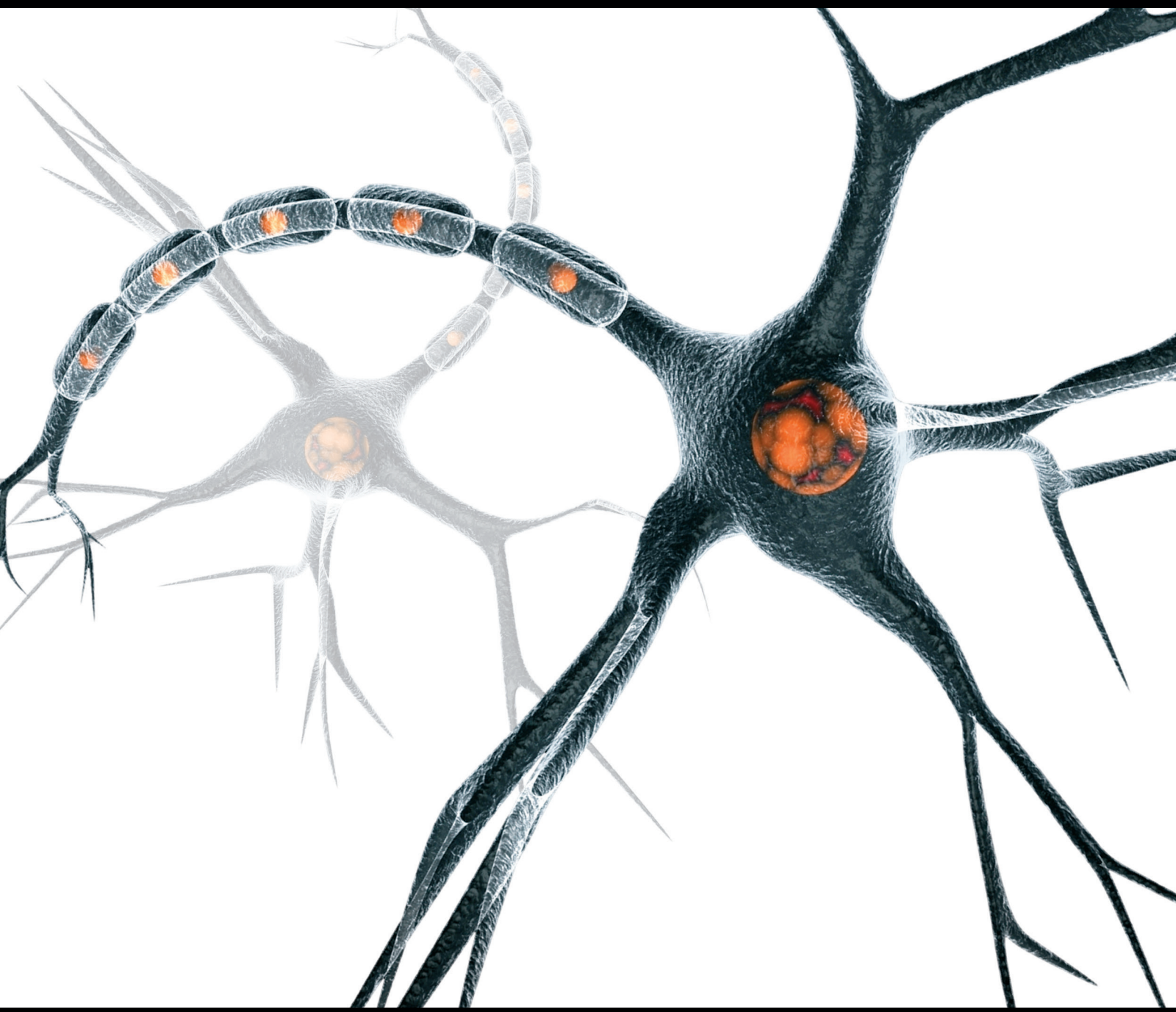
Neural Plasticity

Neural Plasticity after Congenital Brain Lesions

Lead Guest Editor: Simona Fiori
Guest Editors: Andrea Guzzetta, Martin Staudt, and Roslyn N. Boyd



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Editorial Board

Contents

Neural Plasticity after Congenital Brain Lesions
Simona Fiori, Martin Staudt, Roslyn N. Boyd, and Andrea Guzzetta
Editorial (2 pages), Article ID 9154282, Volume 2019 (2019)

Rodent Models of Developmental Ischemic Stroke for Translational Research: Strengths and Weaknesses
Mariangela Gennaro, Alessandro Mattiello, and Tommaso Pizzorusso
Review Article (16 pages), Article ID 5089321, Volume 2019 (2019)

Transcranial Direct Current Stimulation (tDCS) in Unilateral Cerebral Palsy: A Pilot Study of Motor Effect
Emanuela Inguaggiato, Nadia Bolognini, Simona Fiori, and Giovanni Cioni
Clinical Study (10 pages), Article ID 2184398, Volume 2019 (2019)

Impairments of Visuospatial Attention in Children with Unilateral Spastic Cerebral Palsy
Gaétan Ickx, Samar M. Hatem, Inmaculada Riquelme, Kathleen M. Friel, Camille Henne, Rodrigo Araneda, Andrew M. Gordon, and Yannick Bleyenheuft
Research Article (14 pages), Article ID 1435808, Volume 2018 (2019)

Potentials of Ultrahigh-Field MRI for the Study of Somatosensory Reorganization in Congenital Hemiplegia
Simona Fiori, Laura Biagi, Paolo Cecchi, Giovanni Cioni, Elena Beani, Michela Tosetti, Mirco Cosottini, and Andrea Guzzetta
Research Article (11 pages), Article ID 8472807, Volume 2018 (2019)

Time Course of Upper Limb Function in Children with Unilateral Cerebral Palsy: A Five-Year Follow-Up Study
Katrijn Klingels, Sarah Meyer, Lisa Mailleux, Cristina Simon-Martinez, Jasmine Hoskens, Elegast Monbaliu, Geert Verheyden, Geert Verbeke, Guy Molenaers, Els Ortibus, and Hilde Feys
Research Article (9 pages), Article ID 2831342, Volume 2018 (2019)

Transcranial Direct Current Stimulation (tDCS) Paired with Occupation-Centered Bimanual Training in Children with Unilateral Cerebral Palsy: A Preliminary Study
Tonya L. Rich, Samuel Nemanich, Mo Chen, Kathleen Friel, Timothy Feyma, Linda Krach, Tanjila Nawshin, Gregg Meekins, and Bernadette T. Gillick
Clinical Study (14 pages), Article ID 9610812, Volume 2018 (2019)

Impaired Ability to Suppress Excitability of Antagonist Motoneurons at Onset of Dorsiflexion in Adults with Cerebral Palsy
Svend Sparre Geertsen, Henrik Kirk, and Jens Bo Nielsen
Research Article (11 pages), Article ID 1265143, Volume 2018 (2019)

Corticospinal Tract Wiring and Brain Lesion Characteristics in Unilateral Cerebral Palsy: Determinants of Upper Limb Motor and Sensory Function
Cristina Simon-Martinez, Ellen Jaspers, Lisa Mailleux, Els Ortibus, Katrijn Klingels, Nicole Wenderoth, and Hilde Feys
Research Article (13 pages), Article ID 2671613, Volume 2018 (2019)

Reorganization of the Action Observation Network and Sensory-Motor System in Children with Unilateral Cerebral Palsy: An fMRI Study

Giuseppina Sgandurra, Laura Biagi, Leonardo Fogassi, Elisa Sicola, Adriano Ferrari, Andrea Guzzetta, Michela Tosetti, and Giovanni Cioni

Research Article (15 pages), Article ID 6950547, Volume 2018 (2019)

Hindawi
Neural Plasticity
Volume 2019, Article ID 9154282, 2 pages
https://doi.org/10.1155/2019/9154282

Editorial

Neural Plasticity after Congenital Brain Lesions

Simona Fiori,[1] **Martin Staudt**,[2,3] **Roslyn N. Boyd**,[4] **and Andrea Guzzetta**[1,5]

[1]*Department of Developmental Neuroscience, IRCCS Stella Maris Foundation, Pisa, Italy*
[2]*University Children's Hospital, Department of Pediatric Neurology and Developmental Medicine, University of Tübingen, Germany*
[3]*Clinic for Neuropediatrics and Neurorehabilitation, Epilepsy Centre for Children and Adolescents, Schön Klinik Vogtareuth, Germany*
[4]*Queensland Cerebral Palsy and Rehabilitation Research Centre, UQ Child Health Research Centre, Faculty of Medicine, The University of Queensland, South Brisbane, Queensland, Australia*
[5]*Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy*

Correspondence should be addressed to Simona Fiori; s.fiori@fsm.unipi.it

Received 10 April 2019; Accepted 10 April 2019; Published 2 May 2019

In the past decades, interest has grown on the mechanisms of early brain plasticity and their implications for newborns and infants with brain damage. Early brain damage triggers complex processes of adaptive neuroplasticity, which involve various functional systems and are highly influenced by the environment. Understanding the complex process of reorganization of neural functions through adaptive plasticity is a fast-growing field of research that has the potential to prompt more targeted and evidence-based interventions to promote neurodevelopment. The objective of this special issue was to collect scientific reports and literature reviews, from both animal models and human studies, contributing to a better understanding of the characteristics of early adaptive neuroplasticity following early brain damage.

Understanding in vivo mechanisms of damage and plasticity needs animal models. Unilateral brain damage is probably the most studied model for the characterization of brain plasticity. The review by M. Gennaro et al. is aimed at exploring developmental ischemic stroke pathophysiological mechanisms, focusing on key factors contributing to neonatal brain vulnerability and summarizing current available stroke models in animal labs. These models are highly informative to human studies, as they identify mechanisms of neuroplasticity in controlled experimental conditions often using invasive means of investigation. Fortunately, noninvasive neuroimaging and electrophysiological techniques are now widely available and make it possible to describe the specificity of early mechanisms also in humans.

Evidence of early plasticity was found, for example, by C. Simon-Martinez et al. who explored determinants of impaired upper limb sensory and motor functions and corticospinal tract wiring by using transcranial magnetic stimulation and related this to lesion topography as assessed by a visual semiquantitative scale for brain lesion severity on MRI in CP. In an innovative ultra-high-field MRI technique, S. Fiori et al. explored the plasticity of sensory systems in young adults with congenital brain lesions. Their findings support the crucial role of topography of brain damage and reorganized somatosensory areas in relation to deficits of hand sensory and possibly motor function. In another study, S. S. Geertsen et al. explored the electrophysiological mechanisms of coactivation of muscles and high step-to-step variability of gait in adults with early brain lesion and CP, which are the result of a long learning process involving predictive coding of the sensory consequences of movement.

Indeed, different factors interact with brain adaptive plasticity potentials, influencing the natural history of cerebral palsy in the first years. To understand these mechanisms, K. Klingels et al. collected longitudinal functional data over a 5-year time period in children with unilateral CP. They observed increasing limitations in the passive range of motion and improvement in capacity measures, while the

spontaneous use of the impaired limb in bimanual tasks became less effective after the age of 9 years.

Evolution of upper limb function in children with unilateral CP and the factors that influence these time trends can provide guidance in delineating treatment priorities. The effects of new treatment options have recently been the object of growing interest. For example, E. Inguaggiato et al. found an increase in unilateral hemiparetic hand function (manual dexterity) in children and young adults with unilateral CP, with improvements emerging immediately at the end of transcranial direct current stimulation (tDCS) and persisting for at least 90 minutes. The action observation network (AON) was studied by G. Sgandurra et al. in children with unilateral CP. They demonstrated similarities compared to AON in typically developing children and propose an AON paradigm to explain and predict the efficacy of rehabilitation in unilateral CP children and to investigate the effects of plasticity induced by specific rehabilitation programs. They also demonstrated that a more lateralized AON corresponded to a worse impaired hand performance, as an example of maladaptive plasticity. In another paper, T. L. Rich et al. applied transcranial direct current stimulation (tDCS) as an opportunity to open a window for plasticity in unilateral CP, showing that a single application of anodal tDCS over the affected M1 can improve, in a safe and feasible way, possible but inconsistent gains in hand function.

Of course, neural plasticity has not only implications in the organization of motor and somatosensory function. Visual deficits, for example, are increasingly recognized as being part of the clinical picture in most cases. Confirmation comes from the paper of G. Ickx et al. who explored visuospatial attention in unilateral CP and demonstrated clinical differences according to the laterality of brain and lesion timing: children with corticosubcortical lesions more frequently presented visuospatial attention deficits than children with periventricular brain lesion, which might be the results of impacted function or plasticity mechanisms.

Conflicts of Interest

The editors declare that they have no conflicts of interest regarding the publication of this special issue.

Acknowledgments

We would like to thank all the authors who contributed to this special issue. This publication would not be possible without the participation of our expert reviewers, who provided vital constructive feedback and criticism throughout the review process.

Simona Fiori
Martin Staudt
Roslyn N. Boyd
Andrea Guzzetta

Hindawi
Neural Plasticity
Volume 2019, Article ID 5089321, 16 pages
https://doi.org/10.1155/2019/5089321

Review Article

Rodent Models of Developmental Ischemic Stroke for Translational Research: Strengths and Weaknesses

Mariangela Gennaro,[1] **Alessandro Mattiello**,[2] **and Tommaso Pizzorusso**[1,3]

[1]*Institute of Neuroscience, National Research Council (CNR), Via Moruzzi 1, I-56124 Pisa, Italy*
[2]*Department of Clinical and Experimental Medicine, University of Pisa, via Savi 10, I-56126 Pisa, Italy*
[3]*Department of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA, University of Florence, Area San Salvi–Pad. 26, I-50135 Florence, Italy*

Correspondence should be addressed to Tommaso Pizzorusso; tommaso@in.cnr.it

Received 18 May 2018; Revised 19 December 2018; Accepted 6 February 2019; Published 4 April 2019

Guest Editor: Simona Fiori

Cerebral ischemia can occur at any stage in life, but clinical consequences greatly differ depending on the developmental stage of the affected brain structures. Timing of the lesion occurrence seems to be critical, as it strongly interferes with neuronal circuit development and determines the way spontaneous plasticity takes place. Translational stroke research requires the use of animal models as they represent a reliable tool to understand the pathogenic mechanisms underlying the generation, progression, and pathological consequences of a stroke. Moreover, in vivo experiments are instrumental to investigate new therapeutic strategies and the best temporal window of intervention. Differently from adults, very few models of the human developmental stroke have been characterized, and most of them have been established in rodents. The models currently used provide a better understanding of the molecular factors involved in the effects of ischemia; however, they still hold many limitations due to matching developmental stages across different species and the complexity of the human disorder that hardly can be described by segregated variables. In this review, we summarize the key factors contributing to neonatal brain vulnerability to ischemic strokes and we provide an overview of the advantages and limitations of the currently available models to recapitulate different aspects of the human developmental stroke.

1. Introduction

An ischemic stroke is a transient or permanent interruption of the blood supply into the cerebral vasculature and represents worldwide one of the most important causes of death and of long-term disability in the survivors [1]. Although the risk of brain ischemia increases in the elderly, the insult can hit young people, at the perinatal and pediatric ages [2]. Depending on the developmental stage of the affected brain structures, a broad spectrum of clinical signs may arise [2] such as hemiplegic cerebral palsy that represents the most frequent deficit after developmental ischemia, with a prevalence of 90% within the affected children [1].

Despite several studies shedding light on different pathogenic mechanisms underlying the generation, progression, and pathological consequences of the developmental ischemic stroke, the translation from the bench to the bedside of these findings encounters several obstacles.

In translational research, animal models for strokes represent a fundamental tool (a) to understand the molecular mechanisms underlying the short- and long-term physiological responses of all individual neuronal systems and of the whole brain to injury, (b) to set up new therapeutic strategies to salvage and rescue those structures, and (c) to find the best temporal window of intervention with pharmacological and rehabilitation interventions [3, 4]. In this view, notwithstanding the complexity of all cascade events, the choice of a reliable model is a researcher priority to reconcile the existing marked differences between rodents and humans at the level both of the cerebral vasculature [5] and of the nervous system architecture [6]. Keeping in consideration of how hard it is to match developmental stages across

different species, in this review, we aim to summarize developmental ischemic stroke pathophysiological mechanisms, focusing on key factors contributing to neonatal brain vulnerability. We also provide an overview of the models currently used to recapitulate the human developmental ischemic stroke, describing their advantages and limitations.

2. Clinical Features of Perinatal and Pediatric Ischemic Stroke

According to the timing of the stroke occurrence during development, two types of strokes are defined: perinatal and pediatric [2, 7]. The perinatal stroke, also known as neonatal, occurs from the 20th week of fetal life through to the 28th postnatal day and represents a significant cause of death and disability involving as many as 1 in 2,300 live births [1, 7]. By contrast, with a prevalence of 2-13 in 100,000, the pediatric stroke can occur from the twenty-eighth day after birth through to age eighteen [8–12]. Despite their different etiology, ischemia due to vascular (arterial or venous) thrombosis is the main cause of hemiplegia in up to 94% of cases of the perinatal versus pediatric stroke [1, 2, 13–15]. Additional neurological signs including intellectual disabilities, behavioral deficits, language and visual defects, psychiatric disorders, and epilepsy are more frequent after the perinatal stroke with respect to the pediatric condition [1, 2, 7, 9, 14, 16–18].

As stated before, depending on the timing of ischemia occurrence, different structures can undergo prevalent damage. For example, in preterm injured babies, white matter injury is more affected due to the abundance of developing oligodendrocytes that are highly sensitive to excitotoxicity and neuroinflammation [19]. On the other hand, in term babies, who have significantly less oligodendrocyte progenitors, grey matter structures (e.g., the basal ganglia, thalamic nuclei, and cerebral cortex) are the most commonly affected by the injury [20, 21]. In general, the perinatal stroke seems to be associated with a greater risk of worse outcomes [2, 7, 14, 18] when compared to the pediatric stroke scenario. This phenomenon is linked to the existence of different stages of the critical period throughout development in which the brain is differently susceptible to the early damage [2, 7]. Thus, in contrast to "Kennard's principle" by which the younger brain holds a greater capability to recover from injury, it seems that an earlier injury may in some cases more deeply impact the early developing brain, finally disturbing and so disrupting its pattern of maturation. This form of plasticity called maladaptive plasticity could be particularly disruptive for motor circuitry refinement where an aberrant mechanism of plasticity frequently arises [2, 7, 22–25]. Under maladaptation, the affected corticospinal tract does not exert the usual role in the movement control proper of the first few months after birth [24], but rather, an abnormal bilateral pattern of the innervation of the spinal motor neuron is observed, with deleterious consequences for long-term motor function [22–24, 26]. Perinatal and pediatric strokes have long remained undiagnosed or misdiagnosed, because of the difficulty of interpreting the paucity of motor handicaps [7]. In this context, Eyre and others in 2007 suggested that the delay in the emergence of motor signs depended upon the activity-dependent competition between the ipsilateral corticospinal tract (CST) from the undamaged side and the spared CST axons from the damaged side. However, recent efforts in clinical research have been made to find novel tools to detect hemiplegic signs as early as possible. For instance, the assessment of general movements at the neonatal epoch has been pointed out as a promising predictive method to detect the presence of neonatal cerebral infarction in infants [27, 28].

3. Ischemic Stroke Pathophysiology in the Immature Brain

Several experimental and clinical studies have been reviewed on the pathophysiology of perinatal and pediatric ischemic strokes frequently showing the presence of different mechanisms activated upon developmental injury [2, 14, 29]. The severity of damage following the neonatal brain ischemia may depend upon several factors: the type of neuronal cell death mainly activated during development [30], the maturation of the immune system [31], and the developmental stage of the cerebral vasculature [32] (Figure 1).

3.1. Excitotoxicity. Soon after blood flow interruption in the territory of a major brain artery, a failure in energy-dependent processes is generated, with the sudden loss of membrane potential, strong depolarization and Ca^{++} influx due to the activation [33]. As a consequence, neurons and glia undergo ion and water imbalance with the subsequent formation of intracellular edemas and membrane depolarization that leads to glutamate-dependent excitotoxicity that in turn triggers alteration in the brain metabolic profile [34] and death pathways [35]. The immature brain shows unique patterns of cell death activation in response to an ischemic lesion [36–38]. In fact, while necrosis is the prominent mechanism of neuronal cell death in the core lesion in adults, apoptosis is more readily activated in the immature brain. This is in part due to the high expression of key components of apoptotic pathways, such as caspase-3, that have a pivotal role in the programmed neuronal death during brain development [30, 36, 37]. Indeed, in a developing rat model of hypoxia-ischemia (HI), AMP-activated protein kinase (AMPK), a sensor of cellular energy status also involved in chronic inflammatory disorder [39], regulates FOXO3a-mediated neuronal apoptosis through increased expression levels of pro-apoptosis proteins, such as Bim and Caspase-3 [40].

The immature brain displays high excitability that can contribute to excitotoxic injury. This intrinsic high excitability of the immature brain relies mostly on a developmental increase in expression levels of the glutamate receptor [41, 42], both in ionotropic (NMDA) and in metabotropic (AMPA) ones. In fact, experimental evidence in rats suggests that the NMDA receptor density peaks late in the first postnatal week in both the hippocampus and the neocortex, whereas the AMPA receptor density peaks in the second postnatal week at around P10 [43]. Moreover, a different composition of individual receptor subunits of NMDA

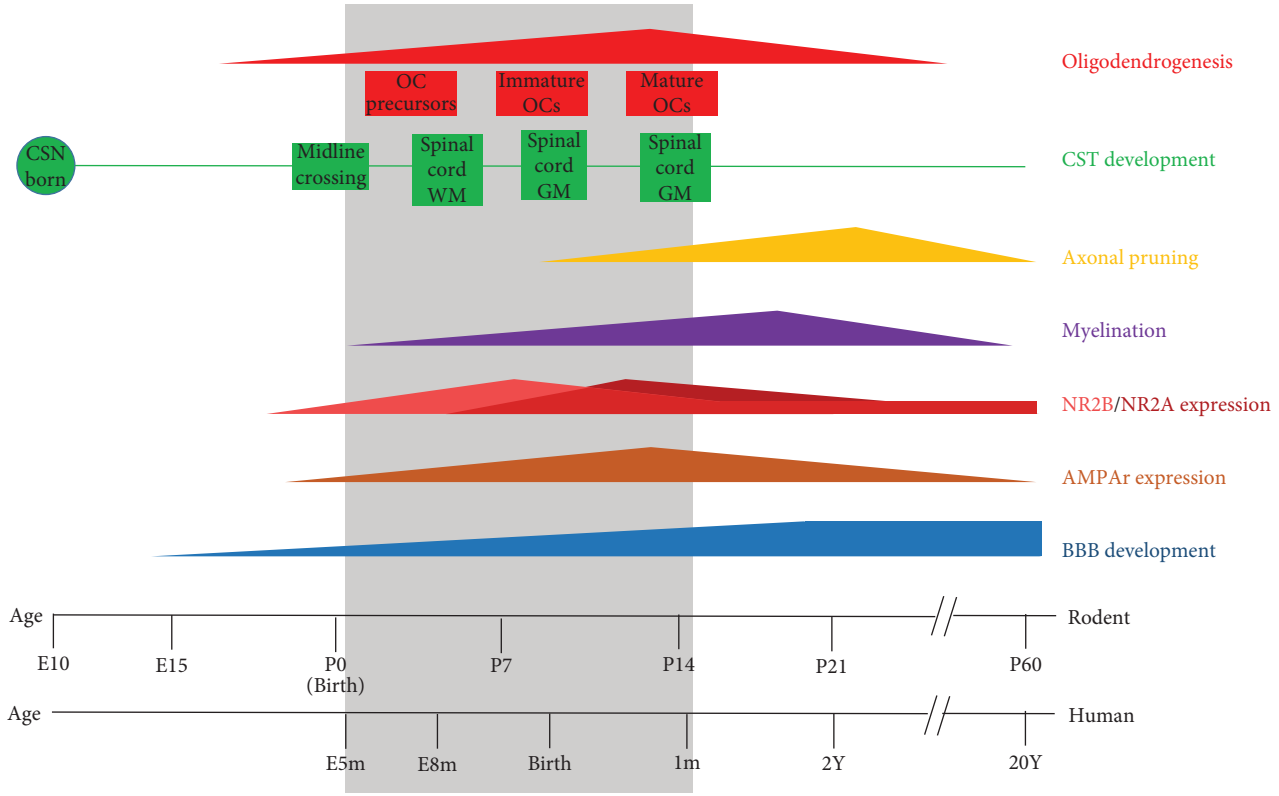

Figure 1: Comparison between the rodent and human development of some molecular, cellular, and structural elements of the nervous system. The grey rectangle depicts the "perinatal" range throughout life. Perinatal insults, such as an ischemic stroke, that hit during this age can interfere with several aspects of neural development.

