associated with motor learning (Ghilardi et al., 2000; Debas et al., 2010; Hardwick, Rottschy, Miall, & Eickhoff, 2013). However, application for tracking motor changes in children with spasticity may result in difficulties: First, the absolute necessity of keeping the head still while producing movements with upper extremities may be too difficult. Second, children with implanted metallic material could be automatically excluded due to the risks associated with the magnetic field.

The aim of this pilot study was to implement these three neurophysiological assessments (TMS, fMRI, DTI) in 2 children with USCP before and after a HABIT-ILE treatment. We aimed to capture neurophysiological changes and to document the complementary relationship between these measures, potential changes associated with therapy, and the feasibility of applying these techniques in children with USCP.

2. Material and methods

2.1. Participants

Two participants with different CST organization were included in this study.

Child 1 was a 6 year old girl presenting with a right hemiparesis consecutive to a left sylvian cerebral vascular accident (CVA) in perinatal period. The child was classified as level II on the Manual Ability Classification System (MACS, Eliasson et al., 2006) and presents a Gross Motor Function Classification System, (GMFCS, Palisano et al., 1997) of level I.

Child 2 was a 9 year old girl presenting with a left hemiparesis. The cerebral palsy is consecutive to a right sylvian CVA. Her MACS is classified as level II and her GMFCS as level I.

2.2. Intensive training

Participants were engaged in a HABIT-ILE treatment 9 h/day during 10 consecutive days (total amount of 90 hours) during school holidays. HABIT-ILE uses structured bimanual tasks that require simultaneous control and coordination of UE and LE movements (Bleyenheuft & Gordon, 2014; Bleyenheuft et al., 2014).

2.3. Functional/behavioral assessments

Before and after the HABIT-ILE session, upper and lower extremity changes were assessed.

On both hands, dexterity was measured with the Jebsen–Taylor test of hand function (JTTHF, Jebsen et al., 1969), pinch force with a pinch dynamometer (Mathiowetz, Wiemer, & Federman, 1986), Stereognosis with the Cooper test (Cooper, Majnemer, Rosenblatt, & Birnbaum, 1995). Manual ability was measured with (1) the ABILHAND-kids (logit) (Arnould, Penta, Renders, & Thonnard, 2004), (2) the Assisting Hand Assessment (AHA; Krumlinde-Sundholm & Eliasson, 2003; Krumlinde-Sundholm, Holmefur, Kottorp, & Eliasson, 2007) and (3) the Pediatric Evaluation of Disability Inventory (PEDI, Haley, Coster, Ludlow, Haltiwanger, & Andrellos, 1992; McCarthy et al., 2002; Vos-Vromans, Ketelaar and Gorter, 2005). Lower extremity assessment included the 6 minute walk test (6MWT), Enright (2003); Geiger et al. (2007); Li et al. (2007) and the ABILOCO-kids questionnaire (Caty, Arnould, Thonnard, & Lejeune, 2008). The Canadian Occupational Performance Measure (COPM) was used to measure changes in the functional goals (Carswell et al., 2004; Law, McColl, Opzoomer, Polatajko, & Pollock, 1990; Verkerk, Wolf, Louwers, Meester-Delver, & Nollet, 2006). More details are provided on these assessments in the supplementary material.

2.4. TMS motor mapping

Single-pulse TMS mapping of the motor cortex was performed before and after ninety hours of HABIT-ILE. The details of setup (Noirhomme et al., 2004) and MEP recording are available in the supplementary material.

In short, TMS pulses were delivered at a frequency of less than 0.1 Hz, with the coil being moved along the head in 2 cm increments between each pulse. Pulses were delivered until either a motor evoked potential (MEP) of the affected upper extremity (UE) was found, or until the hemisphere had been thoroughly mapped in 2 cm increments (<80% maximum stimulator output).

If an MEP of the affected FDI was found, the coil was held at that spot ("hotspot") and the stimulator output was lowered until an MEP could no longer be elicited. The stimulator output at which an MEP of the affected FDI could be elicited from six of ten sequential pulses delivered at a frequency of 0.1 Hz was defined as the motor threshold (MT).

TMS mapping was subsequently performed by delivering single TMS pulses at a stimulus intensity of 110% the MT for the affected FDI. Three to six TMS pulses were delivered to each grid point. Both hemispheres were mapped.

After rehabilitation, TMS mapping was repeated using the same procedures as before rehabilitation. The MT of the affected FDI was found as described above. Three to six TMS pulses were delivered to each grid point on the skull cap, at a stimulus intensity of 110% the pre-rehabilitation MT of the affected FDI.

2.4.1. Data analyses

EMG data were exported from Signal into MATLAB (Mathworks). A MATLAB script was written to identify the onset time, offset time, and magnitude of the MEP for each muscle. Onset and offset time were determined relative to the time of TMS stimulation. The latency of MEP onset was defined as the duration between TMS stimulation and onset of the MEP. The strength of the MEP was defined as the peak-to-peak amplitude of the MEP.

For each grid point in the map, the average MEP strength and latency was calculated. Each grid point was categorized as a digit, wrist, or duel digit-wrist response site by the presence or absence of an MEP in the FDI or WF at that site. The number of digit and wrist sites were tallied. Differences in the number and MEP amplitude of digit and wrist responses before and after rehabilitation were calculated.

Similarly to TMS, children were assessed with imaging before and after intensive training. Imaging consisted of conventional MRI; functional magnetic resonance imaging (fMRI) and diffusion tensor images (DTI) performed at 3T (Achieva, Philips Healthcare, Eindhoven, The Netherlands.

2.5. fMRI

Functional MR images of brain activity were also collected using the same 3T head scanner (with repeated single-shot gradient-echo echo-planar imaging: echo time (TE) = 50 ms, flip angle (FA) = 90°, Inplane Resolution = 1.964 mm × 1.964 mm, slice order descending and interleaved, slice thickness = 3 mm). Repetition time (TR) was 2250 ms, 38 slices (the whole brain is scanned 148 times per run, 6 times per condition per run).