[42, 44] and AMPA [45] due to a developmental regulation of their expression also contributes to increasing the glutamate-dependent excitotoxicity after a perinatal ischemic lesion. The higher expression of NR2B versus NR2A, together with a lower ratio of the GluR2 expression versus other AMPA receptor subunits in the immature neocortex and hippocampus, accounts for increased Ca2+ permeability, which in turn leads to exacerbated excitotoxicity after the injury [46]. An additional factor impinging on glutamate-dependent excitotoxicity after early injury is the intrinsic nature in action of the GABAergic system, which is immature and excitatory during early postnatal brain development [47, 48]. The reduced expression of several endogenous antioxidant enzymes as well as the very high concentration of unsaturated fatty acids, the high rate of oxygen consumption, and the availability of redox-active iron [49] also contributes to cytotoxicity.

3.2. Inflammation. Free-radical formation and activation of the inflammatory cascades also contribute to neuronal cell death after an ischemic injury in the immature brain [29]. Inflammation plays a dual role in perinatal ischemic stroke pathophysiology [50]. It represents a risk factor of perinatal stroke onset; however, it also contributes to protect the brain from injury, by supporting tissue healing [51, 52]. Its detrimental effects could be due to the facilitatory effects of perinatal inflammation on the pathophysiology of ischemia [53, 54], an effect linked to the ability of congenital inflammation eliciting thrombus formation; for a review, see [55]. The immaturity of the immune system at the perinatal age also impinges on the brain pathological response to ischemia [50]. For example, the nonclassical complement activation in term infants as well as in rat pups is downregulated with respect to the mature brain [56]. Furthermore, in adulthood, microglia activation plays a detrimental role in the acute phase of the ischemic lesion as it produces inflammatory mediators such as ROS and releases other toxic molecules [54]. In contrast, during development, microglia can play a reparative role [57, 58], since it actively releases anti-inflammatory cytokines and neurotrophic factors that contribute to resolve inflammation processes protecting viable neurons from apoptotic death [59]. Direct evidence of its protective role comes from two experimental studies where selective pharmacologic depletion of microglial cells two days before inducing tMCAO in P7 rats caused, respectively, a larger infarct size [59] and increased intracerebral hemorrhages [60]. Astrocytes act in concert with microglia in neonatal stroke pathophysiology. Indeed, early after injury, astrocytes actively contribute to the production of proinflammatory cytokines, in association with neurons and endothelial cells [59]. As for an adult stroke, also after neonatal ischemia, astrogliosis is sustained by higher activation of JAK/STAT signaling in both astrocytes and neurons, with a final insulting effect on brain cells [61–63]. In this context, recent work demonstrated that reducing this signaling pathway indirectly either by inhibiting the STAT3 transducer and

activator glycogen synthase kinase 3β (GSK3β) [61] or by blocking JAK2 and downstream STAT3 phosphorylation [63] promotes neuroprotection and reduced inflammatory response after a neonatal stroke. However, other controversial result come from a study carried in a model of hypoxia-ischemia (HI), where it has been shown that reactive astroglia does not exacerbate lesion extension, since GFAP deletion did not affect infarct volume [64]. Similar results were observed in a model of perinatal brain injury [65]. In the chronic phase, astrocytes contribute to limit edema after neonatal brain injury, since astrocyte end-feet in the neurovascular unit increases aquaporin 4 expression, thus facilitating water clearance to the vascular compartment [65].

3.3. Immaturity. Another crucial intrinsic factor contributing to the higher vulnerability of the developing brain to neonatal ischemia is the immaturity of brain microvessels [66]. For example, comparison of protein and transcript contents of the mouse forebrain enriched in microvessels at different ages across development showed an age-dependent increase of proteins and mRNA specific for endothelial cell adhesion, junction pathway, and extracellular matrix as well as for a shift of energy metabolism, transport, and antioxidant effector proteins, all associated with the acquisition of a mature microvessel structure [66]. Brain-blood barrier (BBB) permeability also appears different when compared with the adults both in physiological and pathological conditions [67]. In fact, BBB permeability in the early postnatal age is much lower with respect to the later stage of development, and in response to perinatal ischemic injury, extravasation of albumin at 2 hours after reperfusion is increased from 5- to 25-fold in the rat adult injured brain but only 2-fold in a newborn [67]. It has been proposed that the reduced BBB permeability at the early stage of brain development relies upon a higher expression of several tight junction and basal membrane components in neonates [67], on distinct mechanisms of endothelial cell activation, immature extracellular matrix (ECM) components [66], and neutrophil-endothelial interactions [67, 68]. Altogether, these mechanisms, in addition to preserving BBB integrity, also prevent neutrophil, monocyte, and T and B cell infiltration from the peripheral district to the brain [32]. Taking together, all these data point at the existence of a critical time window of neonate brain vulnerability to early damage that strongly determines the pattern of brain injury.

4. Developmental Ischemic Stroke Models

While several animal models of the adult ischemic stroke have been developed so far, few animal models of perinatal and pediatric strokes are available to recapitulate the mechanisms underlying the onset and the evolution of acute and long lasting deficits in children. In Table 1 a summary of the rodent models of the developmental ischemic stroke, and their assessment, is listed.

4.1. Models of Hypoxia-Ischemia. Over the past three decades, the Levine-Rice model of neonatal hypoxic-ischemic (HI) has been extensively used to generate the human perinatal ischemic stroke and has been characterized through histological analysis as well as behavioral tests (for reviews, see [69–71]). This model is a modification in the pups of the Levine preparation previously performed in the adult rat [72], and it is characterized by one to more hours of unilateral ligation of the common carotid artery followed by reperfusion and recovery. Afterward, whole body hypoxia is practiced by placing animals into a hypoxic chamber containing humidified 8% O_2. This model causes hypoperfusion in the ligated side of the brain, while the nonligated side is exposed to hypoxia alone [73]. Rat pups at P7 have been preferentially used versus mice [74, 75] to study neonatal stroke pathophysiology [14, 55], as well as neuroprotection, regenerative potential of the immature brain [76–79], and the applicable rehabilitative therapies [80]. However, the HI neonatal model generates high variability in infarct size, leading to a multifactorial pathological condition; moreover, model induction strikingly differs from the etiology of hypoxic-ischemic injury in humans and does not cause a consistent focal injury pattern, making study of the injured core and penumbra more challenging [74, 75].

4.2. Models of Occlusion of the Middle Cerebral Artery. Since human perinatal ischemic strokes mainly affect the MCA [81, 82], models developed for adult ischemic stroke were adapted to earlier ages. The MCAO model implies the temporary occlusion of the common carotid artery (CCA), introducing a suture directly into the internal carotid artery (ICA) and advancing the suture until it interrupts blood flow to the MCA [83, 84]. Depending on the duration of MCAO, interruption of cerebral blood flow CBF can be transient or permanent, causing therefore mild to severe brain damage and outcome [83]. Furthermore, not only the infarct size but also reperfusion can be modulated depending on the duration of occlusion [74]. Temporary MCAO in neonatal animals was investigated for the first time by Ashwal et al. [85], who performed this technique in P14-P18 rats. 3 hours of occlusion induced a lesion that affects 40-50% of the total hemisphere, resembling in part a global human pediatric stroke. MCAO was also performed in P7 rats, where disruption of CBF and cytotoxic edema formation were observed in MCA territory, accompanied by subsequent microglia and astroglia infiltration after reperfusion [86]. Unfortunately, this method produced a high mortality rate, with only 21% of rats still surviving after 28 days [87], making difficult any long-term assessment of outcomes. Embolic MCAO was also implemented [86]: the embolus measure was designed according to the rat size and resulted in an infarct affecting 51-56% of the ipsilateral hemisphere [88]. Ninety minutes of the intraluminal filament MCAO model at P20-25, followed by 22 h of reperfusion, was also used to characterize a mouse model of a childhood ischemic stroke [89]. One of the most interesting data obtained in this study is the assessment of sex-specific responses to cerebral ischemia in a juvenile mouse brain. The results showed a lack of gender difference in the response to ischemic injury and a sexual dimorphic neuroprotective role of estrogen [89]. These results greatly differ from what is usually observed in adults, either in humans or in rodent models [90].

Table 1: Summary of main rodent models of the developmental stroke used in translational research.

Animal	Age of lesion induction	Method of induction	Age of assessment	Variables assessed
Rat [40, 49, 63, 73, 79, 80, 180–183, 185, 186]; mouse [49, 61, 63, 64]	P7 [40, 49, 63, 73, 80, 180–183, 185, 186]; P9 [61, 64]; P10 [79]; P3 [175]	Hypoxia-ischemia based on the Levine-Rice method	Up to P11 [189]; P9 [40, 73, 181, 183]; P8 [190]; up to P67 [191]; P12 [63, 175, 182]; P21 [175, 185]; within 3 hr after lesion [186]; P11 and P40 [79]; from P21 to P60 [80]; up to P10 [40]; 6 hr post-HI and at P16 [61]; P31 [64]	Analysis of damage by MRI [73, 189]; analysis of brain edema by histology [192]; behavioral assessment of sensorimotor function [73]; analysis of intracellular calcium accumulation [190]; phosphocreatine, neuronal MAP-2, SNAP-25, and glial GFAP [193]; analysis of lesion volume and of white matter injury by histology [49, 61, 73, 187, 191, 192, 194]; analysis of systemic physiological variables (mean arterial blood pressure, heart rate, PO_2, PCO_2, pH, lactate, and glucose) and of high-energy phosphate and glycolytic intermediates [195]; effects of adiponectin treatment efficacy on the brain infarct area, apoptosis, brain atrophy, and neurological function [79]; investigation of efficacy of combining constraint-induced movement therapy (CIMT) and electroacupuncture on motor asymmetry and on lesion size and astrogliosis [80]; analysis of the role of AMPK signaling in the developing rat brain with HI [40]; analysis of inflammatory activation by immunohistochemistry [187]; assessment of oxidative stress after injury [49]; assessment of JAK/STAT signaling in brain inflammation [61, 63] and neuroprotection [63] by biochemical, molecular, and histological approaches [61, 63]; role of GFAP deletion on astrogliosis after HI and on infarct volume by immunohistochemistry [64]
Rat	P7	Embolus MCAO	Up to P8	Analysis of lesion volume by histology [88]
Rat	P7	MCA electrocoagulation associated with 1-hour left CCAO	Up to P90	Analysis of inflammatory responses by histology [196]
Rat [59, 65, 85, 86, 91, 188, 189]; mouse [60]	P14-P18 [85]; P7 [30, 59, 60, 67, 86, 188, 189]; P10 [65, 91]	Transient MCAO	P8 [85]; up to P90 [188]; P8 [67] up to P10 [60, 189]; P8 [30]; P10 [91]; P8 and P10 [59]; up to P38 [65]	Analysis of lesion volume by histology [59, 65, 85, 86, 91, 197, 198]; analysis of lesion evolution by MRI [59, 65, 67] and neuroprotection assessment [30, 91]; microglia activation by histology [59]; BBB integrity postinjury by histological, biochemical, and molecular techniques [67]; assessment of the role of microglia on hemorrhages by histological, biochemical, and molecular techniques [60]; assessment of brain edema through brain aquaporin-4 expression profiling [65]

Table 1: Continued.

Animal	Age of lesion induction	Method of induction	Age of assessment	Variables assessed
Mouse [30, 89, 90]	P12 [92]; P7 [30]; P20-25 [89]	MCAO	Up to P68 [90]; P8 [30]; 22 hr after lesion [89]	Analysis of lesion volume by histology and behavioral assessment of functional deficits [92]; anatomical analysis of caspase-3 activation in the ischemic core and penumbra [30]; effects of ischemia and estrogen treatment on the proapoptotic gene Bax [89]
Mouse [76, 77]	From P3 to P10 [76]; from P3 to P10 [77]	Chronic hypoxia	P10 and P48 [76]; P18 and P48 [77]	Analysis of injury by histology and unbiased stereological analysis of neurogenesis by BrdU assay [76, 77]
Rat	P7	Photothrombosis	P12 and P25	Study of PTZ-seizure susceptibility by EEG recordings [100]
Rat [103–105]	P14 [103]; P21 [103, 104]; P12 and P25 [105]	ET1 injection: intracortical [103]; intrastriatal [104]; intrahippocampal [105]	From P60 [103]; not specified [104]; up to 22 hr postinjury [105]	Assessment of lesion timing on damage volume, long-term motor outcome, and axonal sprouting of contralesional CST at the red nucleus and spinal cord level using anterograde tracing [122]; MRI analysis of damage extension, CBF volume and metabolic changes, and BBB integrity [123]; assessment of ischemia-induced seizures by video/EEG recordings [124]

Transient MCAO without permanent ligation or cauterization has been applied to P10 rats, comparing the effect of different durations of artery occlusion on the extension of brain injury and on behavioral outcome. In this case, extension of the brain lesion correlated with duration of occlusion, since a 90 min occlusion produced a mild-to-moderate injury pattern affecting the striatum and causing transient locomotor deficits, while 3 h caused a moderate-to-severe injury that often affected the cortex and hippocampus and caused enduring locomotor deficits that outlasted the reperfusion phase [91]. Recently, direct electrocoagulation of the unilateral MCA in P12 CB-17 mice has been used: this model holds a reduced variability both in brain injury and in CBF after 24 h from insult with respect to the HI model. Furthermore, using electrocoagulation as a permanent insult, significant neurofunctional deficits in the rotarod and open field can be elicited [92].

4.3. Models of Thrombotic Ischemia. The photothrombotic stroke is a model of thromboischemia based on intravascular photooxidation of a photoactive dye (in most cases, the rose bengal given through intraperitoneal administration) through brief irradiation of the intact skull by a light beam at a specific wavelength [93]. Depending on the intensity and duration of light illumination, as well as the stereotaxic coordinates chosen, different extensions of the lesion can be produced [6, 94]. Until now, photothrombotic models have been mostly used to study stroke in adults [95–98], and only recently, it has been used to recapitulate the perinatal stroke condition both in neonate piglets [99] and in rats at P7 [100]. Among the advantages of this model is the possibility of creating small size infarcts to target specific regions [6]. However, there are intrinsic disadvantages of this model since, in contrast with human stroke pathophysiology, its nature is only occlusive, and no growth and maturation of the ischemic penumbra and local collateral flow/reperfusion can take place [101].

4.4. Models of ET1 Vasoconstriction. Endothelin 1 (ET1) is a small (21 amino acids) vasoactive peptide produced by the endothelium and smooth muscle cells [102] which acts as a paracrine and autocrine factor [103] constricting vessels [104] through specific receptors (ETRA and ETRB) [105]. ETRA is mainly located on smooth muscle cells, and its activation is thought to be the major contributor to vasoconstriction upon ET1 binding [106]. Instead, ETRB is localized on both the smooth muscle and endothelial cells but is associated with vasodilation, caused by the release of nitric oxide (NO) and prostacyclin from endothelial cells [107]. Other than in vascular cells, the endothelin system (ET system) is also present in the central nervous system [102], where it plays an important role in the case of lesion occurrence. Indeed, after brain injury, ET1 is acutely overexpressed in the cerebrospinal fluid and plasma of humans [108, 109], rats [110], and pigs [111], suggesting that endogenous upregulation is an evolutionary conserved mechanism. However, whether the ET system overactivation may be protective or detrimental for the postlesion outcome is still a matter of debate. Several experimental works indicate that the endogenous ET system upregulation may contribute to lesion pathophysiology. Indeed, postlesion upregulation of either ET1 or ETR expressions correlate with astrogliosis [112], extent of the brain lesion [113], BBB dysfunction [114–116], and inflammation [117]. This evidence is a very important issue to keep in mind when generating ET1 models of ischemia, as it influences the interpretation of experimental results. ET1 can be either stereotaxically injected into parenchymal regions of interest or topically applied on the pial surface of the brain, to constrict local arterioles, or near the MCA [118, 119] reperfusion occurs, but at a much slower rate with respect to the intraluminal suture model. Lesion size can be modulated by varying the concentration or volume of ET1 to achieve reproducible injury [120]. The constant hypoperfusion rate prevents the development of an extensive edema, moving partially away from the human ischemia. On the other hand, this model seems to be more appropriate for chronic long-term studies rather than for studies on the acute effects of a stroke [121].

In contrast to adult stroke studies, very few works have used ET1 to generate models of the developmental stroke thus far [122]. ET1 was previously injected into the striatal area of the juvenile (P21) rat brain to induce a reproducible focal lesion [123], but only anatomical changes in response to ET1 injection were evaluated. Tsenov et al. in 2007 [124] used intrahippocampal ET-1 injection to generate a model of ischemia-induced seizures in immature rats, at P12 and P25, respectively, showing that at both developmental ages, ET1 into the dorsal hippocampus elicited convulsions as well as neuron loss.

4.5. Rodent Models: Similarities and Differences with Human Brain Development. The success of generating reliable models of the human developmental stroke strongly relies upon the ability to get the similarities in the cross-species corticospinal system function and development (for a review, see [125]). Most of the studies use rodent models because they can be easily manipulated to explore the genetic basis of motor development [126] as well as to understand motor learning mechanisms using behavioral and functional approaches [127]. Rodents show some similarities with humans at the CST level [127–129].

Indeed, as in humans, rodents have a CST that projects the full length of the spinal cord [129–131] and is involved in fine movement control [127, 132]. Both in humans and in rodents, CST development is accomplished at the postnatal age [133, 134]. Indeed, temporal changes in the diffusion anisotropy quantified by diffusor tensor imaging DTI in rats from P0 (day of birth) to P56 showed developmental changes in the DTI metrics in multiple gray and white matter structures related to neuronal and axonal pruning and myelination [133]. Furthermore, in the neonatal rat, the corticospinal projection originates from the whole neocortex including the visual cortex, and corticospinal projections also have transient ipsilateral projections that are predominantly pruned when maturity is reached [135].

However, notable differences between the human and rodent developing brain exist. *In primis*, there is a complete

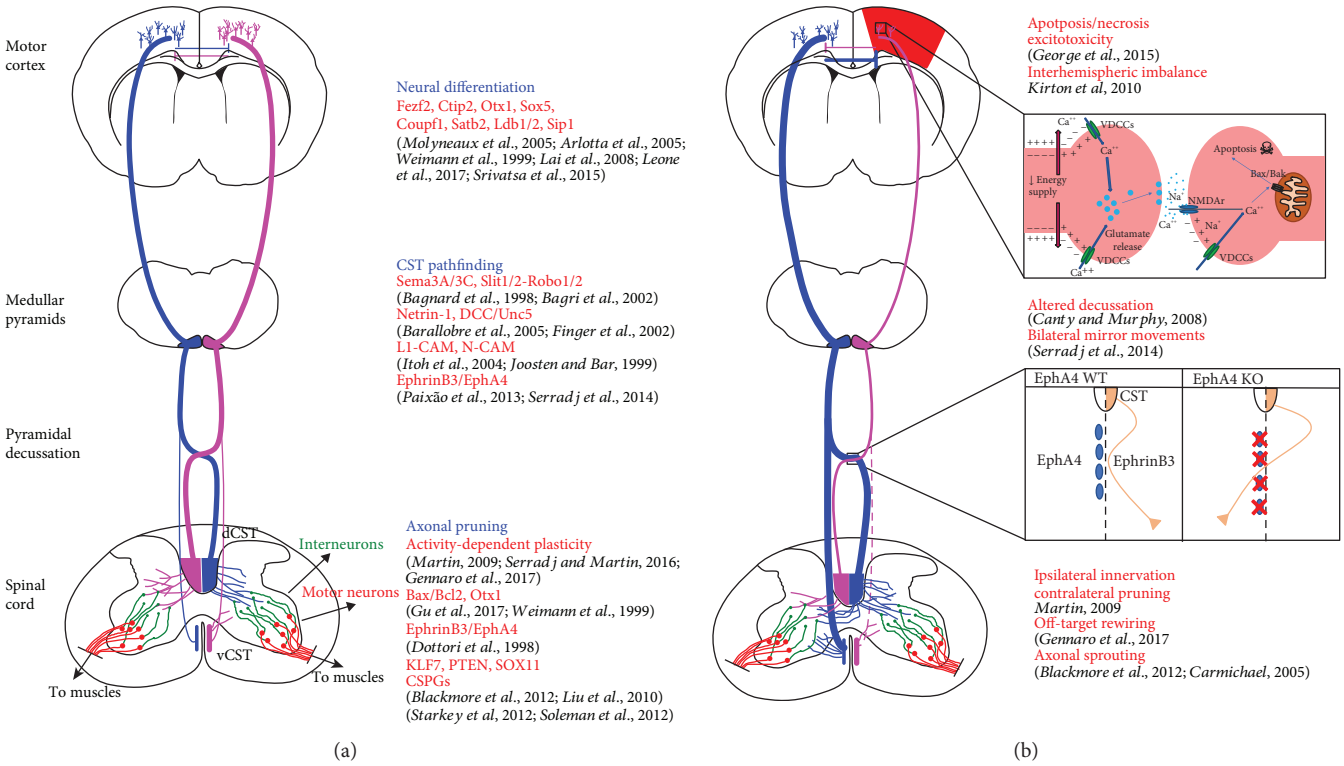

Figure 2: (a) Molecular and environmental factors involved in physiological CST development in rodents. (b) Processes altered after a brain injury that hits during CST development. Insets show some of the mechanisms involved in the acute damage provoked by cerebral ischemia (excitotoxicity, top right) and the factor involved in the axonal pathfinding and midline crossing in the CST development (EphA4/EphrinB3, bottom right).

absence of gyrification in the rodent brain [136]; second, great differences in the CST path are present: rodent CST axons run into the dorsal funiculus and do not establish direct synapses onto spinal motor neurons [137], but rather, the CST almost entirely projects more dorsally to the premotor spinal circuits [134, 138–142]. Concerning brain vasculature, similarities and differences of the circle of Willis between rodents and humans have been reported [5]. Both in rodents and humans, the internal carotid artery irrorates the anterior circulation whilst the posterior cerebral artery supplies the posterior circulation [5]. Moreover, in both species, the internal carotid artery provides the major blood source to the encephalon; however, it is more extended in rodents versus humans since it has a greater number of collaterals which supply a wide cerebral area [5]. This interspecies difference in brain vascular morphology may impinge on the degree of blood flow deprivation induced by different models and accordingly on the entity of neuronal damage.

4.6. Milestones Controlling CST Development across Different Species. Another crucial factor to be kept in mind when generating a rodent model of experimental models of a stroke is the ability to match the age-specific motor behavior repertoire with the progressive steps of CST maturation across species. While the corticospinal system matures, adaptive motor behaviors begin to be expressed [143, 144]. In mammals, CST development begins prenatally while mature motor skills are developed during the first month in the rat [145] and the first 2 to 3 months in cats [25]. Human motor development is incomplete until 12-13 years [146, 147]. As shown in Figure 2, several experimental studies have clarified that the mammal CST maturation process involves the interplay between genetics, neural activity, and experience to allow appropriate circuit formation and acquisition of complex tasks [6, 122, 134, 148–176]. For example, guidance cues such as EphrinB3 and its receptor tyrosine kinase EphA4 ensure the correct CST pathfinding [172], since selective elimination of the EphA4 gene in the mouse forebrain leads to a strong CST bilateral projection to the spinal cord that persists up to adulthood with enduring skilled motor impairments (Figure 2) [168]. Activity- and use-dependent processes subsequently shape the pattern initially established by genetic mechanisms and lead to the withdrawal of less active ipsilateral CST projections while contralateral ones are instead reinforced [23, 24, 141, 166]. Indeed, studies in cats have revealed that blocking motor experience or motor cortex activity causes defects in CS axon remodeling in the spinal cord, leading to permanent impairments in skilled movements [177]. Furthermore, concurrently to CS axon remodeling, motor maps for interjoint muscle synergies also develop during the postnatal stages in cats [155]. Recently, the mechanism by which rodents gradually acquire precise control over their flexor and extensor muscles to allow acquisition of skilled abilities has been elucidated [178]. In this elegant work, Gu et al. showed that maturation of muscle activation patterns controlling skilled movements is acquired

through reorganization of the CS axons controlling antagonist muscles, according to an activity-dependent Bax/Bak-caspase pathway. Deletion of the Bax/Bak proteins selectively in the mouse motor cortex resulted in the lack of activity-dependent pruning of exuberant axon collaterals [178], suggesting therefore the nonapoptotic pathway Bax/Bak as a novel milestone for proper CST motor development in rodents. Thus, across species, motor control development implies a triad of events during the refinement period: loss of transient ipsilateral termination with growth of experience-selected axons to local spinal targets, development of motor cortical motor maps, and finally myelination [179].

Although great insights into the milestones controlling normal maturation of CST across different species have been achieved, a debate on the appropriate matching of age between human and rodent neonates, as well as on how to correlate neuronal events that occur during maturation across these species, still remains open [180]. Some authors suggest that depending upon different criteria, such as brain weight growth [181], white matter myelination [182], corticospinal system development [183], and EEG maturation [184], the human term would include P7-P10 in rodents, with brain development at P7 in rats being more comparable to that of premature or full-term infants [70, 182, 185]. P20 in rodents would correspond to a 2–3-year-old human child [180, 181]. Nonetheless, there are some controversial opinions about which postnatal age in the rodent would recapitulate the term infant stage. For example, in an attempt to generate a model of the human term moderate HI, Quairiaux et al. used rat pups at P3 to characterize the effect of this really early damage on morphological and functional outcome [186]. In this work, in agreement with previous findings [187], the lesion at this early developmental stage caused impairments that mainly involved the somatosensory parietal cortex [186]. The importance of age of ischemia occurrence as a determinant for stroke outcome is underscored by a study that compared the effects of a stroke in the rat motor cortex at two temporally close ages: P14, when CS axons reach a maximum level of spinal cord gray matter innervations [154], and P21, when the CST axon pruning reaches its maximal levels [188]. Focal ischemic lesions at these two ages caused substantially different outcomes: the P14 lesion resulted in being more detrimental than the P21 lesion for long-term motor outcome in association with an extensive but mistargeted CST sprouting at the spinal cord level [122]. These data imply the existence of a strict age-dependent regulation of CST plasticity that can even be maladaptive during development.

5. Conclusions

Despite the variability in the techniques adopted and the developmental stages used to model human developmental ischemic strokes, preclinical studies continue to be extremely useful. Indeed, they inform us about the existence of multiple factors influencing the postinjury functional outcome. The timing of lesion occurrence seems to be critical, as it strongly interferes with CST development and determines the way spontaneous plasticity takes place. Classical studies showed that the effects of visual deprivation during temporal windows of development-designated critical periods dramatically impaired visual acuity maturation resulting in amblyopia. Similarly, a developmental brain injury causing a "deprivation" of activity of CST could also have long-term functional consequences that could strongly depend upon the age of the lesion and the relationship with critical motor periods [23]. The comparison with the current knowledge coming from visual system experience-dependent development suggests that experience-dependent changes could also be exploited to open a window for restorative therapies in the case of early motor system injuries. So far, harnessing post-stroke neural plasticity via electrophysiological and behavioral approaches was found to have beneficial effects promoting significant recovery of motor function, and early intervention after a perinatal ischemic stroke has been shown crucial in preventing maladaptive plasticity [22, 122]. However, future studies should be directed to identify the age-specific molecular programs triggered by developmental injury. Specifically, finding a causal link of the age-specific regulation between genetic factors and environmental molecular cues would help to determine the pattern of sprouting and therefore implement more effective therapeutic strategies aimed at regaining or preserving motor functions. Technological development has dramatically accelerated moving towards cell-specific studies, both at the molecular (e.g., single-cell sequencing from defined populations) and functional (e.g., in-depth in vivo functional imaging and noninvasive stimulation) level. Applying these methods to selectively study the CST and its milieu in models of a juvenile stroke will be fundamental to understand which molecular factors and which pattern of electrical activity can regulate developing CST growth and pruning, with positive consequences on the development of treatments that could also be beneficial in adult models of CST lesions.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Hindawi
Neural Plasticity
Volume 2019, Article ID 2184398, 10 pages
https://doi.org/10.1155/2019/2184398

Clinical Study

Transcranial Direct Current Stimulation (tDCS) in Unilateral Cerebral Palsy: A Pilot Study of Motor Effect

Emanuela Inguaggiato,[1] Nadia Bolognini,[2,3] Simona Fiori,[1] and Giovanni Cioni[1,4]

[1]*Department of Developmental Neuroscience, IRCCS Fondazione Stella Maris, Calambrone, Pisa, Italy*
[2]*Department of Psychology, NeuroMi, University of Milano Bicocca, Milan, Italy*
[3]*Laboratory of Neuropsychology, IRCCS Istituto Auxologico Italiano, Milan, Italy*
[4]*Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy*

Correspondence should be addressed to Giovanni Cioni; gcioni@fsm.unipi.it

Received 11 July 2018; Revised 26 October 2018; Accepted 22 November 2018; Published 14 January 2019

Academic Editor: Sergio Bagnato

Transcranial Direct Current Stimulation (tDCS) is an emerging tool to improve upper limb motor functions after stroke acquired in adulthood; however, there is a paucity of reports on its efficacy for upper limb motor rehabilitation in congenital or early-acquired stroke. In this pilot study we have explored, for the first time, the immediate effects, and their short-term persistence, of a single application of anodal tDCS on chronic upper limb motor disorders in children and young individuals with Unilateral Cerebral Palsy (UCP). To this aim, in a crossover sham-controlled study, eight subjects aged 10-28 years with UCP underwent two sessions of *active* and *sham* tDCS. Anodal tDCS (1.5 mA, 20 min) was delivered over the primary motor cortex (M1) of the ipsilesional hemisphere. Results showed, only following the active stimulation, an immediate improvement in unimanual gross motor dexterity of hemiplegic, but not of nonhemiplegic, hand in Box and Block test (BBT). Such improvement remained stable for at least 90 minutes. Performance of both hands in Hand Grip Strength test was not modified by anodal tDCS. Improvement in BBT was unrelated to participants' age or lesion size, as revealed by MRI data analysis. No serious adverse effects occurred after tDCS; some mild and transient side effects (e.g., headache, tingling, and itchiness) were reported in a limited number of cases. This study provides an innovative contribution to scientific literature on the efficacy and safety of anodal tDCS in UCP. This trial is registered with NCT03137940.

1. Introduction

Unilateral Cerebral Palsy (UCP) represents the most frequent form of CP, affecting about 30%-40% of all children with CP [1]. In general, the upper limb is more involved, impacting daily use of hand in activities such as reaching, grasping, and manipulation of objects. UCP is associated with heterogeneous brain lesions, mainly due to perinatal stroke, and its clinical manifestation is related to timing (acquired vs. congenital, acute vs. chronic) and etiology of brain injury [1]. The hand contralateral to the nondamaged or less damaged hemisphere may be underperforming, compared to typically developing children, and therefore, the terms more-affected and less-affected hand, instead of affected and nonaffected, have been suggested in studies with children with UCP [2]. In order to improve functions of the hemiplegic hand, several types of intervention have been used with some success. Recently, there has been an increasing interest in the use of Noninvasive Brain Stimulation (NIBS) techniques, such as transcranial magnetic (TMS) and direct current electrical stimulation (tDCS), to enhance poststroke motor disorders and neurodevelopmental outcomes.

With tDCS, continuous and weak electric currents (typically 0.5-2.0 mA) are applied over the scalp in order to modulate brain activity [3–5]. On the neuronal level, the primary mechanism of action is a polarity-dependent shift (polarization) of the resting membrane potential. While anodal stimulation generally enhances motor cortical excitability, cathodal stimulation has the opposite

effect, decreasing cortical excitability. This polarization mechanism underlies the acute, short-lasting, and reversible effects of tDCS in humans [6]. Multiple consecutive applications of tDCS are required to induce persistent after-effects with cortical excitability shifts maintained in the long term. Such after-effects involve modification of synaptic microenvironment and are mediated by GABA and NMDA receptors, which subtend synaptic plasticity mechanisms similar to those observed in long-term potentiation (LTP) and depression (LDP) [6, 7]. It is important to remember that the excitatory and inhibitory effects of anodal and cathodal stimulations depend on various factors, most of which are still unknown. Indeed, a growing body of evidence shows that tDCS does not function in a linear manner, so that physiological and behavioral outcomes, in terms of facilitation or inhibition of cortical excitability, depend on the interaction of several factors related not only to technical parameters, such as current polarity and duration, but also to individual and task characteristics, as well as to metaplasticity-related effects [8]. This is especially relevant, and even more complex, in a developing brain [9].

The fact that plasticity-dependent after-effects induced by tDCS are associated with long-term behavioral improvements has fostered clinical research on the therapeutic potential of this technique for the treatment of neurological and psychiatric diseases [10]. With respect to rehabilitation of poststroke motor disorders in adults, two main approaches have been tested in line with a model proposing the existence of a maladaptive interhemispheric imbalance between the two hemispheres after a unilateral stroke [11]. Following this model, poststroke motor recovery may be facilitated by either upregulating the excitability of the lesioned motor cortex (through anodal tDCS) or by downregulating the hyperexcitability of the intact motor cortex (through cathodal tDCS) [12–14]. Though the principal theory differs in many aspects, these two approaches have been adopted also for improving upper limb motor disorders in subjects with UCP. Indeed, current knowledge recognizes, as main components of developmental neuroplasticity following perinatal brain injury, both influences of contralateral and ipsilateral corticospinal projections to the paretic hand, and the intrahemispheric and interhemispheric connections of the lesioned and intact motor cortices. It follows that the damaged as well as the intact motor cortex may represent potential central therapeutic targets for tDCS in UCP [9, 15, 16]. It is also important to consider that this neuromodulation tool can modulate the activity and functional connectivity of large-scale brain networks in both hemispheres, even when the stimulating electrode is applied "unilaterally," over a specific cortical region, such as M1 [15].

In the pediatric population, there is a paucity of research on the therapeutic potential of tDCS, with respect to both clinical efficacy and safety in children and young individuals with neurodevelopmental disorders. Some limited data exist from research conducted on ADHD, autism, epilepsy, and learning disorders [17]; they confirm the feasibility and safety of tDCS in the pediatric population, describing some positive clinical effects obtained in the treatment of these disorders. In individuals with CP, single or multiple applications of anodal tDCS over the primary motor cortex (M1) of the affected, or more affected, hemisphere seem to improve gait and reduce muscle spasticity [18, 19]. An essential central concept that has emerged from therapeutic brain stimulation studies in adults is the need to stimulate motor learning in the injured brain. To facilitate motor recovery, tDCS should be used as an add-on intervention to motor therapies in clinical settings [20–24]. In this regard, some promising effects on manual functions have been obtained applying, during a motor therapy, cathodal tDCS over the intact hemisphere [25, 26]. However, tDCS efficacy for driving upper limb motor recovery in UCP still requires further research.

In this context, the main aim of our pilot, proof-of-principle, study was to evaluate, for the first time, the effect of a single anodal tDCS application over the ipsilesional motor cortex on the unilateral gross manual function of the more affected, hemiplegic, contralesional hand in a small group of subjects with UCP, while also exploring the possible influences of demographic and lesion factors. We measured both the immediate effects of tDCS (i.e., acute effects emerging immediately at the end of the stimulation) and their persistence in the short-term (within 90 minutes poststimulation). We focused on short-term effects since seminal neurophysiological studies in humans have showed that a single application of anodal tDCS for 13 minutes can induce an increase of motor cortex excitability (as indexed by increased amplitude of motor-evoked potentials induced by single-pulse TMS) that persists for a maximum duration of 1.5 hours after stimulation [27]. We adopted a study design similar to that used in stroke adults in the original study by Boggio and colleagues [28], who investigated the possible modulation induced by a single application of tDCS on the motor functions of the paretic hand in stroke adults. As in Boggio's study, this study did not combine tDCS with motor learning tasks. We also assessed tDCS effects on the motor function of the nonhemiplegic hand, its safety and tolerability by monitoring possible side effects and effect on blood pressure and heart rate.

Anodal tDCS was applied for 20 minutes, with an intensity of 1.5 mA. These tDCS parameters (intensity and duration) were chosen in light of previous evidence in stroke adults [6, 8–13, 19]. Both in children with typical development and in children with UCP, current evidence is still insufficient to delineate the optimal tDCS dosage (i.e., current intensity and duration) for modulating motor performance. In studies investigating tDCS effects in pediatric populations, current intensities have ranged from 0.3 to 2.0 mA (most frequently 1 mA), with a duration up to 20 minutes [29]. In children with UCP, only cathodal stimulation, administered as adjuvant to motor therapy, has been used to modulate upper limb motor functions. In this case, it was shown that an intensity equal to or below 1 mA was unable to increase gains of motor training, as compared to the add-on use of sham tDCS (at least with respect to objective motor outcomes) [25, 26, 30]. In a study on healthy children assessing tDCS effects on motor learning, cathodal stimulation at 2 mA was shown to be less effective than anodal and cathodal stimulations at 1 mA [30]. So far, a current intensity of 1.5 mA was never tested in UCP [29], while there is evidence of its efficacy in adult stroke (e.g., [19]).

2. Materials and Methods

2.1. Participants. Participants were selected from the UCP database of the IRCCS Stella Maris Foundation (Pisa, IT), according to the following inclusion criteria: (1) diagnosis of UCP, confirmed by brain MRI indicating congenital unilateral brain lesion (i.e., a lesion that occurs either prenatally or perinatally within 28 days from birth), (2) aged between 10 and 28 years, (3) absence of history of seizures or epilepsy, and (4) no contraindication to tDCS [31–33]. Subjects were excluded if one of the following conditions exists: (1) epilepsy or first degree relative with epilepsy (in some cases the presence of epilepsy was identified after selection from database and therefore subsequently excluded) [33], (2) bilateral lesion, (3) other severe concomitant disabilities, and (4) botulinum toxin for the upper limb within the last 6 months. Contacted participants were also selected on the basis of residence: we excluded subjects that lived more than 100 km from IRCCS Stella Maris Foundation. After section and telephone contact to verify eligibility and potential interest of subjects and their families for the study, eight participants (mean age = 17.5 ± 6.1, range = 10-22 years) were recruited (Figure 1).

Functional hand level was determined according to the Manual Ability Classification System (MACS, Italian translation, 2010) [34]. Clinical and demographic features of participants are reported in Table 1.

This study was conducted according to the Good Clinical Practice and was approved by the Tuscan Region Pediatric Ethics Committee (Florence, Italy) in March 2016. The study began in June 2016 and finished in October 2017. The trial has been registered in the ClinicalTrials.gov (ClinicalTrials.gov Identifier: NCT03137940). Participants were informed that they could voluntarily withdraw from the study at any time.

2.2. Structural MRI. Each participant underwent structural MRIs, on which severity of lesion was classified using a qualitative classification related to timing of brain insult in UCP [35] and a semiquantitative scale for brain lesion severity by a pediatric neurologist (SF), with expertise in neuroimaging [36, 37]. Timing of insult results in three forms of congenital brain lesions [35], corresponding to brain maldevelopments (first two trimesters of pregnancy), periventricular venous infarction (early third trimester), and ischemic stroke (later third trimester). The semiquantitative scale described by Fiori and colleagues [36, 37] is a reliable system for the classification of brain lesion severity in children with CP. According to this scale, brain lesions are represented on a graphical template and raw scores for each region of the brain are systematically calculated, where higher scores represent more severe pathologies (i.e., a larger lesion within a given region as indicated by signal change and missing tissue). Hemispheric score is the sum of lobar scores (maximum score of 12) in each hemisphere. Basal ganglia and brainstem score is the sum of subcortical structures (basal ganglia, thalamus, brainstem, and posterior limb of internal capsule: maximum score of 5) on each side, and the global score is the sum of the right and left hemispheric scores, basal ganglia and brainstem scores, and corpus callosum and cerebellum scores (maximum score of 40).

2.3. Transcranial Direct Current Stimulation: tDCS. tDCS was delivered by a battery-powered constant current stimulator (BrainStim, E.M.S. s.r.l., Bologna, Italy; http://brainstim.it) using a pair of surface saline-soaked sponge electrodes placed on the scalp. The anodal electrode was placed over C3 or C4 (according to the 10-20 electroencephalograph system for electrode placement) in order to stimulate the primary motor cortex (M1) of the damaged hemisphere, with the cathode electrode placed over the contralateral supraorbital area. During active tDCS, a constant current of 1.5 mA was applied for 20 minutes, with a ramping period of 30 seconds at both the beginning and end of stimulation (i.e., fade-in and fade-out phases, respectively).

Sham tDCS was applied with the same parameters and electrode montage as active tDCS, but the current lasted only 30 seconds [38]. Sham and active modes of the tDCS device were set in advance by one of the investigators (NB), who did not participate in data collection, thus keeping both participant and investigator applying tDCS and collecting data blind. This sham procedure is commonly used in clinical investigation [5].

2.4. Outcome Measures

2.4.1. Box and Block Test (BBT). BBT is a highly reliable hand dexterity test [39, 40], composed of a box and divided into two compartments, containing 150 wooden cubes (2.5 cm^3). Participants are instructed to grasp a wooden cube from one side of the box and drop it into the opposite side. Subjects perform a 1-minute trial, grasping and releasing as many blocks as possible and performance is measured by the number of blocks transferred in 1 min. If the subject transfers two or more cubes at the same time, this number is subtracted from the total score. According to BBT instructions, a 15-second practice preceded testing. The test was video-recorded for off-line analyses.

2.4.2. Hand Grip Strength (HGS) Test. The HGS measures (in kg) the maximum voluntary isometric strength of the hand, through a hydraulic hand dynamometer (the mean of three trials was taken as score).

2.5. Safety Questionnaire, Blood Pressure, and Heart Rate. A questionnaire, adapted from Bolognini et al. [24, 41, 42], was used to monitor adverse effects of tDCS; these items are illustrated in Tables 2 and 3 and examined the occurrence of the most common tDCS side effects (e.g., itchiness, headache, and tingling) [31]. Adaptation of the questionnaire consisted in the substitution of some specific terms to make it easier for children to understand; moreover, a specific section for follow-up assessment after 24 hours was inserted to assess day-after changes in mood, daily activities, and quality of sleep.

If an adverse effect was reported, the participant had to rate its intensity (0 = absent, 1 = mild, 2 = moderate, and 3 = severe) and report whether, in their view, the reported sensation was related or not to tDCS (0 = no correlation, 1 = possible, 2 = probably, and 4 = surely). Moreover, at the

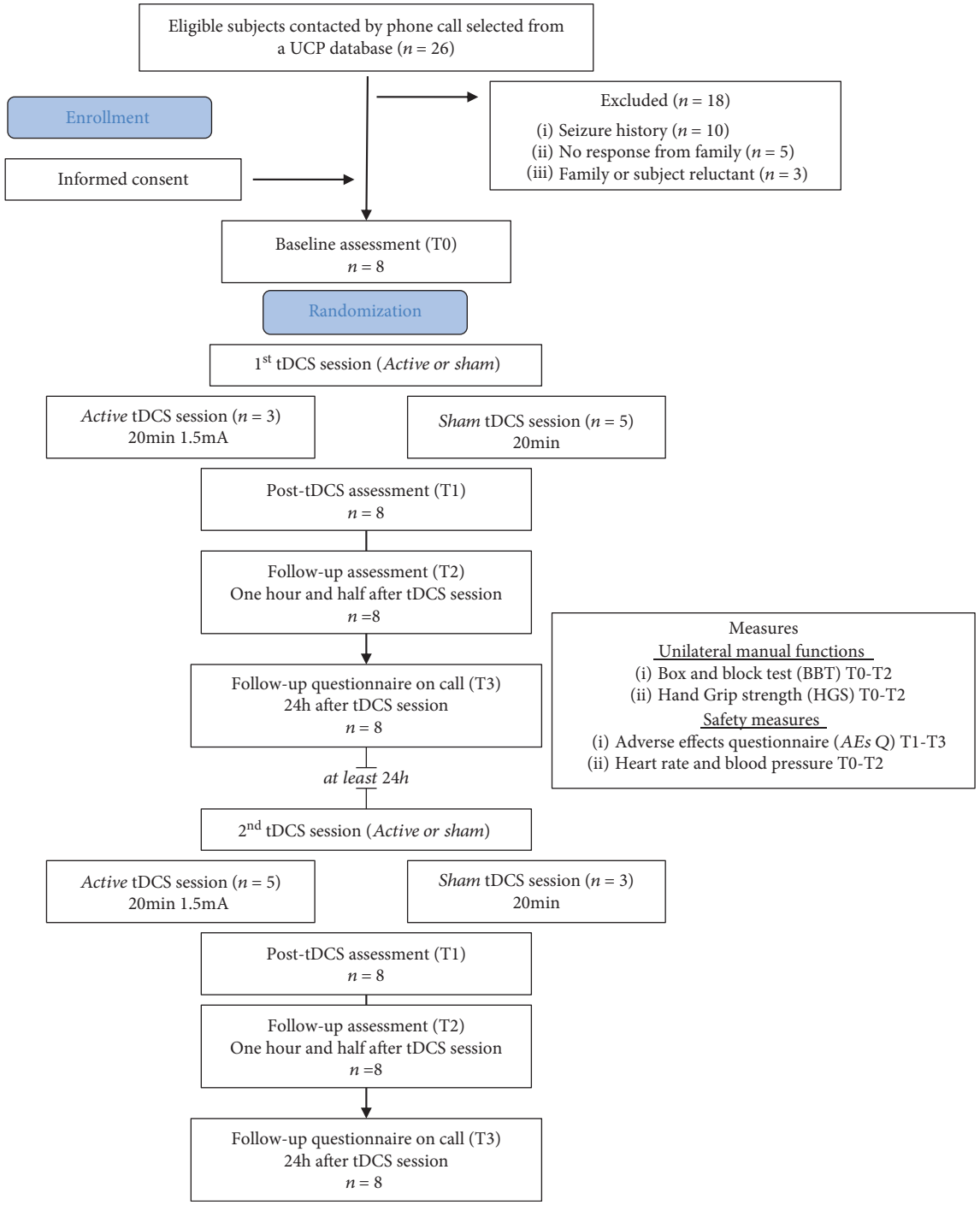

Figure 1: CONSORT diagram and study flow.

end of questionnaire, the experimenter also inquired, in an informal way, about the overall well-being and general feeling of participants and their caregivers with reference to tDCS.