2.5.1. fMRI paradigm

During the fMRI experiments, participants performed 3 runs of 5 min 33 s duration each sequence. There were 3 × 6 oral instructions (1500 ms) provided ("left hand", "right hand", "both hands") per run. After the verbal command, the children

Table 1
Description of behavioral/functional changes.

	Child 1		Child 2	
	Pre	Post	Pre	Post
JTHF MA (s)	1080	915	275	84
JTHF LA (s)	49	48	37	36
Key pinch MA (kg)	1.5	2.83	1.58	2.08
Key pinch LA (kg)	3.66	3.83	5.50	4.83
ABILHAND-Kids (logits)	0.34	2.15	2.89	6.68
PEDI (self-care)	38	58	51	63
AHA (AHA-units)	43	42	66	67
Stereognisis MA (/10)	2	5	3	4
Stereognisis LA (/10)	9	10	10	10
6MWT (m)	390	480	374	520
Abiloco-kids (logits)	3.60	5.90	4.31	5.92
COPM performance (/10)	4.2	7.9	5.7	8.2
COPM satisfaction (/10)	7.8	8.8	5.0	8.8

JTHF: Jebsen–Taylor Test of Hand Function; MA: more affected; LA: less affected; PEDI: pediatric evaluation of disability inventory; COPM: Canadian Occupational Performance Measure.

performed the movement requested: grip and lift a cube (2 cm edge) with left hand, with right hand or grip two cubes simultaneously (one cube in each hand). Pretests showed that after 8 s all our participants finished the movements. The duration between trials was then chosen to be 16.5 s to ensure a comfortable baseline. During all experiments, one experienced physical therapist inside the scanning room checked all the movements and pressed four button boxes to record information about them (start and end of the left and right hand movement). This information was used to create the multiple regression model (General Linear Model; GLM) for the fMRI data analysis.

2.5.2. Data analysis: fMRI runs

The fMRI signal in the various conditions was compared using BrainVoyager QX (Version 2.3, Brain Innovation, Maastricht, The Netherlands). Prior to analysis, the functional data sets were subjected to a series of preprocessing operations. Preprocessing consisted of a linear trend removal for excluding scanner-related signal, a temporal high-pass filtering applied to remove temporal frequencies lower than 3 cycles per run, and a correction for small interscan head movements by a rigid body algorithm rotating and translating each functional volume in 3D space. Data were corrected for the difference between the scan times of the different slices. Data was smoothed in the spatial domain (Gaussian filter: Full Width at Half Maximum = 5 mm). Subsequently, the functional data were analyzed using a multiple regression model (General Linear Model; GLM) consisting of predictors, which corresponded to the particular experimental conditions of each experiment: movement of right hand condition (RC), left hand (LC) and both hands (BC) for the two sessions separately (6 conditions in all, RC_{s1} and RC_{s2} for session 1 and 2 respectively, etc.).[1] The predictor time courses used were computed on the basis of a linear model of the relation between neural activity and hemodynamic response (Boynton, Engel, Glover, & Heeger, 1996). The statistical maps computed were overlaid to the 3D T1-weighted scans in the AC–PC native space. All coregistrations were corrected manually and the corrections of the movement were optimized (sinc interpolation).

2.5.3. fMRI statistical analyses and contrasts of interest

For each child, we performed first whole brain analysis using the following general contrast: $[(RC_{s1} + RC_{s2} + LC_{s1} + LC_{s2} + BC_{s1} + BC_{s2}) > \text{rest}]$ aimed at isolating the areas responding to hand movements comparing to rest and found the sensori-motor cortex. Further, we computed ROI analysis in each area found with this contrast. Follow up contrasts $[(RC_{s2} - RC_{s1}), (LC_{s2} - LC_{s1}), (BC_{s2} - BC_{s1}), (LC_{s2} + RC_{s2} + BC_{s2}) - (LC_{s1} + RC_{s1} + BC_{s1})$ or $(RC_{s2} + BC_{s2}) - (RC_{s1} + BC_{s1}), (LC_{s2} - LC_{s1}) - (RC_{s2} - RC_{s1})$ or $(BC_{s2} - BC_{s1}) - (RC_{s2} - RC_{s1})$[2]] were calculated at the peak of activation. These values were used to compare activation before and after the intensive training.

2.6. DTI

DTI images were acquired at the end of each fMRI session (details of the sequence provided in supplementary material). In order to determine FA of the CSTs, spheres of 3 mm (123 voxels) were created symmetrically in both tracts, their middle centered on the CS fibers at the level of the mid pons, as visualized in a transversal plane passing through the middle cerebellar peduncle (see Fig. 3). The number of voxels presenting a main z direction was then counted, and the value of the FA

[1] We also created a second GLM where the movement parameters were used as confounds. However, it was a bit problematic because head movements were strongly related to hand movements required and we decided to use it only to explore potential areas outside sensori-motor cortex.
[2] For the child 2 the conditions LC have not been used.

in z direction reported for the 123 voxels in each sphere. A determinist tracking was then made from the spheres. Only the fibers with FA > 0.15 and a deviation angle <50° were considered for this tracking.

3. Results

3.1. Child 1

Child 1 showed improvements in upper and lower extremities in all functional assessments except the AHA (see Table 1).

3.1.1. TMS

For child 1, the motor map of the affected hand was found in the contralateral, affected hemisphere.

After 90 hours of HABIT-ILE, substantial changes in the responsiveness and excitability of motor maps were found. In child 1 (contralateral map of the affected hand) there was a 50% increase in the number of digit and wrist responsive sites after rehabilitation. The average amplitude of the MEP of the affected FDI increased from 311.47 μV to 458.73 μV (47.3% increase). The average amplitude of the MEP of the affected WF increased from 262.73 μV to 428.37 μV (63.1% increase).