Blood pressure and heart rate were evaluated using an automatic device (Boso medicus machine; Bosch+Sohn GMBH, Germany).

2.6. Experimental Design. We adopted a crossover, double-blind, sham-controlled design, with all participants undergoing two tDCS sessions, one with active and one with sham tDCS (in a random order across participants). In both sessions, motor functions (BBT, HGS), heart rate, and blood pressure were assessed immediately before tDCS (T0, i.e., baseline), immediately after (T1), and 90 minutes after the end of tDCS (T2) (Figure 1).

The tDCS questionnaire was administered at the end of each tDCS session (T1 and T2; Table 2) and the day after, through a phone call (T3; see Table 3). Since this was a pilot study, with explorative purposes, a sample-size calculation was not performed.

Table 1: Characteristics of the participants.

No.	tDCS order (a = active, s = sham)	Age	UCP side (R = right, L = left)	UCP form [1]	MACS [34]	MRI global severity score [36, 37]
1	sa	22	L	II	3	na
2	sa	27	R	I	1	15
3	sa	17	L	II	2	9
4	sa	11	L	III	3	4.5
5	sa	10	R	III	2	13.5
6	as	12	L	III	2	14.5
7	as	21	R	II	2	8
8	as	20	R	I	3	6
M ± SD		**17.5 ± 6.1**	**4L: 4R**		**2.25 ± 0.70**	**9.5 ± 3.87**

Acronyms: No. = number; M = mean; SD = standard deviation; A = active; UCP = Unilateral Cerebral Palsy; S = sham; R = right; L = left; MACS = Manual Ability Classification System; MRI = magnetic resonance imaging; na = quality of the images not suitable for detailed assessment.

2.7. Statistical Analyses. Statistical analyses were carried out with SPSS (IBM SPSS Statistic version 21).

Considering that each participant underwent two stimulation sessions (active and sham tDCS) and that the evaluations were performed at 3 time points (baseline, T0, immediately and 90 min after tDCS, T1 and T2, respectively), a repeated-measure analysis of variance (rmANOVA) was used to evaluate the effects of within-factor tDCS (active, sham) and Time (T0, T1, and T2) on BBT and HGS scores, separately for the hemiplegic and nonhemiplegic hands. We separately analyzed the two hands since modulation of motor performance of the hemiplegic hand represented our primary outcome. Moreover, we recognized the exploratory nature of this study on a small sample. Effects were also evaluated according to a standardized size-effect index that is partial eta-squared ($p\eta^2$). For significant effects, post-hoc testing was performed and corrected for multiple comparisons (Bonferroni). In every analysis, the significance level was set at $p < 0.05$. All data are expressed as mean ± SE.

Preliminary testing for normality with the Shapiro-Wilk test showed that, in every test (BBT, HGS, blood pressure, and heart rate), data were normally distributed (all $p > 0.09$) in all assessments (T0-T1-T2, of both active and sham tDCS sessions). Moreover, before running the analyses, the sphericity requirements for rmANOVAs were assessed by using Mauchly's test; whenever assumptions were not met, Greenhouse-Geisser correction was used for violations of sphericity.

3. Results

3.1. Box and Block Test and Hand Grip Strength. With respect to the performance of the hemiplegic hand in the BBT, the rmANOVA showed a main effect of Time ($F_{2,14} = 4.13$, $p = 0.039$, $p\eta^2 = 0.37$) and a significant tDCS by Time interaction ($F_{2,14} = 3.76$, $p = 0.049$, $p\eta^2 = 0.39$), while the main effect of tDCS did not reach significance ($F_{1,7} = 1.06$, $p = 0.34$, $p\eta^2 = 0.13$). Post-hoc comparisons showed a significant improvement from baseline only after active tDCS (T0, number of block/min = 18.4 ± 8.1 vs. T1 = 21.9 ± 9.2, $p = 0.037$ and T2 = 21.1 ± 8.1, $p = 0.049$), without difference between the 2 post-tDCS scores (T1 vs. T2, $p = 0.59$). The 3 time points did not differ from each other when sham tDCS was applied (all $p > 0.6$). Importantly, the baseline performance (T0) in the active and sham sessions was comparable ($p = 0.48$) (see Figure 2), excluding possible carry-over practice effects across sessions.

Regarding the nonhemiplegic hand (secondary outcome), no significant effect emerged from rmANOVA: tDCS ($F_{1,7} = 0.15$, $p = 0.71$, $p\eta^2 = 0.02$), Time ($F_{2,14} = 2.72$, $p = 0.1$, $p\eta^2 = 0.2$), tDCS × Time ($F_{2,14} = 0.18$, $p = 0.84$, $p\eta^2 = 0.03$) (see Figure 2).

We further checked for possible carry-over effect induced by receiving active stimulation as first; to this aim we ran a 2-way ANOVA, with the between-subject factor tDCS Order (active first vs. sham first) and the within-subject factor Time (T0 vs. T1): results showed a main effect of Time ($F_{1,6} = 18.76$, $p = 0.005$), confirming significant improvement from anodal stimulation from T0 to T1, but no main effect of tDCS Order ($F_{1,6} = 0.36$, $p = 0.6$), or a significant Time × tDCS Order interaction ($F_{1,6} = 2.69$, $p = 0.15$).

HG test could be administered to only six participants, as two subjects did not perform the test due to severe hand impairment. For both hands, rmANOVA did not show any significant effect (see Figure 3): hemiplegic hand, Time ($F_{1,5} = 0.03$, $p = 0.98$, $p\eta^2 = 0.01$), tDCS ($F_{2,10} = 0.76$, $p = 0.5$, $p\eta^2 = 0.13$), tDCS × Time ($F_{2,10} = 0.22$, $p = 0.8$, $p\eta^2 = 0.04$); nonhemiplegic hand, Time ($F_{1,5} = 0.06$, $p = 0.82$, $p\eta^2 = 0.01$), tDCS ($F_{2,10} = 2.56$, $p = 0.1$, $p\eta^2 = 0.1$), tDCS × Time ($F_{2,10} = 1.8$, $p = 0.2$, $p\eta^2 = 0.2$).

3.2. Blood Pressure, Heart Rate, and tDCS Side Effects. Blood pressure (mmHg) and heart rate (bpm) were analyzed with the same rmANOVA model used for motor scores; for both, results did not show any changes across time points and between tDCS sessions: heart rate, tDCS ($F_{1,7} = 0.08$, $p = 0.79$, $p\eta^2 = 0.01$), Time ($F_{2,14} = 1.93$, $p = 0.18$, $p\eta^2 = 0.03$), tDCS × Time ($F_{2,14} = 0.30$, $p = 0.74$, $p\eta^2 = 0.04$); blood pressure, tDCS ($F_{1,7} = 0.05$, $p = 0.83$, $p\eta^2 = 0.01$), Time ($F_{2,14} = 0.15$, $p = 0.86$, $p\eta^2 = 0.02$), tDCS × Time ($F_{2,14} = 0.97$, $p = 0.40$, $p\eta^2 = 0.03$).

Table 2: Adverse effects' questionnaire (early evaluation).

Adverse Effects Questionnaire (AEs-Q) items	Active ($n = 8$ subjects)				Sham ($n = 8$ subjects)			
	No. of subjects reporting tDCS AEs at T1	Mean intensity range of the effect (0 = absent, 1 = mild, 2 = moderate, 3 = severe)	No. of subjects reporting tDCS AEs at T2	Mean intensity range of the effect (0 = absent, 1 = mild, 2 = moderate, 3 = severe)	No. of subjects reporting tDCS AEs at T1	Mean intensity range of the effect (0 = absent, 1 = mild, 2 = moderate, 3 = severe)	No. of subjects reporting tDCS AEs at T2	Mean intensity range of the effect (0 = absent, 1 = mild, 2 = moderate, 3 = severe)
Headache	2	1	2	1.5	2	1.5	2	1
Neck pain	1	1	0	—	1	1.5	1	1
Scalp pain	1	1	0	—	3	1.5	0	—
Burning	2	1	0	—	3	1	0	—
Tingling	3	1	0	—	3	1.5	1	1
Drowsiness	1	0	1	1	1	1	1	1
Lack of concentration	1	0	1	1	0	—	0	—
Feelings change	1	1	0	—	0	—	0	—
Were you afraid?	No				No			
Would you do it again?	8	Yes			8	Yes		

Data represent the number of subjects, both for active and sham tDCS sessions, that reported the specific adverse effects at T1 (immediately after tDCS) and at T2 (90 min after tDCS); if adverse effects were present, the intensity were reported (0 = absent, 1 = mild, 2 = moderate, 3 = severe).

Table 3: Side effects' questionnaire at 24 h after tDCS session.

Adverse Effects Questionnaire (AEs-Q) items	Active ($n = 8$ subjects)		Sham ($n = 8$ subjects)	
	No. of subjects reporting tDCS AEs after 24 h (T3)	Mean intensity range of the effect (0 = absent, 1 = mild, 2 = moderate, 3 = severe)	No. of subjects reporting tDCS AEs after 24 h (T3)	Mean intensity range of the effect (0 = absent, 1 = mild, 2 = moderate, 3 = severe)
Difficulty falling asleep	0	—	0	—
Night awakenings	0	—	0	—
Early awakenings	0	—	0	—
Insomnia	0	—	0	—
Daytime sleepiness	1	1	1	1
Reduction of activities	0	—	0	—
Hyperactivity	1	1	1	1
Inattention	2	1	1	1
Irritability	1	1	0	—
Restlessness	1	1	1	1
Sadness	0	—	0	—
Euphoria	0	—	0	—

Data represent the number of subjects, both for active and sham tDCS sessions, that reported the specific adverse effects at T3 i.e., 24 h after the tDCS session; if adverse effects were present, the intensity were reported (0 = absent, 1 = mild, 2 = moderate, 3 = severe). The questionnaire was administrated by telephone.

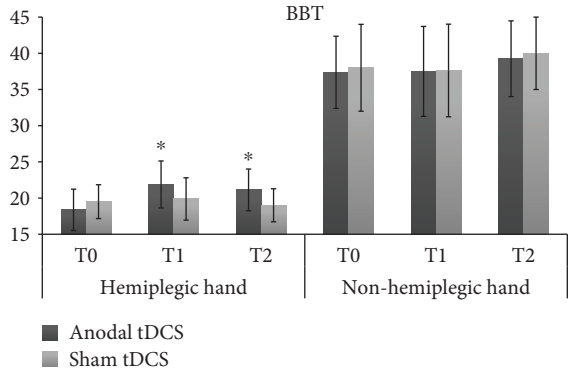

Figure 2: BBT scores (i.e., number of blocks moved in 1 min) for the hemiplegic and nonhemiplegic hands, at each assessment of the active anodal tDCS and sham tDCS sessions. T0 = baseline; T1 = immediately after the end of tDCS; T2 = 90 min after the stimulation session. * = significant change from baseline, $p < 0.05$.

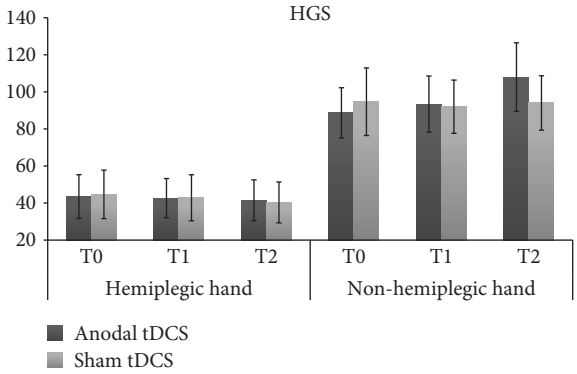

Figure 3: HGS scores (i.e., mean voluntary isometric strength, in kg) for the hemiplegic and nonhemiplegic hands, at each assessment of the real anodal tDCS and sham tDCS sessions. T0 = baseline; T1 = immediately after the end of tDCS; T2 = 90 min after the stimulation session.

No participant reported severe adverse effects following stimulation. With respect to the self-report questionnaire assessing tDCS side effects, as shown in Tables 2 and 3, only a limited number of participants reported transient and slight discomfort after stimulation, but this occurred in a similar number of participants, with comparable intensity, during both active (mean number of participants reporting 1 or more side effect = 1.5; mean total score = 0.75) and sham tDCS (mean number of participants reporting 1 or more side effect = 1; mean total score = 1, vs. active tDCS), as assessed by comparing active and sham tDCS with Wilcoxon test: number of participants reporting side effect, $Z = 0.37$, $p = 0.72$, intensity of the reported side effects, $Z = 1.05$, $p = 0.3$.

3.3. Exploratory Analysis of Demographic and Lesion Effects. Given the heterogeneity of our small sample with respect to age and lesion size (see Table 1), correlation analyses were performed for BBT, where a significant improvement in tDCS was found. In particular, Pearson correlations were used to test the association between improvement for BBT after active tDCS (T1 *minus* T0) and age (mean age = 17.5 ± 6.1 years) and lesion; the latter considering in different size analyses of hemispheric damage (i.e., mean lesion severity score = 6.8 ± 4.5) and of subcortical damage (i.e., mean lesion severity score = 2.3 ± 1.9), their sum (i.e., mean lesion severity score = 8.9 ± 4.9), and only frontal lobe damage (i.e., mean lesion severity score = 1.8 ± 1.2). All correlation analyses did not show any association between improvement brought about by active anodal tDCS and the considered factor: age ($r = 0.20$, $p = 0.64$), cortical lesion ($r = -0.40$, $p = 0.38$), subcortical lesion ($r = 0.36$, $p = 0.42$), cortical-subcortical lesion ($r = -0.17$, $p = 0.72$), and frontal lobe lesion ($r = -0.40$, $p = 0.38$). To further check for possible effects of age and lesion size, analyses of covariance (ANCOVA) were also performed, with Time (T0 and T1)

as within-subject factor and, as linear and interactive covariates, age and abovementioned measures of brain lesion. In every ANCOVA, no significant interaction between Time and covariates was found (all $p > 0.4$).

4. Discussion

The main aim of this pilot study was to explore the effects of a single application of anodal tDCS at 1.5 mA (a current intensity so far never tested in UCP) on the motor performance in individuals affected by UCP, considering also the assessment and recording of possible side effects. Results show that a single application of anodal tDCS over the affected M1 can improve, in a safe and well-tolerated way, unilateral manual function (hand dexterity) of subjects with UCP; improvement emerges immediately at the end of stimulation and persists for at least 90 minutes. It is worth noting, improvement was confined to the hemiplegic hand, while performance of the nonhemiplegic one was not influenced by tDCS.

Regarding HGS, no effect was brought about by tDCS. On the one hand, it should be noted that this test was performed on only six participants (see Results), so the absence of the effect could be related to a smaller sample, as compared to BBT. On the other hand, BBT and HGS measure different aspects of motor behavior; the first measures unilateral gross manual dexterity while the second one measures isometric force of voluntary movements. It follows that anodal tDCS may be more useful in changing functional hand performance, closer to real-world object manipulation, rather than lower motor function, such as muscular contractions, at least with the current parameters (intensity, duration, and polarity), and in the case of a single application.

Maintenance of improvement for BBT after 90 minutes is in line with the neurophysiological evidence showing that a single application of anodal tDCS for more than 10 minutes can induce after-effects on motor cortex excitability that last up to 90 minutes [26]. We did not assess whether such motor improvements were maintained over time, although we speculate a return to baseline performance since the two pre-tDCS assessments did not differ from each other and those participants who received active tDCS as first had a T0 score of the sham tDCS session (15.7) almost comparable to that of the active session (T0 = 15). Since stimulation sessions were performed at least 24 hours apart, this indicates that the effect of tDCS was transient, likely disappearing the day after. However, this aspect deserves further empirical investigation.

Finally, our results apparently show no relationship between tDCS effects at BBT and brain lesion timing, site, and severity. Previous studies have demonstrated that timing and severity of brain lesion are related to hand motor function, assessed by function and activity levels, in children with UCP [37, 43]. However, the heterogeneity of our small sample precludes any definitive statement on the absence of the associations between tDCS effects and individual demographic and brain lesion characteristics. Indeed, different plasticity mechanisms are involved in function recovery after unilateral brain lesions according to the involvement of different brain cells and structures. A better understanding of the possible role of brain lesion-related factors to tDCS effects, also through the use of more advanced imaging techniques, is mandatory in order to adapt intervention strategies. We can only speculate that individual patterns of corticospinal reorganization in UCP might impact tDCS efficacy, especially with respect to the hemisphere stimulated and current polarity, more than brain structural abnormalities [26]. Future studies are needed to verify this hypothesis.

Importantly, in line with previous evidence, during this study no serious adverse effects were induced by tDCS both immediately after and in follow-up (90 minutes and 24 hours after tDCS session), providing a first indication on the safety and good tolerance of anodal stimulation at 1.5 mA for 20 minutes in UCP, when NIBS guidelines for safe application are followed [44, 45]. Some side effects occurred in a limited number of participants, but they were mild and transient, and similar in both the active and sham sessions. Moreover, we did not detect any tDCS-related changes in blood pressure, heart rate, rhythm and quality of nocturnal sleep, mood, and daily activities (the latter also checked the day after the stimulation, at 24 hours).

The main limitations of this study are the small size and high heterogeneity of sample. Although to be viewed as preliminary, the evidence from this study supports the potential facilitatory effects of anodal tDCS in promoting improvement of unilateral manual disorders in UCP and suggests the safety of this stimulation approach in the pediatric neurological population.

Further studies on larger samples of subjects with UCP are needed to confirm and broaden our preliminary findings. From a rehabilitation perspective, it will be of interest to combine multiple sessions of anodal tDCS with a motor training, considering that the cathodal tDCS was unable to increase the motor-learning gains in subjects with UCP [26, 30]. The optimal dosage, timing, and montage of tDCS still need to be fully determined, also for adults. Here we provide an initial evidence of the efficacy of a current intensity of 1.5 mA for anodal stimulation; further studies in UCP are required to verify whether the intensity of 1.5 mA could be more, equal, or less effective than other intensities. Moreover, the influences of timing of brain lesion and type of corticospinal reorganization as well as motor and neurological degree of severity need to be further investigated given that our preliminary findings are inclusive in this regard. In this regard, another major limitation of the present study is the absence of the assessment of the neurophysiological status of our UCP participants, which precluded the evaluation of tDCS effects on cortical responses, as well as of the relationship between tDCS-induced behavioral gains and underlying neurophysiology. In the developing brain with neurologic injury, motor outcomes and tDCS effects are both related to differences in the corticospinal circuitry [15, 26, 46]. Finally, the development of specific guidelines for the application of tDCS in the pediatric population could facilitate recruitment and standardization on the use and management of tDCS [44] and potentially lead to a greater role as a therapeutic tool for neurodevelopmental rehabilitation.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The study was supported by the Italian Ministry of Health Grant RC 2017 and 5/1000 Grant 2015.

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Hindawi
Neural Plasticity
Volume 2018, Article ID 1435808, 14 pages
https://doi.org/10.1155/2018/1435808

Research Article

Impairments of Visuospatial Attention in Children with Unilateral Spastic Cerebral Palsy

Gaétan Ickx,[1] **Samar M. Hatem**,[1,2,3] **Inmaculada Riquelme**,[4,5] **Kathleen M. Friel**,[6,7] **Camille Henne**,[3] **Rodrigo Araneda**,[1] **Andrew M. Gordon**,[8] **and Yannick Bleyenheuft**[1]

[1]*Institute of Neuroscience, Université catholique de Louvain Brussels, Belgium*
[2]*Faculty of Medicine and Pharmacy, Faculty of Physical Education and Physiotherapy, Vrije Universiteit Brussel, Brussels, Belgium*
[3]*Brugmann University Hospital, Brussels, Belgium*
[4]*Research Institute on Health Sciences (IUNICS-IDISBA), University of the Balearic Islands, Palma de Mallorca, Spain*
[5]*Department of Nursing and Physiotherapy, University of the Balearic Islands, Palma de Mallorca, Spain*
[6]*Burke Neurological Institute, White Plains, NY, USA*
[7]*Weill Cornell Medicine, New York, NY, USA*
[8]*Teachers College, Columbia University, New York, NY, USA*

Correspondence should be addressed to Yannick Bleyenheuft; yannick.bleyenheuft@uclouvain.be

Received 22 March 2018; Revised 10 July 2018; Accepted 17 September 2018; Published 17 December 2018

Academic Editor: Michael Borich

Aim. This observational study aimed at assessing the prevalence of visuospatial attention deficits in children with unilateral spastic cerebral palsy (USCP), taking into consideration the affected hemibody and the localization of the brain lesion. *Method*. Seventy-five children with USCP were assessed with four visuospatial attention tests: star cancellation, Ogden figure copy, line bisection, and proprioceptive pointing. *Results*. A majority (64%) of children with USCP presented a deficit in at least one test compared to the reference values. The alterations observed in children with left or right USCP were related to egocentric or allocentric neglect, respectively. Children with cortico/subcortical lesion presented more often visuospatial attention deficits than children with periventricular lesion. Visuospatial attention deficits were not associated with brain lesion locations. *Interpretation*. Visuospatial attention deficits are prevalent in children with USCP and should be taken into account during their rehabilitation process. The present results shed new light on the interpretation of motor impairments in children with USCP as they may be influenced by the frequent presence of visuospatial deficits.

1. Introduction

Cerebral palsy (CP) results from brain lesions occurring during prenatal, perinatal, or early postnatal life. Cerebral palsy's overall prevalence is 2 per thousand live births and highest in children born before 28 weeks of gestation [1]. One of the most common subtypes of CP is unilateral spastic cerebral palsy (USCP) which represents up to 34% of all cases [1–5]. The main consequence of USCP is motor impairment which depends on the timing, size, and localization of the lesion as well as on the child's cerebral reorganization and recovery [6]. Additional impairments include deficits in sensory and cognitive function as well as sensory-motor integration [7, 8]. Visuospatial attention is the capacity of someone to attend to and to process stimuli in his surrounding space [9]. Visuospatial attention deficits are likely to be present in children with USCP, probably at least in part, influenced by the impact of the motor deficit over the attentional system [10], though they scarcely have been studied.