3.1.2. fMRI

3.1.2.1. Behavioral results. During the first fMRI assessment, over the 18 trials programmed with the right (affected) hand across the 3 runs, child 1 succeeded in only 11 trials. With the less-affected hand, 16 out of 18 trials were correctly performed. Finally, 12 out 18 bimanual trials were successful during this pre-training assessment.

At post-training assessment, with the same paradigm, all trials but 2 were completed with the right/more-affected hand (16 out of 18). All left hand (18/18) and bimanual trials (18/18) were correctly completed.

3.1.2.2. fMRI results. At q(False Discovery Rate) < 0.05 (t = 3.54) and cluster size >20 mm^3, we reported 8 clusters for the contrast [$(RC_{s1} + RC_{s2} + LC_{s1} + LC_{s2} + BC_{s1} + BC_{s2}) >$ rest] (see Table 2 and Fig. 1). We found clusters in the primary motor cortex (M1), primary sensory cortex (S1), the putamen and premotor cortex on the right hemisphere. On the right side we also found the left inferior and the superior frontal gyrus to be activated. The largest region was found in the supplementary motor area (SMA). On the left, we only discovered a cluster in the motor/premotor area.

Then, we computed ROI analysis in each area found. For the BC, we found an increase between before and after treatment in almost all clusters (all but right BA6 and one of the two cluster found in right M1). For the LC, the activation was significantly increased in the left motor/premotor cortex and in the SMA. Finally for the RC, we discovered an increase of activation in the right BA44. In the right BA6, the activity for the right hand is almost significantly higher in the first session than in the second (p = 0.063).

We did also the contrast $(LC_{s2} + RC_{s2} + BC_{s2}) - (LC_{s1} + RC_{s1} + BC_{s1})$ in a whole brain analysis with movement parameters used as confounds of non interest in the general linear model (see remark 1). At $p < 0.0025$ (t = 3.032) and cluster size >20 mm^3, we found 5 clusters (see Table 3 and Fig. 2). Globally, the differences between before and after treatment were similar for all conditions showing an increase of activation.

3.1.3. DTI

Over the 123 voxels of the left sphere, this child presented 94 voxels with a preferential z direction. Ninety-three of these 94 voxels presented a FA higher than 0.3 (see Fig. 3D).

Over the 123 voxels of the right sphere, 117 had a main direction in z. 116 out of 117 of these fibers presented a FA higher than 0.3 (see Fig. 3C).

Table 2
Child 1 – Contrast: $(RC_{s1} + RC_{s2} + LC_{s1} + LC_{s2} + BC_{s1} + BC_{s2}) >$ rest.

Clusters*	Size (mm^3)	t – Peak of activation	$RC_{s2} - RC_{s1}$	$LC_{s2} - LC_{s1}$	$BC_{s2} - BC_{s1}$	$(RC_{s2} + LC_{s2} + BC_{s2}) - (RC_{s1} + LC_{s1} + BC_{s1})$	$(RC_{s2} - RC_{s1}) - (LC_{s2} - LC_{s1})$
Right precentral gyrus, BA4	197	4.432	0.987	0.562	0.003	0.122	0.609
Right postcentral and precentral gyri, BA3 and BA4	1102	4.858	0.278	0.858	0.288	0.398	0.247
Left precentral gyrus, BA4–6	20	3.717	0.289	0.025	0.035	0.021	0.285
SMA, BA6	1200	4.211	0.574	0.014	0.002	0.009	0.086
Right superior frontal gyrus, BA6	33	3.793	(−)0.063	0.437	0.298	0.979	(−)0.016
Right superior frontal gyrus, BA10	106	4.187	0.932	0.218	0.023	0.125	0.299
Right inferior frontal gyrus, BA44	528	4.603	0.041	0.508	0.000	0.007	0.201
Right putamen	410	4.707	0.163652	0.254208	0.005	0.023	0.808

* Found at q(FDR) < 0.05 and cluster size >20 mm^3, columns 2–5 presenting p-values of a t-test. In case a contrast provided a decrease in activation, the p-value is preceded by a (−) sign.

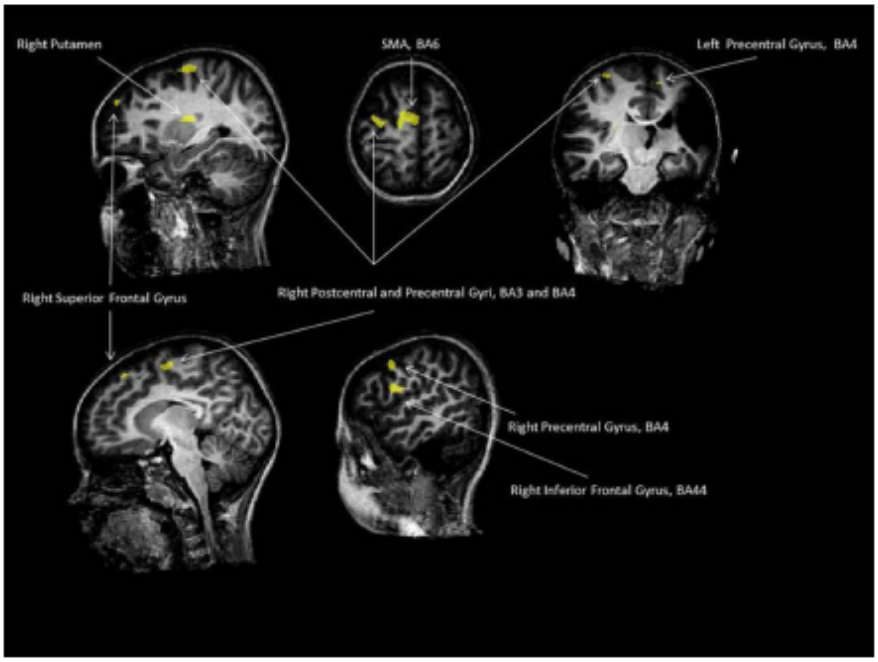

Fig. 1. Child 1/at q(False Discovery Rate) < 0.05 ($t = 3.54$) and cluster size > 20 mm^3, the clusters for the contrast [($RC_{x1} + RC_{x2} + LC_{x1} + LC_{\varnothing} + BC_{x1} + BC_{x2}$) > rest].