Visuospatial attention deficits have been widely studied in adult patients with acquired brain lesions and are mainly observed in lesions of the right hemisphere. They lead to hemineglect of the contralesional body and hemispace in 10 to 33% of patients [11–13]. Neuroimaging studies have shown a relationship between hemineglect and lesions located in the right temporoparietal junction (TPJ), as well

as in certain areas of the frontal, parietal, and temporal lobe [14, 15]. In visuospatial attention, different frames of reference—either egocentric or allocentric—can be distinguished. Egocentric neglect is described with regard to the body midline of the patient (i.e., the patient neglects stimuli presented on one side of the hemispace referred to his own body midline) and allocentric neglect is described with regard to the midline of an object in the peripersonal or extrapersonal space (i.e., the patient neglects stimuli on one side of the object's midline). The egocentric visuospatial representation is important for movement planning and motor control during direct interaction between body and objects, while the allocentric representation is important for determining spatial references in the environment. The interaction between the allocentric and egocentric visuospatial representations allows for spatial processing [16, 17]. Both allocentric and egocentric visuospatial representations show a progressive maturation with age in typically developing children, with only the egocentric visuospatial representation reaching maturity upon adolescence [14, 18]. After stroke in adults, dissociations may appear between egocentric and allocentric neglect [14]. These dissociations may differ in function of the physical distance between the subject and the visuospatial attention test [19]. Despite the relevance of both ego-and allocentric spatial representations, studies in children with USCP have interpreted visuospatial assessments mostly with regard to the egocentric reference frame (cancellation tasks, figure copy, drawing, and exploration tasks). To our knowledge, no studies specifically have assessed allocentric visuospatial attention in a large sample of children with USCP.

Few studies have reported lateralized visuospatial attention deficits in children with early brain lesions. Trauner [20], in a study with a large sample of children with early brain lesions ($n = 60$) and typically developing ($n = 36$) children, reported evidence of spatial neglect in two-thirds of children with both left and right brain lesions. In this study, a board with toys was presented to toddlers and the localization of toys touched by the child was recorded. Other studies [21–23] also reported the presence of spatial neglect in children with a right or left early brain lesion using, for example, the teddy bear cancellation test. Another study focused on children with early left brain damage [24] and reported the presence of a correlation between the reorganization of language function in the right hemisphere and visuospatial performance in the star cancellation test. Yousefian et al. [25] observed contralateral neglect in children with perinatal stroke using the clock drawing test in comparison with a control group. Differences were reported according to the side of lesion and the age of children: younger children (6–8 years) with right hemispheric lesions had error patterns similar to adult patients with right hemispheric lesions [25]. Visuospatial attention appears as a dynamic process maturing with age in children with CP as well as in typically developing children [18, 25]. While contralateral neglect in adults is mainly observed with right hemispheric lesions, it may occur in children with right or with left hemispheric lesions. This suggests differences in the anatomical distribution and brain reorganization of visuospatial abilities between the developing and mature brain.

Independently from contralateral neglect, children with CP also have been reported to present deficits of executive functions and more specifically of global attentional control. One study showed attentional deficits in children with CP, as well as a lower performance of inhibition, working memory, and general executive function [26]. Another study reported the presence of global executive deficits in children with CP based on the Rey-Osterrieth complex figure and subtests of different executive functioning assessments [27]. Attentional deficits were reported as nonlateralized, though some differences were observed between children with left and right CP in inhibition/switching tasks.

Furthermore, visuospatial attention has been shown to be acquired in function of locomotor experience. Previous research has shown in toddlers that visuospatial attention improves with the development of walking abilities [28]. In children with CP, brain plasticity and reorganization are correlated with several functions such as motor abilities, language, and vision [29–33].

The aim of the present study is to investigate the prevalence of visuospatial attention deficits in a large sample of children with USCP, using both ego- and allocentric tests with regard to the affected hemibody. We hypothesized that many children with USCP would show abnormal values in both ego- and allocentric visuospatial attention tests. Detecting the presence of these deficits appears as important to tailor the rehabilitation process to each child and thus to improve his/her ability in everyday motor activities.

2. Method

2.1. Participants. Children with USCP ($n = 75$) were recruited by the MSL-IN Lab (Institute of Neuroscience, Université Catholique de Louvain, Brussels, Belgium) and the Center for Cerebral Palsy Research (Teachers College, Columbia University, United States) during four consecutive years (2013–2016). All children were participants of an intensive rehabilitation program and were assessed for the present research before starting the intensive rehabilitation programs.

The inclusion criteria were as follows: (1) aged between 5 and 18 years, (2) ability to grasp light objects and lift the more affected arm 15 cm above a table surface, (3) ability to follow instructions and complete testing, (4) attending school in the same grade as their typically developing peers of the same age, (5) Manual Ability Classification System [34] levels I, II, or III, and (6) Global Motor Function Classification System levels I, II, or III [35]. Exclusion criteria were as follows: (1) uncontrolled seizures, (2) orthopedic surgery or botulinum toxin injections less than twelve months before or within the study period, and (3) possibility of treatment/testing interference because of uncorrected visual problems (as described by their physician). No formal cognitive assessment was performed or used as inclusion criterion in the present study. Participants and caregivers provided informed consent. The study was approved by the Institutional Review Boards of the Teachers College, Columbia University, and of the Université Catholique de Louvain.

Brain MRI and ophthalmological assessments were not performed as part of this study. Given that the children were

participants of an intensive rehabilitation program off-site from the hospital, there was no simple access to the technologies needed for brain imagery and ophthalmological evaluations within the time frame of the present study. Therefore, when previous MRI was available, brain lesions were classified by a neuroradiologist using the criteria of Krägeloh-Mann and Horber [36], allowing to define the origin/timing of their brain lesion (cortical malformation, periventricular lesion, or cortical/subcortical lesion). In addition, the localization of the lesion in the brain was described. In a subsample of the study population and not performed as part of this research, a previous detailed ophthalmological examination by an ophthalmologist (Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium) was available and retrieved from the medical file. This examination included assessment of visual function (visual acuity, visual field defect with Goldmann visual field perimetry, color perception, and refractive error), binocular vision (binocular vision with Worth's test and Bi-prism test, near point of convergence, eye motility assessment with Broad H, stereopsis and stereoacuity with TNO test, and strabismus with cover test), and finally ophthalmological health (examination of anterior and posterior segment). More specifically, in the Goldmann visual field perimetry, children have to maintain fixation on a central point; the fixation is controlled by a trained perimetrist, while a visual stimulus is moved around the patients' visual field. The children have to report by pressing a button, whether they can see the target or not. The visual field of the child is then plotted [37]. In the broad H test, the children are asked to follow a target (a penlight) which is moved in an H pattern to the edge of the binocular field. The ophthalmologist has to record any misalignments of the patients' eyes which could indicate eye motility deficits [38].

2.2. Assessment Tools. Four assessments of visuospatial attention were used: star cancellation, Ogden figure copy, line bisection, and proprioceptive pointing. The four visuospatial tasks were chosen for the following reasons: (1) reference values are available in same-aged typically developing children [18]; (2) the same tasks can be used in adults, ensuring the possibility of a follow-up in the transition from childhood to adulthood; (3) the tasks can be performed single-handedly with the less affected hand, limiting a bias due to sensorimotor deficit; (4) the tasks do not require any other material than paper and pencil; and (5) the tasks are language-independent. The latter two reasons were important in this study as children were included both in Belgium (French) and the US (English). Results of the visuospatial attention assessments were considered as abnormal when lying outside the range of normal values previously described for each age category [26].

2.2.1. Star Cancellation. The test consists of an A4 sheet of paper with stars of two different sizes as well as distractor words which are semirandomly distributed. The child is asked to cancel all small stars. The following variables are recorded: the number of stars omitted on each side (left, right) and the total number of omitted stars [39]. The absolute difference between the number of left omitted stars and right omitted stars also is computed. The variable used to determine if a child with USCP presents with an abnormal value compared to reference values is the total number of omitted stars. Star cancellation mainly assesses egocentric neglect [19].

2.2.2. Ogden Figure Copy. This test consists of a drawing copy task. The child is asked to copy a figure (a house and 4 trees). The score ranges from 0 (no omissions) to 4 (multiple omissions) [40] and is the variable used to determine if a child with USCP presents with an abnormal value compared to reference values. Ogden figure copy assesses both ego-and allocentric neglect [41].

2.2.3. Line Bisection. The line bisection test consists of 2 pages with 10 lines of different lengths on each page. The child is asked to indicate the middle of each line by making a mark with a pencil. The deviation from the center, in percentage of half the line length, is computed with the following formula: deviation = $(b - a)/a^{*}100$, where a is half length of the line and b is the distance between the beginning of the line and the mark made by the child [42]. The variable used to determine if a child with USCP presents with an abnormal value compared to reference values is the average deviation (in percentage) from the center of each line. Line bisection test assesses allocentric neglect. An error towards the paretic side of space is recorded as a negative value.

2.2.4. Proprioceptive Pointing. The child is blindfolded and seated in front of a table. A paper sheet with angled graduation lines (deviation in degrees) from a central point is aligned with the body midline of the child. The child is asked to point straight ahead on the table by moving his finger [43]. The pointing is performed three times. The variable recorded is the average deviation (mean of the three pointings in degrees) with regard to the child's body midline. This variable is used to determine if a child with USCP presents with an abnormal value compared to reference values. Proprioceptive pointing assesses egocentric neglect. A deviation towards the paretic side of space is recorded as a negative value.

3. Statistical Analysis

The descriptive statistic is as follows: a child with USCP was considered to have an abnormal value for any of the visuospatial attention tests if his/her result was outside the age-corrected reference values published previously [18].

Chi-square tests were used to investigate the association between demographic characteristics and the presence of abnormal visuospatial attention assessments, as well as to investigate the association between different abnormal visuospatial attention assessments. Chi-square tests were used to investigate the association between the presence of abnormal visuospatial attention assessments in different age groups (13 age groups from 5 to 17 yrs).

Chi-square tests were used to investigate the association between the presence of abnormal visuospatial assessments in children with left USCP vs. children with right USCP, in

children taking antiepileptic drugs vs. children not taking antiepileptics, and in children with predominant periventricular brain lesions vs. children with cortico/subcortical brain lesions as well as between the different localizations of lesions.

Post hoc Bonferroni was used to correct for the multiplicity of tests.

Student t-test was used to compare intrasubject differences between omissions on one side and the other side of hemispace for the star cancellation test.

The statistical analysis package SPSS was used for all analyses. Significance level was set at $p \leq 0.050$.

4. Results

4.1. Participants. The demographic and clinical data of the study sample are summarized in Table 1. The sample consisted of 75 children with USCP from 5 to 17 years old (mean = 9 y 3 m, SD = 2 y 11 m, 42 boys and 33 girls): 45 children with right USCP and 30 children with left USCP. Children were classified following the Manual Ability Classification System [34] as levels I ($n = 16$), II ($n = 50$), or III ($n = 9$). Brain lesions as observed on available MRI ($n = 69$) were subcortical and cortical lesions of frontal/parietal/temporal areas (n = 4), subcortical and cortical lesions of frontal/parietal/temporal areas and insula ($n = 13$), subcortical and cortical lesions of frontal/parietal/temporal/occipital areas and insula ($n = 9$), subcortical and cortical lesions of parietal/occipital/temporal areas ($n = 3$), subcortical and cortical lesions of parietal/temporal areas ($n = 9$), and subcortical without cortical lesions ($n = 31$). Ophthalmological examinations were available in a subsample of 13 children. Three children had a visual field defect as measured using Goldmann visual field perimetry (2 hemianopsia, 1 quadranopsia; see Table 2), and 3 children had a deficit of eye motility [37]. Two of those three children had at least 1 abnormal result upon testing of visuospatial attention. Due to the small size of the subsample, no further statistical analyses were performed. Six children were taking antiepileptic drugs and had no clinically observable seizures in their recent medical history or at the time of testing.

The individual clinical data and individual results of the visuospatial attention assessments are shown in Table 2.

4.2. Prevalence of Visuospatial Attention Deficits in Children with USCP. Sixty percent of the children presented with abnormal values of at least one visuospatial attention test. 28% of the children with USCP presented with abnormal values of two or more visuospatial attention tests. 10.7% of the children presented with abnormal values of three or more visuospatial attention tests. No association was found between age groups and the presence of abnormal visuospatial assessments (chi-square, all $p > 0.390$). No significant association was observed between the MACS level and the presence of abnormal visuospatial attention assessments (chi-square, all $p > 0.054$). No significant association was observed between the GMFCS level and the presence of abnormal visuospatial attention assessments (chi-square, all $p > 0.402$).

Differences in the percentage of children with deficits were observed depending on the timing of the lesion: compared to children with periventricular lesions, a larger percentage of children with corticosubcortical lesions presented with visuospatial attention deficits ($\chi^2(3,\ n = 32) = 16.655$; $p = 0.001$). The prevalence of abnormal visuospatial attention assessments was not different between the different brain lesion localizations (chi-square, all $p > 0.612$).

No differences were observed for the prevalence of abnormal visuospatial attention assessments between children taking antiepileptic medication and those without (chi-square, all $p > 0.663$).

A significant association was observed between the prevalence of abnormal star cancellation and the prevalence of abnormal Ogden figure copy ($\chi^2(1,\ n = 14) = 11.193$; $p = 0.010$). No other significant associations between abnormal visuospatial attention assessments were found (all $p > 1.000$).

Figure 1 shows the prevalence of abnormal values for one, two, three, or more visuospatial attention tests in the whole sample of children with USCP as well as in children classified by lesion timing. Figure 2 shows the prevalence of abnormal visuospatial attention assessments classified by lesion localization.

4.3. Prevalence of Abnormal Findings in Each of the Visuospatial Attention Tests in Children with USCP. The prevalence of abnormal values in each of the four visuospatial attention tests in children with USCP is described in Figures 3 and 4.

4.3.1. Star Cancellation. 18.7% of the children (number of tested children = 75) presented with abnormal values. The absolute difference between left and right omitted stars was significantly different from "zero," indicating that children omitted more stars on one side than on the other (children with left USCP: $t(1, 29) = 2.769$; $p = 0.01$; children with right USCP: $t(1, 44) = 4.100$; $p < 0.0001$) (Figure 4). When the prevalence of abnormal values was compared between children with left and right USCP, children with a left USCP presented significantly more abnormal values for left omitted stars than children with right USCP ($\chi^2(1,\ n = 30) = 4.559$; $p = 0.033$) (Figure 5). The prevalence of abnormal values was not significantly different between children with periventricular or corticosubcortical lesion for the total number of omitted stars: $\chi^2(1,\ n = 32) = 2.294$; $p = 0.130$. In the number of right omitted stars, the prevalence of abnormal values was significantly larger in children with corticosubcortical lesions than in children with periventricular lesions ($\chi^2(1,\ n = 32$) = 49.095; $p < 0.001$). The prevalence of abnormal values did not differ by lesion location (all $p > 0.300$).

4.3.2. Ogden Figure Copy. 25.3% of the children (number of tested children = 75) presented with abnormal values. The prevalence of abnormal values was not significantly different between children with right and left USCP: $\chi^2(1,\ n = 30$) = 0.084; $p = 0.773$. The prevalence of abnormal values was significantly higher in children with corticosubcortical lesions than in children with periventricular lesions ($\chi^2(1,\ n = 32$) = 9.590; $p = 0.002$) (Figure 4). The prevalence of abnormal values did not differ by lesion location (all $p > 0.600$).

Table 1: Demographic and clinical characteristics of children with unilateral spastic cerebral palsy.

	More affected upper extremity		
	Left	Right	All
Age	9 y 5 m (3 y)	9 y 1 m (2 y 11 m)	9 y 3 m (2 y 11 m)
Gender (*n*)			
Female	9	24	33
Male	21	21	42
Lesion timing (*n*)			
Brain malformation	4	2	6
Periventricular white matter lesion	14	17	31
Cortical/subcortical lesion	10	22	32
NA	2	4	6
MACS (level)			
Level I	7	9	16
Level II	22	28	50
Level III	1	8	9
GMFCS (level)			
Level I	27	37	64
Level II	3	8	11
Total (*n*)	30	45	75

MACS: Manual Ability Classification System; GMFCS: Global Motor Function Classification System.

4.3.3. Line Bisection. 44% of children (number of tested children = 75) presented with abnormal values. Twenty-five children were above the upper bound of the reference range (i.e., bisection deviated towards the nonparetic hemispace), and 8 children were below the lower bound of the reference range (i.e., bisection deviated towards the paretic hemispace). Children with right USCP had abnormal values more often than children with left USCP ($\chi^2(1, n = 45) = 6.427$; $p = 0.011$; children with right USCP = 51.1% and children with left USCP = 33.3%). In the line bisection test, the prevalence of abnormal values was not significantly different in function of lesion timing ($\chi^2(1, n = 32) = 2.807$; $p = 0.094$). The prevalence of abnormal values did not differ by lesion location (all $p > 1.00$).

4.3.4. Proprioceptive Pointing. 10.6% of children (number of tested children = 75) presented with abnormal values: 7 children deviated towards the nonparetic hemispace, and 1 child deviated towards the paretic hemispace. The prevalence of abnormal values was not significantly different between children with right and left USCP: $\chi^2(1, n = 30) = 0.037$; $p = 0.848$. The prevalence of abnormal values was not significantly different between children with predominant white matter lesions and predominant grey matter lesions: $\chi^2(1, n = 32) = 1283$; $p = 0.257$. The prevalence of abnormal values did not differ by lesion location (all $p > 1.00$).

5. Discussion

The aim of this study was to investigate the prevalence of visuospatial attention deficits among children with USCP using both ego- and allocentric tests, taking into consideration the affected hemibody. A majority of children with USCP presented with abnormal visuospatial attention as 60% of our sample scored outside the reference values for at least one visuospatial attention test. In addition, the results indicated a difference between children with left and right USCP. Children with a left USCP showed predominantly an egocentric impairment and children with a right USCP showed mainly an allocentric deficit. Lesion timing also had an influence on the prevalence of visuospatial attention deficits: children with corticosubcortical lesions presented more frequent visuospatial attention deficits than children with periventricular brain lesion. A significant association was observed between an abnormal star cancellation test and an abnormal Ogden figure copy in children with USCP, as previously reported in typically developing children [18, 44].

The originality of the present study lies within the large school-aged population of exclusively children with USCP, investigating both ego- and allocentric visuospatial attention. Previous studies assessing visuospatial attention abilities included children with all types of acquired brain lesions and used a limited number of tasks: the Teddy Bear cancellation task [21] or a spatial exploration task [20] or the clock drawing test [25]. The present study confirms previous findings in egocentric visuospatial attention assessments [20–23], while giving a more complete overview of visuospatial attention deficits in children with USCP.

More than half of the children participating in this study presented with abnormal values for at least one visuospatial attention test and almost one-third of the sample for two or more tests. The presence of visuospatial attention deficits and in particular neglect of one side of space could be relevant for the rehabilitation process in children with USCP. Evidence shows that visuospatial attention interacts with motor function, for instance, during eye-limb coordination

Table 2

Subject	Age	Total omitted stars (*n*)	Ogden figure copy (score)	Line bisection: average error (%)	Proprioceptive pointing: average error (%)	MACS (level)	GMFCS (level)	Lesion timing	Lesion side	Lesion localization	Antiepileptic drug	Ophthalmologic deficits
1	5	4	1	20.8	−3.0	1	1	PWM/PVL	Left	c	NA	NA
2	5	16	4	−8.7	3.3	2	1	GMI	Right	e	NA	NA
3	6	3	0	−18.5	−4.3	2	1	GMI	Right	a	NA	NA
4	6	27	4	−0.1	2.5	1	1	No MRI	Right	a	NA	NA
5	6	0	0	−0.5	3.0	1	2	GMI	Left	c	NA	NA
6	6	12	4	−1.5	1.0	2	2	GMI	Left	c	z	O
7	6	9	3	38.5	4.0	2	1	GMI	Left	d	NA	NA
8	6	1	0	6.2	0.8	1	1	PWM/PVL	Left	f	NA	NA
9	6	8	2	−3.2	1.8	2	1	GMI	Left	f	NA	NA
10	6	0	0	−1.5	0.3	2	2	PWM/PVL	Left	g	NA	O
11	6	4	1	16.0	−1.0	3	1	PWM/PVL	Left	g	NA	NA
12	6	41	4	32.9	1.7	2	1	GMI	Left	g	NA	NA
13	6	3	2	19.0	7.5	1	1	PWM/PVL	Left	g	NA	NA
14	6	3	1	3.0	27.0	2	1	PWM/PVL	Right	g	NA	NA
15	6	1	1	11.8	6.0	1	1	PWM/PVL	Right	g	NA	NA
16	7	2	1	7.9	0.3	2	1	GMI	Left	b	NA	O
17	7	1	0	6.5	−5.8	2	1	GMI	Left	b	NA	NA
18	7	5	1	19.4	−5.5	2	1	GMI	Left	c	NA	NA
19	7	1	0	9.5	2.5	2	1	PWM/PVL	Left	c	NA	NA
20	7	8	2	14.8	6.8	2	2	GMI	Left	d	NA	H/St
21	7	1	1	3.2	0.5	2	1	GMI	Left	d	NA	NA
22	7	6	1	12.0	2.5	2	1	GMI	Left	d	NA	NA
23	7	5	2	8.5	1.3	2	1	GMI	Left	f	NA	NA
24	7	2	0	−10,8	−0,5	2	1	MCD	Right	f	z	NA
25	7	0	0	−4.2	5.3	1	1	MCD	Right	f	NA	NA
26	7	5	2	13.3	1.0	2	1	PWM/PVL	Left	g	NA	Exo/M-1
27	7	6	2	4.5	−0.3	2	1	No MRI	Left	g	NA	NA

Table 2: Continued.

Subject	Age	Total omitted stars (*n*)	Ogden figure copy (score)	Line bisection: average error (%)	Proprioceptive pointing: average error (%)	MACS (level)	GMFCS (level)	Lesion timing	Lesion side	Lesion localization	Antiepileptic drug	Ophthalmologic deficits
28	7	2	1	−10.1	−4.3	2	2	GMI	Right	g	NA	Q/Exo-R Hyper
29	7	9	0	−7.2	4.0	1	1	PWM/PVL	Right	g	NA	O
30	7	4	1	−8.7	6.7	3	1	PWM/PVL	Right	g	NA	NA
31	7	25	4	−30.6	0.0	2	1	MCD	Right	g	NA	NA
32	7	6	0	−3.9	−0.8	2	1	PWM/PVL	Right	g	NA	NA
33	8	0	1	−4.8	−3.5	2	1	GMI	Right	b	NA	St/Eso
34	8	14	2	−17.8	−0.7	2	1	PWM/PVL	Right	c	NA	NA
35	8	0	0	−7.1	−0.5	2	1	GMI	Right	c	NA	NA
36	8	0	0	−2.7	5.0	3	1	GMI	Left	d	NA	NA
37	8	4	1	−6.3	6.3	2	1	GMI	Right	f	NA	NA
38	8	0	0	13.8	−1.0	2	2	GMI	Left	g	NA	NA
39	8	0	0	3.7	0.3	1	1	PWM/PVL	Left	g	NA	NA
40	8	0	0	4.5	1.8	1	1	PWM/PVL	Left	g	NA	NA
41	8	9	1	−9.1	−15.0	1	1	PWM/PVL	Right	g	NA	NA
42	8	31	2	−5.4	11.3	2	1	GMI	Right	g	NA	NA
43	9	7	0	8.0	4.3	3	1	GMI	Left	c	NA	NA
44	9	1	0	9.5	1.0	3	1	GMI	Left	c	NA	NA
45	9	0	1	−5.1	0.5	3	1	No MRI	Left	c	NA	NA
46	9	2	2	7.5	4.0	3	1	GMI	Left	d	NA	NA
47	9	4	0	−0.4	−0.4	2	2	MCD	Left	e	z	O
48	9	0	0	1.2	1.3	2	1	PWM/PVL	Left	g	NA	NA
49	9	5	0	11.5	−0.8	2	1	PWM/PVL	Left	g	NA	NA
50	10	2	2	28.0	4.8	2	2	GMI	Left	d	y	NA
51	10	0	1	−0.6	−1.8	2	2	GMI	Right	e	y	NA
52	10	0	0	−0.9	−0.8	2	2	PWM/PVL	Left	g	NA	O
53	10	2	0	−1.3	6.0	2	1	PWM/PVL	Left	g	NA	NA

Table 2: Continued.

Subject	Age	Total omitted stars (*n*)	Ogden figure copy (score)	Line bisection: average error (%)	Proprioceptive pointing: average error (%)	MACS (level)	GMFCS (level)	Lesion timing	Lesion side	Lesion localization	Antiepileptic drug	Ophthalmologic deficits
54	10	2	0	8.2	1.3	1	1	PWM/PVL	Left	g	NA	NA
55	11	1	0	−2.6	−0.5	2	1	No MRI	Left	a	NA	NA
56	11	3	0	6.4	1.8	1	1	GMI	Left	b	NA	NA
57	11	0	0	−2.8	0.0	1	1	GMI	Right	c	NA	O
58	11	1	0	−8.5	−4.0	2	1	GMI	Right	d	NA	NA
59	11	0	0	−1.0	2.3	2	2	PWM/PVL	Right	g	NA	NA
60	11	1	0	−5.2	−1.8	2	1	PWM/PVL	Right	g	NA	St/L-Hyper/M1/M-2
61	11	1	0	−11.4	1.8	2	1	PWM/PVL	Right	g	NA	NA
62	12	13	2	−0.6	−1.5	2	1	PWM/PVL	Left	a	NA	NA
63	12	1	2	−7.6	−1.8	2	1	No MRI	Right	a	NA	NA
64	12	0	0	−0.1	1.3	2	1	MCD	Left	f	NA	NA
65	12	0	0	2.3	−3.7	2	1	GMI	Left	f	NA	NA
66	12	4	0	2.0	−4.0	1	1	PWM/PVL	Left	g	NA	NA
67	12	0	0	−6.8	−1.7	1	1	MCD	Right	g	NA	NA
68	13	0	1	15.8	9.3	3	1	GMI	Left	c	NA	NA
69	13	1	0	6.3	0.0	2	1	PWM/PVL	Right	g	NA	NA
70	14	0	0	−2.6	0.0	2	1	PWM/PVL	Right	g	NA	NA
71	15	1	0	7.6	0.0	2	1	No MRI	Left	a	y; x	NA
72	15	0	0	3.2	1.5	2	1	PWM/PVL	Left	g	NA	NA
73	16	0	0	−6.8	−3.5	2	1	PWM/PVL	Right	d	NA	H/St/ESO/M-3
74	17	0	1	−12.8	1.0	2	1	PWM/PVL	Right	c	NA	NA
75	17	0	0	0.8	−4.7	3	1	GMI	Left	f	NA	NA

Lesion timing: MCD: brain malformation; PWM/PVL: periventricular white matter lesion; GMI: cortical/subcortical lesion. Lesion localization: a: no MRI; b: subcortical and cortical lesions of frontal/parietal/temporal areas; c: subcortical and cortical lesions of frontal/parietal/temporal areas and insula; d: subcortical and cortical lesions of frontal/parietal/temporal/occipital areas and insula; e: subcortical and cortical lesions of parietal/occipital/temporal lesion; f: subcortical and cortical lesions of parietal/temporal lesions; g: subcortical without cortical lesions. Antiepilectic drug: z: valproate; y: carbamazepine; x: lamotrigine. Ophthalmologic deficit: NA: no available data; O: no deficit; H: hemianopsia; Q: quadranopsia; St: stereoscopic vision deficit; Exo: exotropia; Eso: esotropia; R Hyper: right hypertropia; L Hyper: left hypertropia; M-1: eye motility deficit in the left eye; M-2: eye motility deficit in the right eye; M-3: presence of gaze-evoked nystagmus.

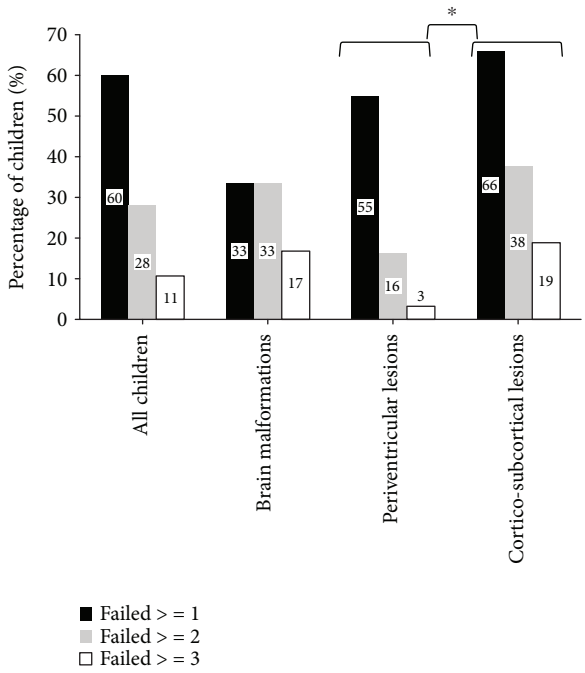

Figure 1: Percentage of children presenting with a visuospatial attention deficit in the whole sample.

[10]. In this way, an early motor deficit could have an impact on the development of the attentional system [45], for example, children with spastic diplegia have shown impairments in visual orientation tasks [46]. Ideally, a global deficit of attention or executive functioning should have been ruled out by a control task. This was not possible in the present study because children were included as participants of an intensive rehabilitation program and were subjected to a large number of assessments during the limited amount of time available before the start of rehabilitation program. However, a global attentional problem appears as improbable for two reasons: (1) visuospatial attention deficits were lateralized and (2) very few children showed abnormal results in all of the four visuospatial attention assessments.

As with other executive functions, visuospatial attention may develop with age. In typically developing children, the performance on some visuospatial attention tasks was shown to mature with age [18]. Previous studies have shown that younger children with CP may present with more visuospatial neglect than older children [23, 25]. The absence of any effect of age on the prevalence of visuospatial attention deficits in the present findings does not preclude that age still may influence their visuospatial abilities in children with CP. The present study was not designed to examine an effect of age: the age range of the included children was too narrow and the visuospatial attention assessments were administered only once in each subject. Hence, no age-related differences were observed.

Visuospatial attention deficits were more frequently observed in children with corticosubcortical lesions than in children with periventricular lesions. Previous studies suggested that children with cortico/subcortical lesion generally present with larger lesions than children with periventricular lesions. Also in these children, more associations are observed between lesion characteristics and clinical outcomes [6, 47]. Mailleux et al. [6] reported frequent and stronger associations between lesion characteristics (size, localization, and extent) and motor function in children with cortico/subcortical lesion than in children with periventricular lesions. Impaired upper extremity function [47] is also more common in CP children with cortical/subcortical lesion compared to periventricular lesions. This overall larger prevalence of deficits in children with cortico/subcortical lesions could be explained by the timing of the lesion. Cortico/subcortical lesions typically arise at the end of the 3rd trimester of gestation [36]: the later the lesion, the less likely it may allow for efficient reorganization/rewiring of affected functions in the brain.

Visuospatial attention deficits were observed in children with right as well as with left brain lesions. This clinical picture is very different from the one in adults demonstrating mainly hemineglect with right brain lesions [48] due to the lateralization of visuospatial abilities within the right hemisphere [49]. Similar results in children either with left or right brain lesions have been reported previously by Thareja et al. [23]. The fact that left brain lesions can lead to an alteration of visuospatial abilities in children with USCP can be explained by the important cerebral reorganization occurring after an early brain lesion. This observation may be explained by the "crowding hypothesis" [24, 50]: a left hemispheric lesion can shift the areas related with language from the left to the right hemisphere, thus affecting visuospatial function. Lidzba et al. [24] highlighted a correlation between the reorganization of language function in the right hemisphere and visuospatial performance in children with early cerebral lesions.

Differences in the type of hemineglect were observed between children with left and right USCP. In the star cancellation test (assessing mainly egocentric neglect), children with left USCP omitted more stars on the left side than on the right side and were more often outside the normative values for the number of left omitted stars than children with right USCP. On the other hand, children with right USCP more frequently presented with abnormal values of the line bisection test compared to children with left USCP, suggesting more often allocentric visuospatial impairment [19]. It has been suggested that different brain substrates are linked to egocentric and allocentric neglect: egocentric neglect being linked to the fronto-parieto-temporal network, while allocentric neglect being related to the parieto-temporo-occipital network [51]. Specifically, egocentric representation has been related with activation in the medial part of the left superior parietal lobe and the allocentric representation with activation in the right parietal lobe, occipitotemporal cortex, and hippocampal regions [52]. Besides the side of hemispheric lesion, specific characteristics of the brain lesion and postlesional brain reorganization and development also may explain the differential visuospatial attentional impairments: larger brain lesions have been observed in children with right USCP than in children with left USCP [53, 54]. However, in the present study, the presence of abnormal

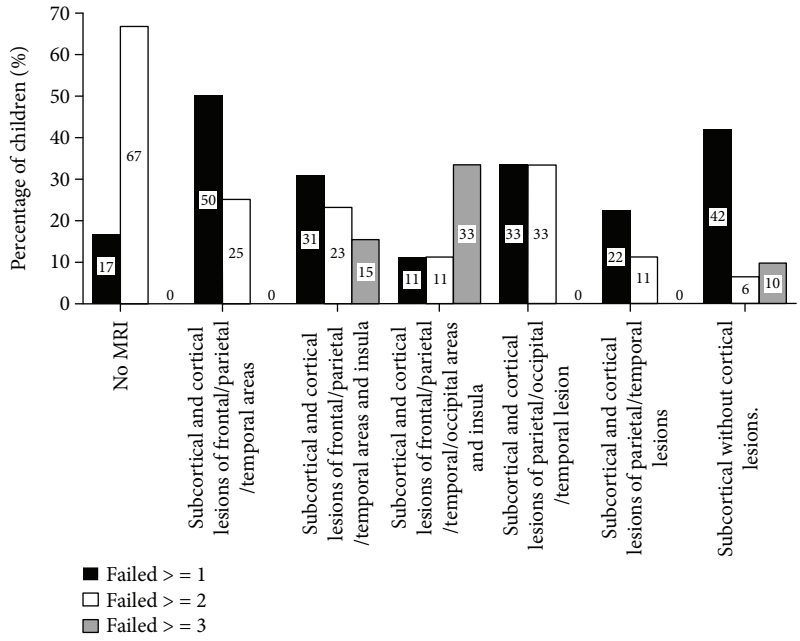

Figure 2: Percentage of children presenting with a visuospatial attention deficit in function of lesion localization.

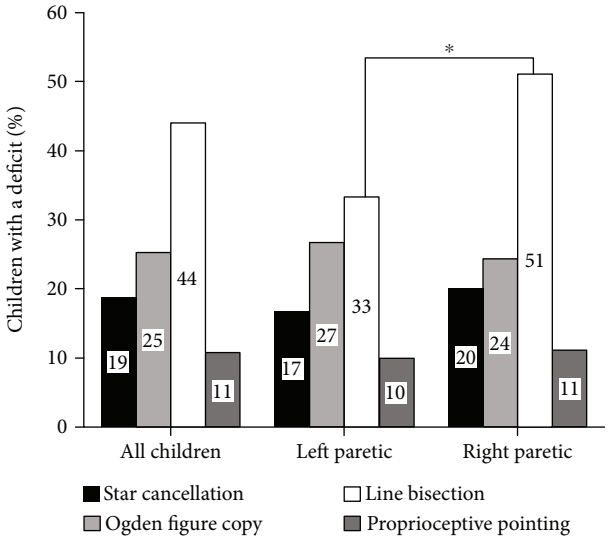

Figure 3: Percentage of children with USCP with abnormal values in each visuospatial attention test for the whole sample and for children with left or right USCP. Chi-square $^{*}p < 0.050$.

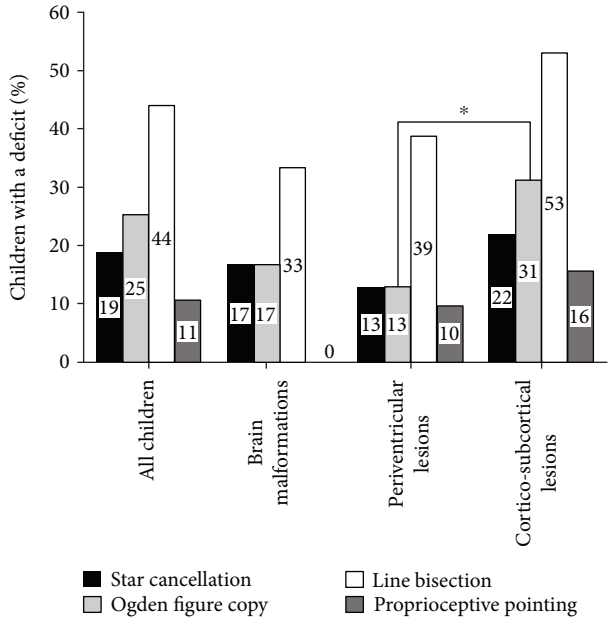

Figure 4: Percentage of children with USCP with abnormal values in each visuospatial attention test for the whole sample and for children with brain malformation, periventricular lesion, or corticosubcortical lesions. Chi-square $^{*}p < 0.050$.

visuospatial assessments was not related to the localization of brain lesions. It must be noted that brain MRIs were not acquired for this study specifically and lesion localizations were interpreted post hoc from available MRIs. More relevant imaging data including fMRI probably could clarify how lesion characteristics and brain reorganization and maturation relate to the development of visuospatial abilities in children with CP.

Among the limitations of the study, it is important to acknowledge the partial availability of ophthalmological examinations in the study sample. Previous studies have investigated the development of visual abilities through childhood as well as the importance of measuring such abilities in children with CP [55, 56]. It is not possible to establish the relationship between visual impairment and visuospatial attention deficits with only 20% of the study sample having received an ophthalmological examination during clinical

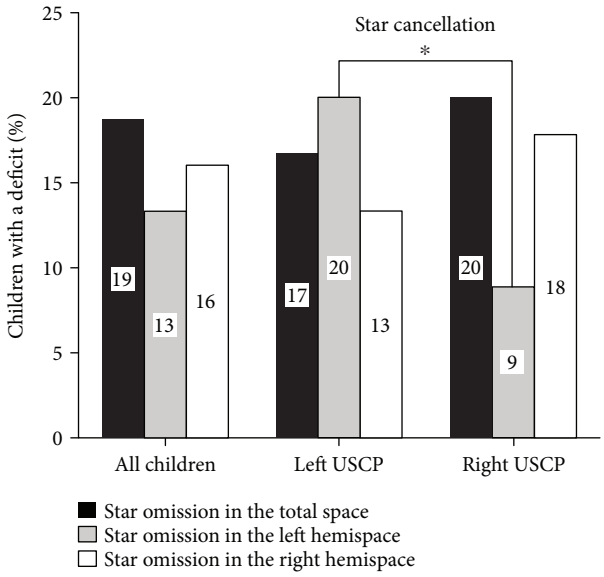

Figure 5: Percentage of children with USCP with abnormal findings in the star cancellation test for each hemispace (star omission in the total space, star omission in the left hemispace, and star omission in the right hemispace). Chi-square $^{*}p < 0.050$.

follow-up. Furthermore, visual field testing is rarely performed in a clinical setting and even more seldom in young children. We can only speculate on how potential visual field defects may have influenced visuospatial attention in our study sample. Adults with hemianopsia performing the line bisection test show an inverse pattern compared to adults with hemineglect [57–61]. Indeed, hemineglect patients bisect towards the ipsilesional side of the lines, whereas hemianoptic patients bisect towards the contralesional side of the lines [57, 58]. Deficits in oculomotor function may also impair visuospatial attention. Correct saccadic movements have been described as important for the development of visuospatial attention [10]. Ego et al. [62] have described a relatively preserved oculomotor function in children with CP which evolves with age to reach almost the same performance as typically developing children. It appears as less probable that deficits in oculomotor function would impair the possibility to scan the environment and lead to deficits of visuospatial attention. Future studies should include a complete ophthalmological examination for all study subjects to exclude underlying impairments of vision as a substrate for visuospatial attention deficits.

In addition, although children of this study attended school in the same grade as their typically developing peers of the same age, intellectual quotient (IQ) per se was not tested and thus we cannot exclude an influence of IQ on our results. This is especially a concern since an interplay between cognitive function and visuospatial abilities has been previously described [10, 27, 63].

This study gives a better insight in the prevalence of visuospatial attention deficits in children with USCP and highlights that visuospatial deficits are common among children with USCP and more frequent in children with cortico/subcortical lesions than in children with periventricular lesions. In order to properly diagnose these deficits, both egocentric and allocentric visuospatial attention tests are needed. Children with right and left USCP do not present the same type of visuospatial attention deficits: left USCP is more linked to egocentric neglect while right USCP is more linked to allocentric neglect. Also, visuospatial attention deficits observed in children with CP were different from those reported in adult patients. This may be due to the nature of brain lesions as well as the process of dynamic brain (re)organization [24, 32]. Though the present results did not indicate any relationship between age and visuospatial abilities in a cross-sectional sample of children with CP, future studies should further investigate the evolution of visuospatial attention deficits in the function of lesion characteristics and brain development in children with cerebral palsy. Indeed, it is possible that spatial deficits observed at a young age may become clinically insignificant at a later stage [18, 23, 25]. Future research should thus include medical imaging in combination with visuospatial and other neuropsychological assessments in a longitudinal perspective. The present findings may help in improving the rehabilitation of children with USCP as visuospatial abilities are critical for motor skill learning and motor control. Depending on the side of the brain lesion, children may show differential responses related to the lateralization aspect of these deficits. Different rehabilitation interventions have been described in adult patients such as vestibular stimulation or prismatic rehabilitation [64–66]. Prismatic rehabilitation has been reported as feasible in children with USCP [43]. Future studies should therefore investigate the effectiveness of prismatic rehabilitation applied to children with USCP for improving visuospatial neglect and possibly motor skill learning.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

What this paper adds. (i) Insight on the prevalence of visuospatial deficits among children with USCP. (ii) Highlights differences in visuospatial abilities between children with left and right USCP and their relationship with allocentric and egocentric visuospatial representations.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

This work was funded by the Foundation JED Belgique. Samar M. Hatem is a postdoctoral research fellow of FRS-FNRS Belgium (FNRS–SPD).

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Hindawi
Neural Plasticity
Volume 2018, Article ID 8472807, 11 pages
https://doi.org/10.1155/2018/8472807

Research Article

Potentials of Ultrahigh-Field MRI for the Study of Somatosensory Reorganization in Congenital Hemiplegia

Simona Fiori,[1] **Laura Biagi**,[2,3] **Paolo Cecchi**,[4] **Giovanni Cioni**,[1,5] **Elena Beani**,[1] **Michela Tosetti**,[2,3] **Mirco Cosottini**,[4,3] **and Andrea Guzzetta**[1,5]

[1]*Department of Developmental Neuroscience, IRCCS Fondazione Stella Maris, Calambrone, Pisa, Italy*
[2]*Laboratory of Medical Physics and Biotechnologies for Magnetic Resonance, IRCCS Fondazione Stella Maris, Calambrone, Pisa, Italy*
[3]*Imago7, Pisa, Italy*
[4]*Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy*
[5]*Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy*

Correspondence should be addressed to Laura Biagi; laura.biagi@fsm.unipi.it

Received 18 May 2018; Revised 3 August 2018; Accepted 4 September 2018; Published 25 November 2018

Academic Editor: Yasuo Terao

Reorganization of somatosensory function influences the clinical recovery of subjects with congenital unilateral brain lesions. Ultrahigh-field (UHF) functional MRI (fMRI) with the use of a 7 T magnet has the potential to contribute fundamentally to the current knowledge of such plasticity mechanisms. The purpose of this study was to obtain preliminary information on the possible advantages of the study of somatosensory reorganization at UHF fMRI. We enrolled 6 young adults (mean age 25 ± 6 years) with congenital unilateral brain lesions (4 in the left hemisphere and 2 in the right hemisphere; 4 with perilesional motor reorganization and 2 with contralesional motor reorganization) and 7 healthy age-matched controls. Nondominant hand sensory assessment included stereognosis and 2-point discrimination. Task-dependent fMRI was performed to elicit a somatosensory activation by using a safe and quantitative device developed ad hoc to deliver a reproducible gentle tactile stimulus to the distal phalanx of thumb and index fingers. Group analysis was performed in the control group. Individual analyses in the native space were performed with data of hemiplegic subjects. The gentle tactile stimulus showed great accuracy in determining somatosensory cortex activation. Single-subject gentle tactile stimulus showed an S1 activation in the postcentral gyrus and an S2 activation in the inferior parietal insular cortex. A correlation emerged between an index of S1 reorganization (distance between expected and reorganized S1) and sensory deficit ($p < 0.05$) in subjects with hemiplegia, with higher distance related to a more severe sensory deficit. Increase in spatial resolution at 7 T allows a better localization of reorganized tactile function validated by its correlation with clinical measures. Our results support the S1 early-determination hypothesis and support the central role of topography of reorganized S1 compared to a less relevant S1-M1 integration.

1. Introduction

Over the last years, the risk of somatosensory impairment in children with unilateral cerebral palsy (CP) has been increasingly recognized, becoming a consistent target for both evaluation and intervention. Studies on tactile dysfunction in unilateral CP report a variable prevalence of deficits ranging from 42 to 90% of children, with stereognosis and two-point discrimination (2PD) as the most frequently impaired aspects [1-4]. A tactile dysfunction has a negative impact on the quality of movements, limits the ability of the child to interact with the environment, and, most importantly, contributes to the progressive functional impairment of the affected upper limb secondary to the so called "learned nonuse" [5-7].