Tracking from left and right spheres allowed the delineation of the respective left and right CST at the first iteration (see Fig. 3A).

Unfortunately, as this child's pre-camp DTI was affected by movement artifacts that could not be corrected for, it was not possible to compare pre and post intensive rehabilitation DTI.

3.2. Child 2

Child 2 showed improvements in all functional assessments (see Table 1).

3.2.1. TMS

For child 2, no TMS-evoked responses could be found in the affected hemisphere, even at a stimulus intensity of 90% maximum stimulator output. However, when the contralesional (unaffected) hemisphere was stimulated, MEPs in both hands were evoked showing that the affected hand was controlled via ipsilateral connections from the unaffected motor cortex.

3.2.1.1. Changes in motor maps after rehabilitation. In this map (ipsilateral map of the affected hand), there was a 233% increase in the number of digit and wrist responsive sites after rehabilitation. The average amplitude of the MEP of the affected FDI increased from 482.04 μV to 2888.83 μV (499.3% increase). The average amplitude of the MEP of the affected WF increased from 309.04 μV to 3475.34 μV (1024.6% increase).

Table 3
Child 1 – Contrast: ($LC_{x2} + RC_{x2} + BC_{\varnothing}$) – ($LC_{x1} + RC_{x1} + BC_{x1}$).

Clusters*	Size (mm^3)	t – Peak of activation	$RC_{x2} - RC_{x1}$	$LC_{x2} - LC_{x1}$	$BC_{x2} - BC_{x1}$	($RC_{x2} + LC_{x2} + BC_{\varnothing}$) – ($RC_{x1} + LC_{x1} + BC_{x1}$)	($RC_{\varnothing} - RC_{x1}$) – ($LC_{\varnothing} - LC_{x1}$)
Right orbitofrontal cortex, BA 11	244	3.536	0.019	0.002	0.001	0.000	0.548
Right orbitofrontal cortex, BA 11	125	3.437	0.086	0.000	0.001	0.000	0.101
Right cingulate gyrus, BA32	261	3.829	0.015	0.000	0.002	0.000	0.315
Left superior frontal gyrus, BA6	188	3.504	0.001	0.007	0.006	0.000	0.484
Left superior frontal gyrus, BA6	117	3.279	0.000	0.005	0.31	0.000	0.276

* Found at $p < 0.0025$ and cluster size >20 mm^3, columns 2–5 presenting p-values of a t-test. In case a contrast provided a decrease in activation, the p-value is preceded by a (–) sign.

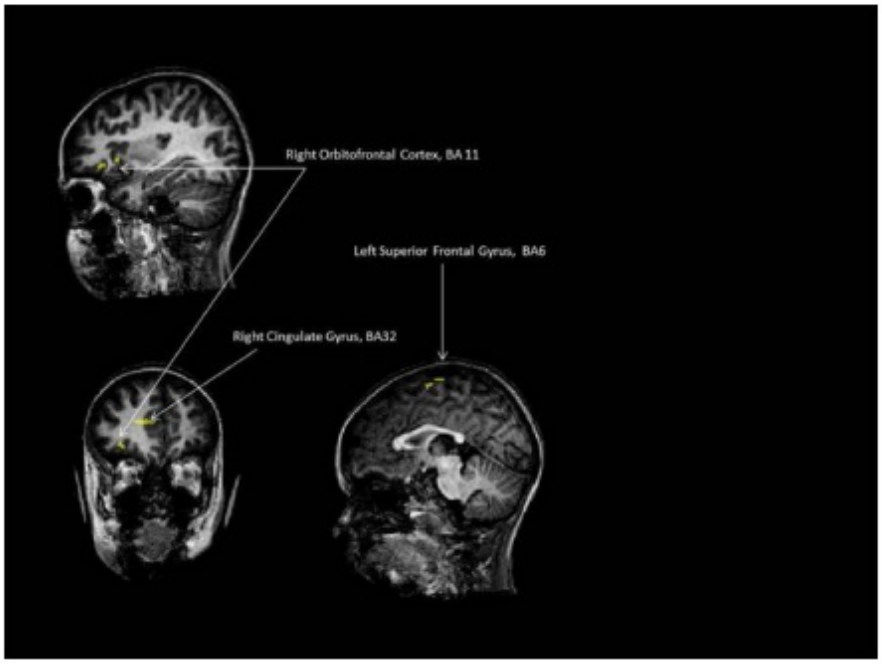

Fig. 2. Child 1/clusters obtained with the contrast $(LC_{s2} + RC_{s2} + BC_{s2}) - (LC_{s1} + RC_{s1} + BC_{s1})$ in a whole brain analysis with movement parameters used as confounds of non interest in the general linear model, at $p < 0.0025$ ($t = 3.032$) and cluster size > 20 mm³.