Besides its clinical recognition, there is a growing interest in the understanding of the neuroplastic mechanisms of the somatosensory system after congenital brain lesions, alongside with the better-known reorganization of the corticospinal system. Reorganization of the afferent thalamocortical

sensory tracts to primary sensory cortex (S1) has been hypothesized to be related mostly to the capability of ascending fibers to bypass pre- or perinatal lesions and reach the expected cortical destination in the postcentral gyrus [8]. This mechanism seems however imperfect, resulting in some degree of sensory deficit as demonstrated by several studies [9-11].

A significant contribution to the study of brain reorganization of the somatosensory system in unilateral CP was traditionally provided by functional MRI studies, although with significant limitations in spatial resolution. Indeed, studies on sensorimotor reorganization with clinical-field MRI (i.e., 1.5 or 3 tesla) are unable to accurately circumscribe the primary sensory area at a single-subject level, including the distinction, within the perirolandic region, between primary sensory and primary motor activation [12, 13].

The increased availability of ultrahigh-field (UHF) MRI (≥7 tesla) constitutes a unique opportunity for the study of the relationship between structure and function in the human brain, as clearly shown by the first studies in healthy subjects [14]. Compared to lower field MRI, UHF MRI has an increased spatial resolution, with expected increase of sensitivity and specificity of fMRI activation [15]. To date, however, no studies have explored the capability of UHF fMRI in the characterization of brain plasticity in hemiplegic subjects with congenital brain lesions.

We here preliminarily investigated the potentials of UHF fMRI for the study of somatosensory reorganization in adolescents or young adults with congenital hemiplegia. In particular, we aimed to test the following hypotheses: (i) in the affected hemisphere (i.e., the hemisphere contralateral to the hemiparetic side), S1 activation is dislocated from the expected area and (ii) the degree of the dislocation of the reorganized S1 correlates with the severity of the somatosensory deficit.

To test our hypotheses, we performed task-dependent fMRI at 7 T by applying a passive, gentle tactile stimulation in hemiplegic and in control subjects, through an automated 7 T MRI-compatible device developed ad hoc. As the control study, we used a sensory task consisted of passively brushing of fingers by means of a toothbrush [9].

The device showed the capability of a reliable specific activation of a tactile postcentral region at 7 T. The coordinates of the activation obtained in each hemiplegic subject were compared with the expected site of activation (S1 localizer) as defined through a group analysis performed on controls. Finally, in the hemiplegic subjects, the degree of the dislocation of S1 was correlated to the severity of tactile sensory deficits as assessed by stereognosis and 2PD.

2. Materials and Methods

2.1. Subjects. Ten adolescents or young adults (8 males, mean age of 26 ± 7 years) with congenital hemiplegia (7 right hemiplegic subjects) were recruited for the study. Subjects were selected from a registry of patients with congenital hemiplegia treated at IRCCS Fondazione Stella Maris.

In order to allow for good levels of collaborations during the experiment, only subjects with an IQ above 70 and no reports of psychiatric comorbidities were considered eligible. Contraindications to MRI were considered as exclusion criteria. Seven right-handed, healthy subjects (3 males, mean age of 29 ± 6 years) were enrolled from the community as controls.

The research project was approved by the Pediatric Ethics Committee of the Tuscany Region (Florence, Italy) and the Italian Ministry of Health and was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Written informed consent in accordance with the authorized protocol was obtained from all adult subjects and from parents or guardians for juvenile participants.

2.2. Clinical Assessment. Hemiplegic subjects received a detailed clinical assessment, which included motor and sensory evaluations. Sensory function of the nondominant hand was assessed by using 2-point discrimination (2PD) and stereognosis, which are known to have good clinometric properties and clinical utility [6]. 2PD describes the distance (in millimeters) below which two points of touch stimuli within one dermatome cannot be distinguished anymore, with higher values reflecting stronger impairment. Stereognosis describes the percentage of objects correctly identified during manipulation with the nondominant hand, with lower values reflecting stronger impairment [16]. Motor function of the nonhemiplegic hand was assessed by using the Wolf motor function test (WMFT) as previously described, by including a quality dimension and a time dimension [17, 18], and the assisting hand assessment (AHA) [19]. The reorganization of primary motor and primary sensory functions was also assessed by motor and somatosensory evoked potentials (MEP and SEP). A lesion severity score was applied to the 1.5 T datasets of hemiplegic subjects [20] in order to determine the possible impact of brain lesion extension on S1 reorganization and clinical assessment.

2.3. Data Acquisition. Data were acquired on a 7 T Discovery MR950 MRI system (GE Healthcare, Milwaukee, WI, USA), equipped with a 2-channel transmit/32-channel receive coil (Nova Medical, Wilmington, MA, USA). Functional images were acquired to accurately identify S1 at a single-subject level and, only for the control group, to identify the expected site of activation (S1 localizer) at a group level. We used a T2∗-weighted gradient echo (GRE) echo-planar imaging (EPI) sequence (TR = 2000 ms, TE = 21.5 ms, flip angle = 60°, field of view (FOV) = 192 mm × 192 mm, matrix = 128 × 128, isotropic voxel = $1.5 \times 1.5 \times 1.5\ mm^3$). Thirty-two slices (about 5 cm coverage) were placed in order to cover primary and secondary sensory cortices. Each functional series was composed by 160 time points (volumes) and 5 additional initial dummy scans, for a total acquisition time of $5'30''$. A whole-brain GRE-EPI sequence was acquired with the same parameters of functional series, except for a longer TR (6000 ms), allowing the complete coverage of the brain. One single volume of 90 slices was acquired after 3 dummy scans in 24 seconds. We acquired also a 3D FSPGR T1-weighted sequence (TR/TE = 5.9/2.1 ms; flip angle 12°, isotropic voxel = $1 \times 1 \times 1\ mm^3$, acquisition time = $4'50''$). Structural images

were also acquired, for spatial coregistration of functional 7 T data with anatomical 1.5 T images.

Subjects were also assessed on a 1.5 T Signa HDxt (GE Healthcare, Milwaukee, WI, USA) MR scanner equipped with an 8-channel array coil (Invivo Corporation, Gainesville, FL, USA). In particular, three-dimensional structural images were acquired using a 3D FSPGR T1-weighted sequence (time of repetition (TR)/time of echo (TE) = 11.9/3.6 ms; flip angle 10°, isotropic voxel = $1 \times 1 \times 1$ mm^3). Since 3D T1-weighted whole brain images at UHF may present signal inhomogeneities that could fail brain segmentation, we used structural images acquired at lower magnetic field for brain segmentation, as well as for the normalization of single-subject brains of control groups in a common space [21].

2.4. Functional Tasks. Two different sensory tasks were performed. The first sensory task consisted of a gentle tactile stimulation delivered on the thumb and the index finger of each hand separately, by applying a tactile stimulator developed ad hoc (Linari Engineering, Pisa, Italy). In detail, the tactile stimulator consisted of an MRI-compatible pneumatic system with two pumps (range of pressure = 0 ÷ 0.2 MPa). The pumps were located in the scanner engine room led by a unique remote control in the console room. Each pump was connected to a little vesicle through plastic pipes, filled with air. Each vesicle was applied to the distal phalanx of the index (F1) or thumb (F2) finger. Pumps had been set with a pressure of 0.1 MPa and an inflation/deflation rate of 1 Hz, so as to have about one "touch" every second.

A second sensory task was used as the control study, to compare the gentle tactile to standard task, commonly used in such patients and in clinics [9, 22]. This task consisted of passively brushing hand index and thumb fingers (F1 and F2, respectively), by means of a toothbrush, at a frequency of about 1 Hz.

The experiment consisted of 4 functional series, 2 for each task performed on either hand. fMRI series were built using a block design format (block duration = 20 s), alternating the sensory stimulation to rest according to the following: F1 → rest → F2 → rest. This scheme was repeated 4 times for each sensory stimulus. To the purposes of this paper, a comprehensive activation of F1 and F2 stimulations was considered for the data analysis (see below).

Subjects were asked to keep their eyes closed during rest or stimulus delivery; occlusive earplugs attenuated ambient scanner noise throughout rest and activation periods. Before each functional series, a brief test of task was performed in each subject to have confirmation of sensory stimulus delivery; as well at the end of each series, subjects were asked to confirm the stimulus perception.

2.5. Data Analysis. Data analysis was performed with BrainVoyager (Brain Innovation, Maastricht, The Netherlands), by using ad hoc scripts written in MATLAB (MathWorks, Natick, MA, USA). First, each functional series was visually inspected, looking for motion spikes or heavy movement periods. Functional data preprocessing included mean intensity adjustment to compensate for interscan intensity differences, temporal interpolation, and resampling to compensate for slice-dependent time differences (sinc function), 3D motion correction (rigid body transformation, sinc interpolation), and high-pass temporal filtering (GLM-Fourier approach, two cycles).

The 1.5 T 3D T1-weighted images were transformed into the AC-PC coordinate system by applying a six-parameter rigid transformation and turned into Talairach's space. In hemiplegic patients, transformations were calculated on a half-artificial brain, by replacing the lesioned hemisphere with the healthy one, flipped on the sagittal plane.

Functional data were coregistered to the "whole brain" GRE-EPI dataset by using a rigid body alignment, considering that EPI acquisition induces same distortions on images. Moreover, in order to coregister the whole-brain GRE-EPI data to the 7 T structural images, an affine transformation (9 parameters; 3 for translation, 3 for rotation, and 3 for FOV scaling) was automatically calculated, visually inspected and manually corrected by two experienced raters (LB and PC). Finally, a rigid body transformation was calculated to align the 7 T structural images to the analogous acquired at 1.5 T.

In order to preserve UHF spatial resolution, in hemiplegic subjects, the analyses were conducted in the native space, using the inverses of above transformations or rather aligning anatomical images to functional ones, keeping these unvaried. Similar approach was used for 3D visualization of reconstructed surface representation (mesh); 1.5 T T1-weighted images in ACPC space were automatically segmented in order to obtain segmented cortical boundary. The inverses of above spatial transformations were applied to the segmented volumes, to import the segmentation in the space of functional data. Finally, manual correction was used to edit little imperfections.

In controls, spatial transformations were applied to functional images, in order to perform group analysis in a common space.

Blood oxygenation level-dependent (BOLD) responses were analyzed using a general linear model (GLM) approach, modelling the regressors of interest (by convolving a boxcar function for each stimulation block with two gamma functions for the hemodynamic response) and six spurious movement regressors (outputs of the 3D motion correction procedure). The contrasted activity for gentle tactile stimulation of both fingers versus the rest condition ((F1 + F2) > rest) was used to investigate the reorganization of somatosensory cortices.

The same contrast for "brush" stimulation of both fingers versus the rest condition ((F1 + F2) > rest) was used as the control test, to compare results.

First-level statistical analyses were performed using a threshold at $p < 0.005$ ($t > 2.85$) and cluster size > 10 mm^3, to generate individual subject's maps in native space. With respect to the tactile stimulation, for each hemisphere (contralateral and ipsilateral to the stimulated hand), two specific regions of interest (ROIs) were considered (S1 and S2). By applying the spatial transformations previously described, in order to compare individual variability of activation, the ROIs of each hemiplegic subject were transferred into

Table 1: Characteristics of subjects with hemiplegia.

Patient	Age	Sex	Side of lesion	2PD	Stereognosis	MEP	SEP	WMFT quality	WMFT time*	AHA	Lesion severity#	Lesion type
1	21	M	R	5	100	C	I	4.13	2.27	67	5	II
2	36	M	L	5	100	I	I	5	1.05	89	8.5	IV
3	19	M	L	9	50	I	I	3.67	5.08	70	14	III
4	20	M	L	10	33	I	I	1.47	2.61	38	10	III
5	28	M	L	7	17	I	I	2.67	2.18	59	4	III
6	26	M	R	2	100	C	I	4.53	1.59	84	18.5	I

Abbreviations: 2PD: 2-point discrimination; MEP: motor evoked potentials; SEP: somatosensory evoked potentials; DI: dislocation index; AHA: assisting hand assessment; M: male; L: left; R: right; C: contralesional; I: ipsilesional. *Expressed in sec. #Out of 40 [20].

Talairach's space and the individual center of mass calculated. In controls, following spatial normalization, the coregistered functional datasets were used for a second-level multisubject analysis, by using a fixed-effect (FFX) GLM-based analysis and a statistical threshold corrected for false discovery rate (FDR) $q < 0.05$ corresponding to a $p < 0.001$. For control group activation, the center of mass of each ROI was calculated. In patients, the vector between the expected and single-subject S1 center of mass was determined, as the measure of reorganized S1 dislocation and its length (in millimeters) was assumed as a "dislocation index." A dislocation index was calculated as well for the activation elicited by the gentle tactile stimulus of the preserved hand in hemiplegic subjects. Standard deviations (Δx, Δy, Δz) of the expected center-of-mass coordinates were used to calculate the radius, r_{CG}, of a sphere describing the expected activation area, according to the following:

$$r_{CG} = 1.5 \times \sqrt{\Delta_x{}^2 + \Delta_y{}^2 + \Delta_z{}^2}. \quad (1)$$

A paired t-test was performed to assess differences in the mean dislocation indices of the dominant and nondominant hand-related activation.

S1 dislocation index was related to sensory deficit assessed by 2PD and stereognosis and to severity of the lesion in the hemisphere contralateral to the nondominant hand by using a one-tailed Pearson correlation index.

3. Results

Of the ten enrolled subjects with hemiplegia, two refused to perform 7 T MRI after performing 1.5 T MRI and withdrew from the study without providing explanations, as allowed by the consent agreement. Two further datasets obtained at 7 T were excluded from the following analysis because of the presence of excessive movement artifacts during functional acquisition, which failed the post hoc correction process. Data from six subjects (4 with right hemiplegia and 2 with left hemiplegia, mean age 25 ± 6 years, range $= 19 \div 36$ years) were thus available for analysis.

3.1. Clinical Assessment. Clinical characteristics of the six subjects are reported in Table 1, including clinical sensory and motor characteristics and somatosensory and motor reorganization assessed by evoked potentials.

According to the timing of lesion [23], structural MRI showed brain maldevelopment in one subject (unilateral extensive polymicrogyria with an interhemispheric cyst), periventricular white matter lesion in one subject (i.e., focal venous infarction), cortical and deep grey matter lesions in 3 subjects (focal stroke, <28 days of life), and early acquired brain injury in one subject (focal stroke, around 3rd month of life). All hemiplegic subjects but one had pure unilateral brain lesions. The only subject with bilateral lesions (patient 5, Table 1) had a watershed infarction with very mild white matter abnormalities in the hemisphere ipsilateral to the dominant hand. Despite the presence of focal lesions, anatomical landmark for hand sensorimotor areas ("hand knob") was successfully identified bilaterally in all subjects (Figure 1) but one (patient 6), the one with extensive polymicrogyria. Brain lesion severity scores [20] are reported in Table 1.

3.2. Identification of Primary and Secondary Somatosensory Areas in Control Subjects. The gentle tactile stimulation of dominant hand fingers in controls determined a monolateral activation in the left postcentral gyrus (Broadmann area (BA) 3-1), located at the Talairach's coordinates $[x, y, z] = -57 \pm 5,\ -17 \pm 3,\ 44 \pm 5$, and represented in the left column of Figure 1. This area was identified as the "expected S1 area," and the dislocation index of each hemiplegic subject was calculated according to its center of mass. Group analysis showed also bilateral activation in the inferior parietal lobule (BA 40-2, averaged coordinates $= \pm 53 \pm 3,\ -25 \pm 3,\ 35 \pm 7$), classified as S2 areas, and monolateral activation in the right precentral gyrus (BA 6).

3.3. Identification of Primary and Secondary Somatosensory Areas in Hemiplegic Subjects. In hemiplegic subjects, fMRI activation at 7 T was carefully checked by three experienced raters (MC, LB, and SF). Thanks to the gentle tactile stimulus, S1 activation foci were successfully mapped in the native space of each subject (Figure 1). ROI coordinates, transformed into Talairach's space, are reported in Table 2. For all hemiplegic subjects, the activation of S1 was clearly unilateral in the hemisphere contralateral to the stimulated hand.

Activation in the inferior parietal lobule was detected bilaterally in 4 out of 6 patients, while was detected

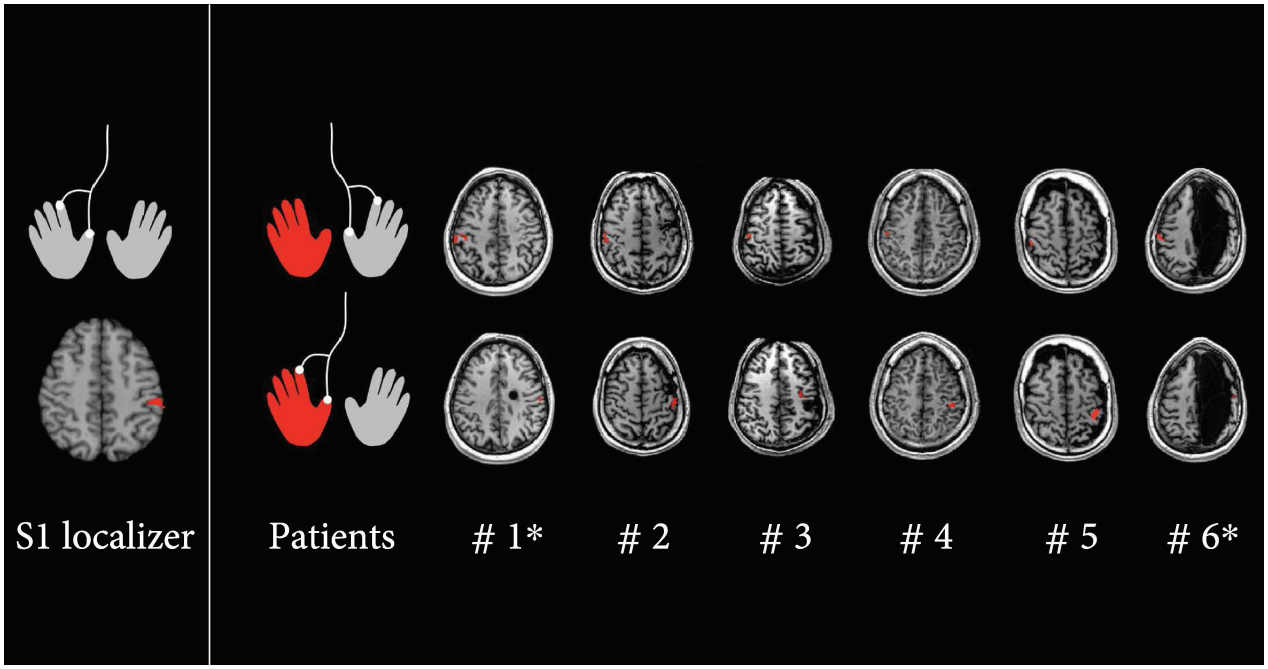

Figure 1: S1 activation rendered on T1 axial images for control group analysis (S1 localizer) and single-subject analysis (patients). The gentle tactile stimulation in the dominant hand elicits a contralateral S1 activation in the group analysis (S1 localizer). Single-subject analyses reveal that the gentle tactile stimulation elicits a unilateral S1 contralateral activation pattern for both nondominant (red) and dominant (grey) hands (patients). *Right brain lesion.

Table 2: Centre of mass localization, extension, and peak Z-score ($Z*$) for primary (S1) and secondary (S2) somatosensory areas, identified by the gentle tactile stimulation of the paretic hand for each single subject.

Area	Sub	Side	Talairach's coordinates			Cluster size (mm^3)	Peak's Z-score
			x	y	z		
S1	1	c	58 ± 2	−20 ± 3	38 ± 5	439	3.72
	2	c	−45 ± 3	−26 ± 6	53 ± 5	1251	6.31
	3	c	−32 ± 3	−17 ± 4	45 ± 4	675	5.80
	4	c	−42 ± 2	−33 ± 2	46 ± 1	87	3.30
	5	c	−41 ± 5	−38 ± 5	48 ± 4	1595	6.16
	6	c	50 ± 3	−13 ± 1	49 ± 3	174	4.68
S2	1	c	56 ± 5	−16 ± 4	24 ± 2	785	5.56
		i	−57 ± 3	−37 ± 4	18 ± 4	381	3.48
	2	c	−47 ± 3	−19 ± 2	19 ± 3	347	4.90
	3	i	50 ± 3	−16 ± 3	39 ± 1	119	3.89
	4	c	−50 ± 3	−25 ± 2	25 ± 1	223	3.83
		i	53 ± 5	−32 ± 2	19 ± 2	339	3.72
	5	c	−49 ± 3	−24 ± 2	20 ± 3	501	5.34
		i	51 ± 3	−24 ± 2	12 ± 2	152	4.22
	6	c	58 ± 3	−11 ± 6	22 ± 7	1390	6.16
		i	−64 ± 2	−7 ± 3	4 ± 1	151	6.54

Talairach's coordinates are provided as the value and standard deviation, based on all voxels of the region of interest. Abbreviations: c: contralateral to the stimulated hand; i: ipsilateral to the stimulated hand.