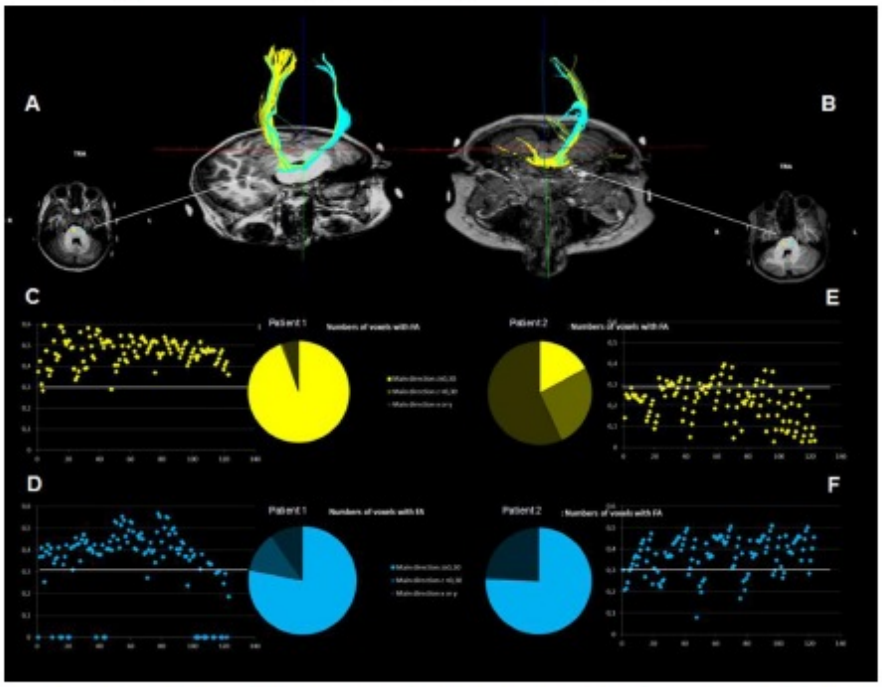

Fig. 3. In order to determine FA of the CSTs, spheres of 3 mm (123 voxels) were created symmetrically in both tracts, their middle centered on the CS fibers as visualized in a transversal plane passing through the middle cerebellar pedunde (see picture in transversal plane). The number of voxels presenting a main z direction was then counted, and the value of the FA in z direction reported for the 123 voxels in each sphere. A deterministic tracking was then made from the spheres. Only the fibers with FA > 0.15 were considered for this tracking and a deviation angular of less than 50° was required. The upper panels (A and B) represent the tracking for children 1 and 2 respectively. The lower panel delineates the fractional anisotropy of the fibers for right (yellow) and left (blue) CST respectively in both children. Fig. 4a: Child 2/at same q.

3.2.2. fMRI

3.2.2.1. Behavioral results. During the first assessment, child 2 succeeded in all trials (18/18) programmed for the right (less-affected) hand across the 3 runs. With the more-affected hand, only 7 out of 18 trials were correctly performed (the child systematically used the opposite hand to help during the task). Finally, 14 out 18 bimanual trials were successful during this pre-training assessment.

At post-training assessment, with the same paradigm, all trials were completed separately with the more-affected hand (left 18/18) and less-affected hand (right 18/18). In bimanual trials 16 out of 18 trials were correctly completed.

3.2.2.2. fMRI results. At same q(FDR) and same cluster size than child 1, we reported 35 clusters for the contrast [$(RC_{s1} + RC_{s2} + LC_{s1} + LC_{s2} + BC_{s1} + BC_{s2}) >$ rest] but with large overlap (73,653 voxels activated in total; see Fig. 4a). Consequently we increased the statistical threshold to p(Bonf) < 0.001 and cluster size $> 20\,mm^3$ allowing a separation between the sensori and motor regions. We defined 14 clusters (see Table 4 and Fig. 4b). S1 was found on both hemispheres contrary to M1 and premotor cortex only found on the left (healthy) hemisphere of the brain. SMA, left inferior and superior parietal lobules were also activated.

Further, we computed ROI analysis in each area found with this contrast. Due to the fact that this child did not succeed during the first session of the more-affected (left) hand task without help of the less-affected hand (for 11 on 18 trials), we decided to focus analysis on the right-hand and two-handed conditions. For the BC we found difference between activity after and before treatment in right S1, left M1 and in the superior parietal lobule (see Table 4). For the RC, a difference was observed in this last cluster only. No interaction was observed in any cluster.

Contrary to the first child, we didn't find a difference with the contrast [$(LC_{s2} + RC_{s2} + BC_{s2}) - (LC_{s1} + RC_{s1} + BC_{s1})$] at $p < 0.0025$ in a whole brain analysis with movement parameters used as confounds of non interest (see remark 1). Nevertheless we found 2 clusters (in BA11 and BA9) at $p < 0.05$ ($t = 1.96$) and cluster size $> 50\,mm^3$ (see Table 5 and Fig. 5). The difference between before and after the treatment was mainly driven by the BC for the two clusters (without consideration of the LC) (Table 5).

3.2.3. DTI results

Over the 123 voxels of the right sphere, this child had 53 voxels with a preferential z direction. Twenty-one of these 53 voxels had a FA higher than 0.3 (see Fig. 3E).

Over the 123 voxels of the left sphere, 111 had a main direction in z. Ninety six out of 111 of these voxels had a FA higher than 0.3 (see Fig. 3F).

A mean FA was calculated in the z direction on all the voxels of the sphere. Before intensive training, the mean FA was of 0.385 in the z direction in the left sphere and of 0.345 in the right sphere. This value increased after intensive training in the left sphere (to 0.403) while it did not increase in the right sphere (0.245).

Tracking from the left sphere allowed the delineation of CST on the left side at the first iteration (see Fig. 3B). Tracking on the basis of the right sphere also allowed to track a projection to the left CST. Whatever the number of iterations of the program to track the fibers, no delineation of a CST on the right side was possible, as represented in the tracking image (Fig. 3B).

4. Discussion

Two children with USCP, presenting different CST post-lesion reorganization (child 1: contralateral connectivity, child 2: ipsilateral connectivity) were assessed before and after a 90 h HABIT-ILE intervention using three different neurophysiological assessments: DTI, fMRI, and TMS.

Child 1 had a motor map of the affected hand in the contralateral (affected) hemisphere, as attested by TMS and DTI investigations. DTI showed that while the CST was partly damaged, the remaining fibers were of good quality, allowing connectivity to the affected upper extremity muscles. TMS investigations showed an increase of both affected hand cortical map and cortical excitability of this map. fMRI presented changes consistent with TMS, but further allowed the demonstration of changes in sensory and associative areas.

Child 2 had a motor map of the affected hand in the unaffected motor cortex solely, as shown by the TMS cortical mapping and by the DTI investigations. Thus an ipsilateral projection was present from the unaffected hemisphere that controlled the affected hand. Despite partial results (due to inability to achieve the task with the affected hand at the start), fMRI highlighted an increase of activation in left motor (BA4) and visuo-motor (BA7) areas. In the ipsilesional side, solely BA3 presented an increased activation after HABIT-ILE. After the intervention, child 2 could perform the task easily with the more affected hand.

Interestingly, child 2 was undergone CST reorganization (i.e., ipsilateral innervation) achieved better functional scores than child 1 (preserved contralateral organization). This is not consistent with previous studies suggesting a better function of the affected hand when the motor map is located in the affected hemisphere (Holmström et al., 2010; Staudt et al., 2004). Furthermore, contrary to what has been previously observed with CIMT (Kuhnke et al., 2008), both children improved functionally and in the motor cortical areas controlling their affected hand. The two cases studied in this paper suggest thus

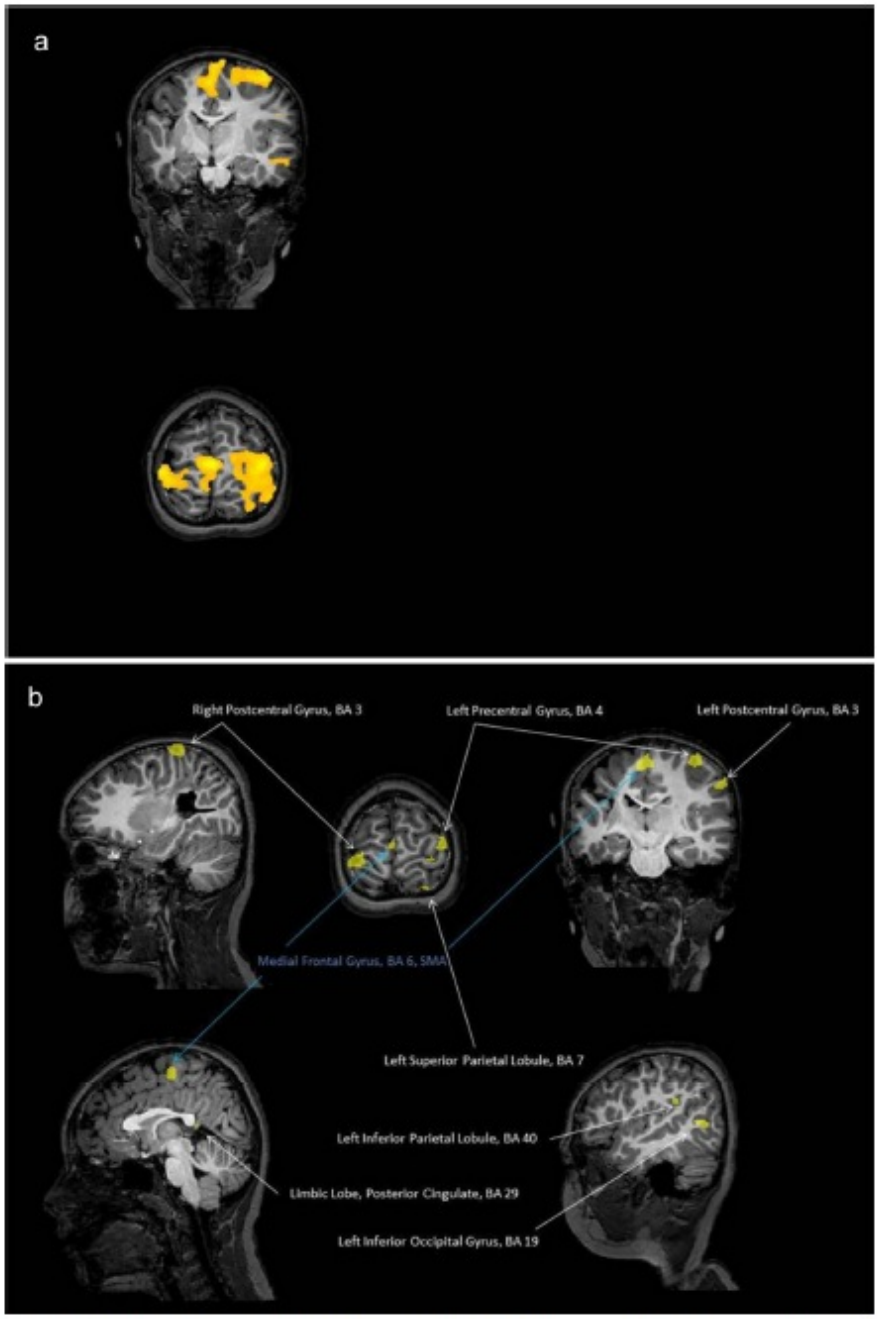

Fig. 4. (a) Child 2/at same *q*(FDR) and same cluster size than child 1, we report 35 clusters for the contrast [$(RC_{x1} + RC_{x2} + LC_{x1} + LC_{x2} + BC_{x1} + BC_{x2}) >$ rest] but with large overlappings (73,653 voxels activated in total). (b) Child 2/at statistical threshold to *p*(Bonf) < 0.001 and cluster size >20 mm^3, 14 clusters were defined for the contrast [$(RC_{x1} + RC_{x2} + LC_{x1} + LC_{x2} + BC_{x1} + BC_{x2}) >$ rest] (see Table 4).

that whatever the organization of the CST after the lesion, children encountered functional/behavioral changes after HABIT-ILE, and these improvements were mirrored by cortical changes.

The cortical organization of descending pathways could be identified both with DTI and TMS. Previous papers have shown that both conventional MRI and DTI could quantify indexes of symmetry estimating the CST dysgenesis, and that these

Table 4
Child 2 – Contrast: $(RC_{x1} + RC_{x2} + LC_{x1} + LC_{\varnothing} + BC_{x1} + BC_{x2}) >$ rest.

Clusters*	Size (mm^3)	t – Peak of activation	$RC_{x2} - RC_{x1}$	$BC_{x2} - BC_{x1}$	$(RC_{\varnothing} + BC_{x2}) - (RC_{x1} + BC_{x1})$	$(BC_{\varnothing} - BC_{x1}) - (RC_{\varnothing} - RC_{x1})$
Right postcentral gyrus, BA3	1635	9.116	0.287	0.747	0.407	0.492
Right postcentral gyrus, BA3	26	6.557	0.588	0.041	0.124	0.169
Left precentral gyrus, BA4	906	7.809	0.776	0.246	0.39	0.424
Left precentral gyrus, BA6	117	7.095	0.28	0.742	0.651	0.195
Left precentral gyrus, BA4	20	6.191	0.82	0.045	0.185	0.105
Left postcentral gyrus, BA3	271	7.287	0.229	0.117	0.099	0.745
Left postcentral gyrus, BA3	109	6.486	0.852	0.898	0.851	0.956
Medial frontal gyrus, Brodmann area 6 – SMA	905	8.769	0.664	0.273	0.363	0.547
Left superior parietal lobule, BA7	99	6.642	0.333	0.049	0.08	0.364
Left superior parietal lobule, BA7	140	6.433	0.007	0.040	0.005	0.541
Left inferior parietal lobule, BA40	67	6.262	0.847	0.334	0.49	0.48
Limbic lobe, cingulate gyrus, Brodmann area 24	75	6.308	0.189	0.053	0.053	0.573
Limbic lobe, posterior cingulate, Brodmann area 29	36	6.602	0.405	0.545	0.391	0.83
Left inferior occipital gyrus, BA19	262	6.731	0.399	0.397	0.317	1

* Found at p(Bonf) < 0.001 and cluster size > 20 mm^3.

indexes correlated with functional abilities of the paretic hand (Bleyenheuft et al., 2007; Bleyenheuft & Thonnard, 2010; Duque et al., 2003; Friel et al., 2014) but not change in hand function after intensive bimanual training (Friel et al., 2014). While conventional MRI systematically underestimates the CST dysgenesis, it might be considered in clinics as a good approximation of the consequence of the lesion on the CST (Bleyenheuft et al., 2007). However, an index of symmetry does not allow investigation of the quality of the fibers and the extent to which the motor pathways may have undergone reorganization post-lesion (Guzzetta et al., 2007; Staudt, 2010). As illustrated in our two cases, to innervate muscles of the more affected hand, the key may be not the quantity of CST fibers maintained, but the intrinsic quality of these fibers. To investigate quality of the fibers, a DTI analysis with a delineation of CST fibers can be useful. The interest of such information resides in the potential differential treatment that could be proposed as a function of the cortical organization.

In this pilot study, all assessments were performed pre- and post-intensive training. While comparison of DTI parameters could be done only in one child, due to technical reasons, some changes in FA were observed. Recent papers in the literature have pointed the potential changes in DTI parameters, interpreted as spontaneous recovery (Jang & Yeo, 2014) or due to intensive training (Kwon et al., 2014). However in the absence of data describing measurement variability between two measurements of DTI performed in the same subject at different time points (without treatment), we are cautious regarding this observation.

When DTI cannot be performed (due to the age of the child, inability to stay still, or some exclusion criteria) TMS can provide a good alternative to determine CST organization. While it should be verified in a larger sample, the matching between CST reconstruction with DTI and TMS data was very consistent in these children. More specific than the cortical organization, TMS allowed investigating changes in cortical excitability. In both children, the motor areas underwent a huge increase in cortical excitability. In the child with maintained crossed descending pathways, both the affected and unaffected motor areas controlling the hands had an increase of 50–60% in the average amplitude of the MEP. For the child with both motor areas located in the unaffected hemisphere, the average amplitude of the MEP was greatly increased in the area dedicated to the more affected (increase of 499%) and to the less affected hand (increase of 1025%).

TMS also allowed investigation of the number of digit and wrist responsive sites for the affected hand after rehabilitation. Paralleling the results of cortical excitability, the number of responsive sites increased after intervention, showing an expansion of the motor map related to the affected hand in both children. However, once again, this motor map extension was larger (233%) in the ipsilateral map of the affected hand of child 2, than in the contralateral map of the affected hand in child 1 (50% increase). This could be linked to a different potential of cortical changes depending on CS organization. After CIMT the difference highlighted in cortical modulation was already described as different (Juenger et al., 2013). However CIMT showed an increase of activity in motor areas controlling the affected hand when this area is contralateral to the

Table 5
Child 2 – Contrast: $(LC_{x2} + RC_{x2} + BC_{\varnothing}) - (LC_{x1} + RC_{x1} + BC_{x1})$.

Clusters*	Size (mm^3)	t – Peak of activation	$RC_{x2} - RC_{x1}$	$BC_{\varnothing} - BC_{x1}$	$(RC_{x2} + BC_{x2}) - (RC_{x1} + BC_{x1})$	$(BC_{\varnothing} - BC_{x1}) - (RC_{\varnothing} - RC_{x1})$
Right superior frontal gyrus, BA9	51	2.63	0.235	0.011	0.026	0.212
Orbitofrontal cortex, BA 11	53	2.33	0.261	0.000	0.004	0.016

* Found at $p < 0.05$ and cluster size > 50 mm^3.

Fig. 5. Child 2/the contrast $[(LC_{x2} + RC_{x2} + BC_{x2}) - (LC_{x1} + RC_{x1} + BC_{x1})]$ at $p < 0.0025$ in a whole brain analysis with movement parameters used as confounds of non interest.

affected hand and a decrease of activation when this area is ipsilateral to the affected hand (Juenger et al., 2013). TMS investigations also showed a decreased cortical excitability after CIMT in this last group. The authors suggested that this was probably linked to an inhibitory S1–M1 interaction: the activity in S1 (maintained in the impaired hemisphere) induced by the exercise would have an inhibitory action on the synaptic activity of M1 through inhibitory interhemispheric pathways. However, children improved in both cases. The authors suggested that the decrease of activation might correspond to a better refinement of the motor cortex. The contrast of the results we have in child 2 for bimanual training suggest a highly different neuromodulation in children with ipsilateral organization when treated with unimanual or bimanual intensive therapy. While in both interventions S1 of the affected hand (preserved in the ipsilesional hemisphere) presented more excitability after the treatment and a behavioral improvement was observed, the consequences of the treatments on ipsilateral motor maps were totally opposite. This raises the importance of comparing in ipsilateral children the amount of functional and cortical direct and long term benefit of CIMT versus bimanual intensive therapies.

Functional magnetic resonance imaging can be of great help in this context since it allows investigating the changes in other fields than the motor response. Especially of interest here are the sensory areas related to the affected hand and the associative areas and their neuromodulation after a bimanual intensive training. While the child 1 with a preserved contralateral organization did increase cortical activation after intervention for nearly all clusters evidenced at the first analysis in the "both hands" condition, both left and right cortical changes were separately observed. Interestingly, the motor changes of this child were consistent with TMS observation both for the affected and non-affected side, showing increases in activation. Changes were also noticed in the SMA for "both hands" and "left hand" use, as well as in BA10 (both hands condition), well known for being involved in executive functions and memory recall (Costa et al., 2011). In child 2, with "ipsilateral" reorganization, all changes were observed on the unaffected hemisphere, except for the modulation in BA3, with an increased activation of sensory area preserved in the lesional hemisphere. An increase in the M1 (BA4) of the unaffected hemisphere was observed consistently with TMS changes for the both hands condition. BA7, known to be related to visuo-motor coordination and especially to correction of the movement when grasping an object (Vingerhoets, 2014), presented also more activation after the intensive rehabilitation treatment in the "both hands" and "right hand" conditions. Unfortunately, the left hand condition could not be analyzed in this child since at pre-assessment, despite training, she was systematically using the less affected hand to give the object to the more affected and to take it out of the hand. This raises the one important problem of performing motor fMRI with children with CP: some of them have difficulty performing isolated movements with the hands, and therefore compromise the interpretability of fMRI data. In the future, devices allowing to grip and move objects without need of visual feedback and without risks of drop should be specifically designed.

If movements parameters are used as confounds of non-interest, as we presented in Tables 3 and 5 for children 1 and 2 respectively, some other higher level changes might be highlighted. In our 2 children, changes in activation were detected under these corrections when using both hands in BA11 added for child 1 to increased activations in BA32 and BA6. Child 2 showed in addition changes in BA9. All these areas are known to be connected to the concept of reward. BA11 has been described as having an important role in the reward of mediated behaviors, notably in psychiatry (Goldapple et al., 2004; Rogers et al., 1999). Furthermore its activation has been described as inversely proportional to the demanding aspect of the task (Pochon et al., 2002) suggesting here that the task being less demanding after the intensive training, more activation could be dedicated to emotional information. BA32 is described in the literature as activated by reward and reward anticipation (Knutson & Cooper, 2005; Pochon et al., 2002; for review see Haber & Knutson, 2010), especially in "self-related (personally relevant contextual value) processing along an affective dimension" (Lane et al., 1998; Posner & Rothbart, 1998). Finally BA9 has also been described as part of reward process, notably when some tasks are associated with high monetary reward (Pochon et al., 2002). Altogether these results suggest that after the intensive HABIT-ILE treatment, children felt rewarded when performing the task. Though this change in the reward circuit could be attributed either to the increased success performing the task or to the positive reinforcement implemented during the intensive intervention, this effect is of great interest because this change in the reward circuit activation may promote long-term use of both hands in everyday life activities. This unexpected finding illustrates the additional interest of fMRI for investigating changes in higher (and sometimes non-motor) level that may occur during intensive rehabilitation processes using motor learning.

In this pilot study, both imaging and TMS were applied in school age children who were awake and alert. This creates some difficulties specific to children that has to be considered for implementing studies on cortical changes. For imaging, the three main challenges are anxiety of the children, movement of the head and ability to perform the motor task. From 6 years old, with careful explanation to the children about what to expect, a visit to the MRI facility before participating, a short video to introduce children to the anticipated noise and training outside of the MRI prior to the actual imaging, children tolerated assessments without specific complaints. The task (picking a block) was too difficult to be performed by one child in the MRI during the first examination, resulting additionally in some head movements during the task. In our subsequent work a task was designed where objects were placed in the hand of the children, which solved the problem of inability to perform the task and drastically reduced associated head movements. Therefore the choice of the task and testing it prior to imaging appears to be crucial to obtain data of good quality. However, for younger children, the MRI and especially the fMRI is not likely to be easily performed. Therefore TMS might be more feasible. In our 2 children, no adverse events were reported during TMS. The main difficulty for the children was the length of the exam that had to be interrupted either for going to the toilet or drinking. Provided that no prior seizure history is reported, TMS thus would be easier to apply in younger and more affected children who have difficulty performing precise movements in the fMRI without associated head movements.

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Appendix A. Supplementary data

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