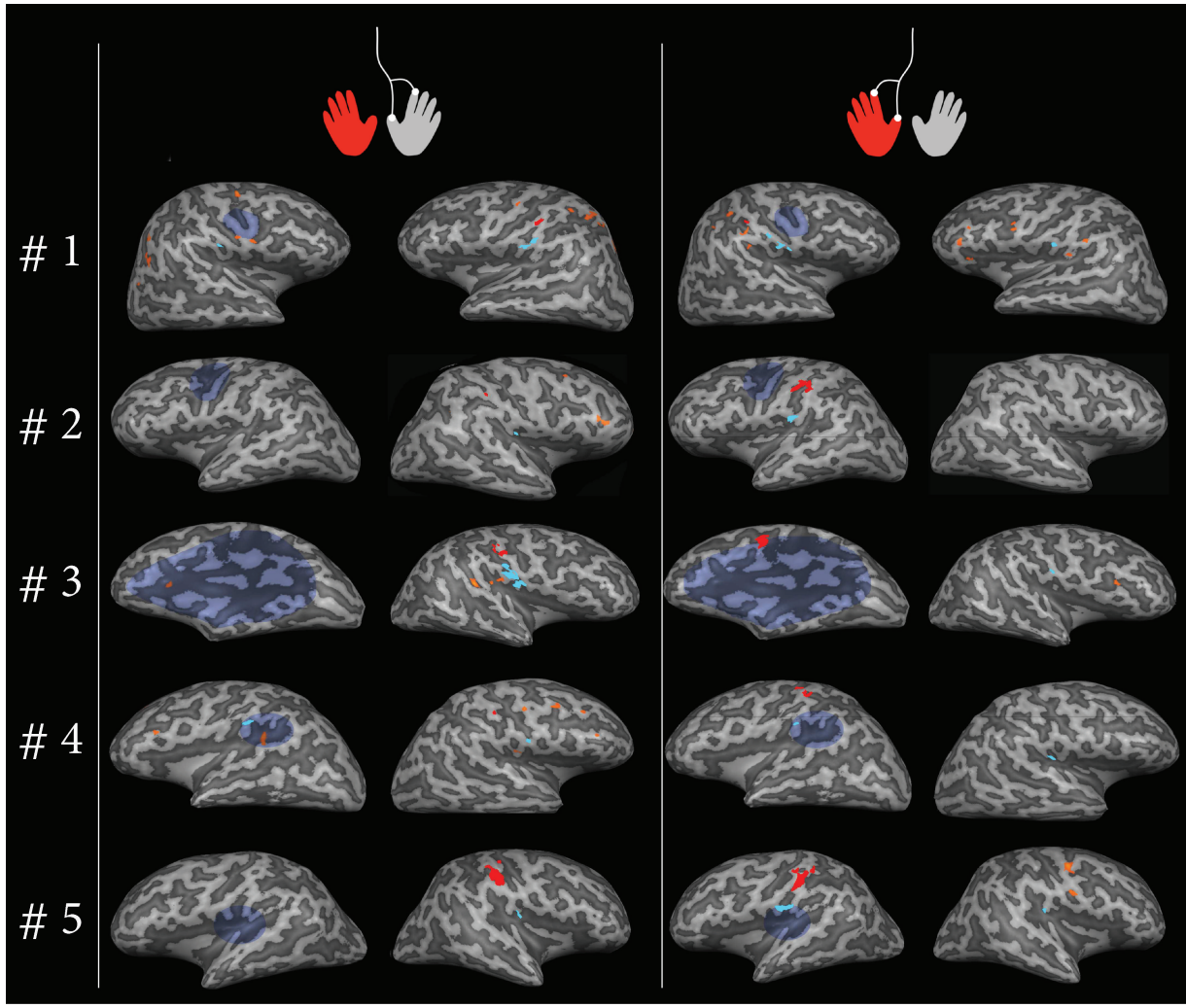

Figure 2: Activation foci for tactile stimulation of both hands, represented on inflated cortices in the native space of each single subject. Subjects' identifiers are shown in the left column, accordingly to Tables 1 and 2. For subject #6, segmentation failed due to the presence of extensive polymicrogyria in lesioned hemisphere, so the representation is missing in this figure. For each subject, the lesion is represented in colored blue transparency on the inflated cortex, approximately corresponding to its anatomical projection on brain surface. S1 responses to tactile stimulation are represented in red, while S2 responses are represented in cyan. Further activation in addition to S1 and S2 for the stimulation of the dominant hand was the following (middle column): #1 HH: PrCG BA 4, IPL BA 40, SPL BA 7, SOG BA 19; LH: PrCG BA 4 and BA 6, Pcu BA 19, MTG BA 39, MOG BA 19. #2 HH: MFG BA 6, IFG BA 46; LH: none. #3 HH: STG BA 22, IPL BA 40; LH: IFG BA 45. #4 HH: PrCG BA 4, MFG BA 6, MFG BA 9, STG BA 22; LH: IPL BA 40, MFG BA 9. #5 HH: none; LH: none. #6 (not represented) HH: IPL BA 40, STG BA 22; LH: none. For the stimulation of the nondominant hand (right column): #1 HH: PrCG BA 6, MFG BA 9-46, IFG BA 46, STG BA,22; LH: IPL BA 40. #2 HH: none; LH: none. #3 HH: IFG BA 44; LH: none. #4 HH: none; LH: none. #5 HH: PrCG BA 4, PrCG BA 6; LH: none. #6 (not represented) HH: IPL BA 40, PrCG BA 6; LH: IPL BA 40, STG BA 22. Abbreviations: HH: healthy hemisphere; LH: lesioned hemisphere; BA: Broadmann area; PrCG: precentral gyrus; MFG: middle frontal gyrus; IFG: inferior frontal gyrus; IPL: inferior parietal lobule; SPL: superior parietal lobule; Pcu: precuneus; SOG: superior occipital gyrus; MOG: middle occipital gyrus; STG: superior temporal gyrus; MTG: middle temporal gyrus.

ipsilaterally or controlaterally in one subject each (Table 2 and Figure 2). Further foci of activation in each subject for gentle tactile task are detailed in Figure 2.

Brushing stimulus elicited a similar activity pattern, except for the activation of ipsilateral S1 and medial frontal gyrus (BA 6) (Figure 1 in Supplementary Materials). Indeed, brushing determined huge activation blobs with no anatomical separation between S1 and S2, neither at FDR nor at Bonferroni multiple comparison correction.

3.4. Dislocation of the Activation in the Hemiplegic Subjects. The S1 dislocation vector for each hemiplegic subject and its position within the sphere of radius $r_{CG} = 11.5$ mm, describing the expected activation, were presented in Figure 3. For the nonhemiplegic hand stimulation, the sphere included the S1 dislocation vector of all patients, while for the hemiplegic hand, the sphere included the S1 dislocation vector of only two of them. S1 dislocation indices of both hands were reported for each single hemiplegic subject in

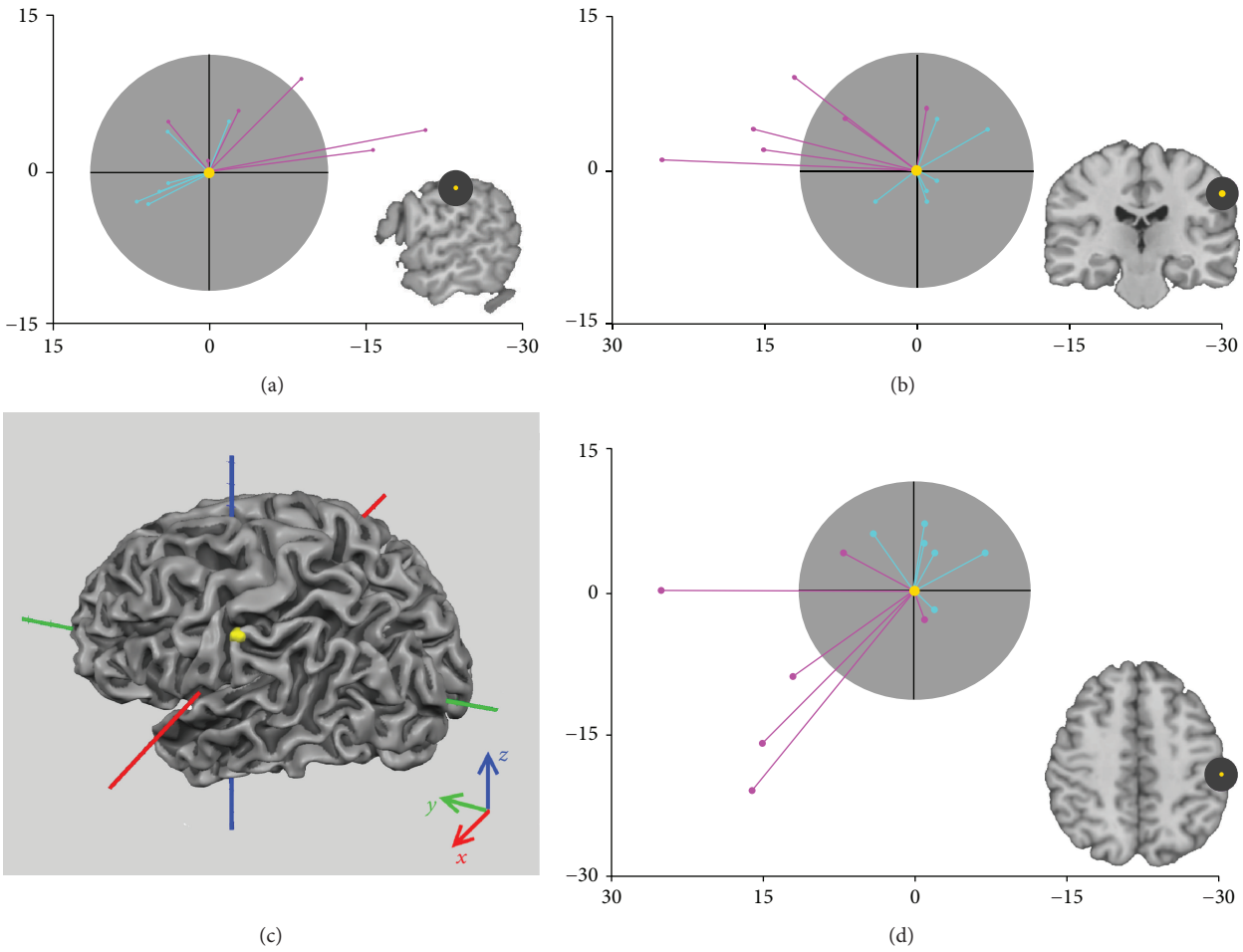

Figure 3: S1 dislocation vectors of each patient for the gentle tactile stimulation of both nondominant (pink vectors) and dominant (cyan vectors) hands, projected in sagittal (a), coronal (b), and axial (d) planes. The yellow point represents the position of S1 resulting from the group analysis ($x = -57$, $y = -17$, $z = 44$) and is set to zero. The sphere of expected localization (<1.5 standard deviation (SD)) in control brains (grey) has radius $r_{CG} = 11.5$ mm. Color code of axis respects the convention $[x, y, z] \rightarrow$ RGB (x = red, y = green, z = blue). Axis scales are in millimeters. In the bottom-left corner (c), the three dimensional representation of S1 control group ROI (yellow) is overlapped on the mesh of the white/grey matter boundary of a standard brain (Colin27 brain, [24]). All dominant hand vectors are within a 1.5 SD, as well as 2 out of 6 hemiplegic subjects. Not the posterior-mesial gradient for S1 dislocation.

Figure 4(a). Mean dislocation indices of the nondominant hand and the dominant hand in hemiplegic subjects resulted significantly different ($p < 0.05$).

3.5. Correlation with Clinical Measures. A significant correlation emerged between reorganized S1 dislocation index and sensory measures, both at stereognosis ($p = 0.012$, $R = -0.868$) and 2PD ($p = 0.041$, $R = 0.756$). In particular, a bigger distance between actual and expected S1 correlated with a more severe tactile deficit (Figure 4(b)).

4. Discussion

This is the first study that uses UHF fMRI for the study of somatosensory reorganization in congenital hemiplegia, by applying a reliable gentle tactile stimulus. We were able to confirm our initial hypotheses. Firstly, our results showed a greater S1 variability in the hemisphere contralateral to the hemiparetic side than in the ipsilateral. Secondly, the degree of dislocation of the reorganized S1 correlated with the severity of tactile deficit.

As hypothesized, S1 activation was identifiable in the native space of each hemiplegic subject; neither normalization nor smoothing was applied for functional images in each single-subject analysis, in order to maximize the potential for spatial localization obtained at UHF fMRI. Indeed, our data have a voxel size of about 3.4 mm^3 compared to previous studies where resolution ranged from 27 to 36 mm^3 [13, 12]. Somatosensory activation due to the gentle tactile stimulus activated a small area (mean activation among subject ~ 0.7 cm^3) that was anatomically identified as S1 in the postcentral gyrus in the hemisphere contralateral to the gentle tactile stimulus of the two hand fingers. This active area found in somatosensory cortex has similar characteristics, in terms of location and dimension, to activities detected in precedent investigations of finger somatotopy at 7 T [14, 25,

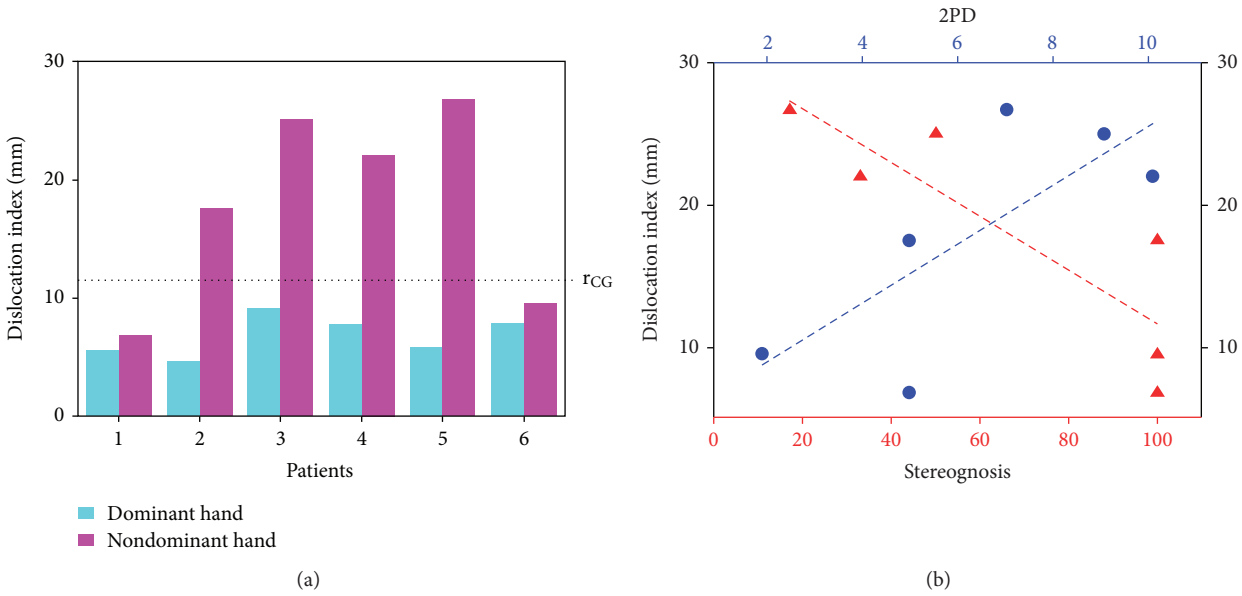

FIGURE 4: (a) Dislocation indices for nondominant (pink bars) and dominant (cyan bars) hands. All dominant hand S1 dislocation indices are inferior to the radius, r_{CG} (expected localization according to the control group analysis) as well as 2 out of 6 nondominant hand indices. The latter are of the 2 subjects with no sensory deficit (see Table 1 for clinical details). (b) Correlation between dislocation index and sensory deficit assessed at 2PD and stereognosis. A significant correlation emerged between dislocation index and sensory deficit, with a greater distance being associated to a worse sensory deficit. Abbreviation: 2PD: 2-point discrimination.

26]. Both in control and hemiplegic subjects (in the latter case, S1 will be referred to as "reorganized S1" when elicited in the hemisphere contralateral to the nondominant hand), this activation was clearly unilateral. On the contrary, the activation determined by brushing presented a bilateral pattern in two out of six patients and in four out of seven controls (control group analysis in Figure 1 in Supplementary Materials). Moreover, brushing stimulus elicited larger areas (mean activation ~ 3.2 cm^3), making the segregation of S1 from S2 harder as well as the distinction between S1 (postcentral gyrus activation) and M1 (precentral). These discrepancies can be attributed in part to the higher specificity of the gentle tactile stimulus, in part to physiological differences in the cortical processing of the two stimuli. A further area was clearly identified with a variable pattern (ipsilateral, bilateral, or contralateral to the stimulation side) in the posterior parietal insular cortex, referred to as S2. The activation due to the brushing determined a similar variable pattern, with larger activation.

Recent studies on somatotopy of healthy subjects at 7 T [25, 14, 27-29] employed some form of mechanical and/or electrical stimulation, difficult to apply in the clinical setting. Human touch was also used as stimulus in a 7 T fMRI study to investigate cortical representation of individual fingers [26], with the limitation of reproducibility. The device that we applied in the current study has the advantage of an automated, predetermined, and reproducible stimulation, which is administered to the subject with no collaboration required (with the exception of the general compliance to an MRI exam). This is particularly useful in hemiplegic subjects in which motor deficit, musculoskeletal constraints, or mirror movement can negatively impact on motor activation and image quality in fMRI. Furthermore, by requiring no collaboration by the subject, the device can be potentially applied also to younger ages or, theoretically, to sleeping subjects. Our results thus support the utility of the device for the fMRI study of somatosensory reorganization.

Although limited to the hemisphere contralateral to the hemiplegic side, reorganized S1 showed a certain degree of variability, as previously demonstrated at lower field MRI [13]. Our and previous findings support the hypothesis that interhemispheric reorganization of the primary somatosensory area (S1) is uncommon in congenital brain lesions [13, 8]. Separately and differently from S1, S2 showed a varied pattern of activation, with pure ipsilesional S2 activation found in 1 subject and pure contralesional S2 activation found in 1 subject, while all the remaining subjects had a bilateral S2 activation. These results agree with previous studies, which hypothesized a broader pattern of S2 localization [13, 12].

In order to check if certain grey matter plasticity was possible for S1, despite limited to the lesioned hemisphere, we tried to give the measure of S1 dislocation, by using an expected spherical volume into which allocate the normal probability to have S1 in healthy subjects (radius, r_{CG}). For reorganized S1, we found that only 2 subjects fall into r_{CG}, which is the probability of being in the expected S1. Conversely, in the hemisphere ipsilateral to the nondominant side, S1 always resulted within an r_{CG} radius. In the previous fMRI study, Wilke et al. [13] compared the topography of S1 in a group of subjects with congenital hemiplegia. They demonstrated a greater variability of S1 location around

the central sulcus in the lesioned hemisphere compared to the hemisphere contralateral to the preserved hand. Compared to our results, they did not include any quantitative measure of S1 dislocation nor their findings were validated by clinical measures. Furthermore, our findings add to the previous literature in that a posterior-mesial pattern for somatosensory reorganized function can be identified (see Figure 3).

Due to the higher spatial resolution and accuracy of S1 mapping at 7 T fMRI, our results measured a relevant distance defining a sphere of 2 cm^3 volume as the expected area to relocate reorganized S1. Due to this marked extension, we may thus assume that a certain degree of cortical plasticity has occurred out of a predetermined somatosensory area in at least 4 out of 6 hemiplegic subjects in our sample, limited to the ipsilesional hemisphere.

Although some degree of cortical plasticity can occur, our results also support previous hypotheses on early determination of S1 [8], if some somatosensory predetermined tissue is preserved. Conversely to Juenger et al. [8], our sample included two earlier occurred lesions, which showed the better tactile clinical profile (patients 1 and 6). Interestingly, one of these two subjects had a very extended polymicrogyria (patient 6) on the side contralateral to the hemiparesis, with a very small amount of apparently preserved cortical grey matter on structural images. However, in that small amount of available normal cortical tissue, it was able to accommodate somatosensory tactile mapping, thus reinforcing the concept of highly defined determination of that area, allowing no sensory deficit on the nondominant hand [30].

A significant correlation emerged in our study between S1 dislocation index and the two measures we used to assess somatosensory deficit. It has been reported in studies on congenital unilateral brain lesions that motor function, especially fine motor control, is worse when M1 reorganization is ipsilateral to the hemiplegic side, likely due to the segregation of motor and somatosensory areas in two different hemispheres [31, 9, 11]. In our sample, we have two subjects with contralateral motor reorganization assessed with MEPs. Interestingly, those subjects have the closest distance to the expected S1 and have better sensory function, with no substantial differences in motor function compared to the group with ipsilesional reorganization. If this observation will be confirmed in a bigger sample of subjects, it would support the hypothesis that the functionality of reorganized S1 depends more on the distance from the expected somatosensory region of the cortex than the distance from M1 [13]. Previous findings and ours add to the previous literature, in the sense that a clinical meaning is given to S1 early determination. It needs to be noticed that, as expected, all subjects with contralateral reorganization have perinatal arterial ischemic lesion. This has been already hypothesized to play a role in limiting white matter ascending thalamocortical afferents to S1 [32]. Finally, as we might have expected a possible impact of brain lesion size on S1 reorganization, we assessed brain lesion severity by using a recently developed semiquantitative system [20]. Interestingly, no relationship emerged between the dislocation index and brain lesion severity. Studies on a larger sample with quantitative measurements of lesion volume will confirm this finding.

In order to further support the relevance of sensory deficit for motor outcome in our sample, we explored at post hoc the relationship between sensory deficit and motor impairment. In particular, we found a correlation between stereognosis and quality dimension at WMFT ($p = 0.015$, $p = 0.856$) and AHA ($p = 0.045$, $p = 0.753$) and between 2PD and quality dimension at WMFT ($p = 0.036$, $p = -0.773$) and AHA ($p = 0.038$, $p = -0.765$). Our results further support a possible influence of tactile deficit on motor control.

The principal limitation of this study is the sample size. The number of subjects included in this sample was limited due to the low prevalence of the disease and the psychological and physical compliance required by the MRI exam; also, two subjects were excluded for excessive movement artifacts, which may be an issue at UHF MRI. However, it has been suggested to consider cautiously but positively, significant results in small cohorts [33]. The small number of subjects does not allow for including the hemiplegic side and sex as a covariate in the analysis. Similarly, due to the limited number of subjects in the final dataset, the potential effect of motor reorganization has not been systematically considered in the analyses but only speculated in the discussion based on single-subject findings. Finally, no comparison between S1 and M1 reorganization was conducted, which might be influenced differently by specific factors.

5. Conclusions

For the first time, our study uses UHF fMRI for the study of somatosensory reorganization in congenital hemiplegia, by applying a reliable gentle tactile stimulus. Since the use of UHF MRI is still limited, it is of utmost importance to identify the possible fields for its application, i.e., to define the added value of UHF MRI as compared to conventional MRI. In fact, the increased signal sensitivity at 7 T allows obtaining more reliable BOLD signals in single subjects, compared to lower field strengths, also shortening acquisition duration and block repetitions [25], which is highly recommended in the clinical and pediatric setting. We believe that this initial demonstration of the potentials of UHF in studying adaptive brain plasticity in young adults might foster further research in larger samples of subjects with congenital brain damage and at younger ages.

Data Availability

All the data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflict of interests.

Acknowledgments

This study has been partially funded by the University of Pisa (Bando Progetti di Ricerca di Ateneo 2015, PRA2015) to MC.

Supplementary Materials

Figure 1 represents the activation in the primary somatosensory area elicited by the brushing task, for control group analysis (group analysis) and single-subject analysis (patients). The brushing stimulation in the dominant hand elicits a bilateral S1 activation in the group analysis of controls (S1 localizer). Bilateral representation of S1 was found also in single-subject analysis carried on patients. In particular, 2 out of 6 patients had bilateral activation for the brushing stimulation of the dominant hand (grey, top row, patients #1 and #5) and the nondominant hand (red, bottom row, patients #1 and #4). *Right brain lesion. *(Supplementary Materials)*

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Hindawi
Neural Plasticity
Volume 2018, Article ID 2831342, 9 pages
https://doi.org/10.1155/2018/2831342

Research Article

Time Course of Upper Limb Function in Children with Unilateral Cerebral Palsy: A Five-Year Follow-Up Study

Katrijn Klingels,[1,2] **Sarah Meyer**,[1] **Lisa Mailleux**,[1] **Cristina Simon-Martinez**,[1] **Jasmine Hoskens**,[1] **Elegast Monbaliu**,[1] **Geert Verheyden**,[1] **Geert Verbeke**,[3] **Guy Molenaers**,[4] **Els Ortibus**,[5] **and Hilde Feys**[1]

[1]*KU Leuven, University of Leuven, Department of Rehabilitation Sciences, Leuven, Belgium*
[2]*UHasselt, BIOMED, Rehabilitation Research Center (REVAL), Hasselt, Belgium*
[3]*KU Leuven, University of Leuven, Leuven Biostatistics and Statistical Bioinformatics Centre, Leuven, Belgium*
[4]*KU Leuven, University of Leuven, Department of Musculoskeletal Sciences, Leuven, Belgium*
[5]*KU Leuven, University of Leuven, Department of Development and Regeneration, Leuven, Belgium*

Correspondence should be addressed to Katrijn Klingels; katrijn.klingels@uhasselt.be

Received 21 March 2018; Accepted 16 July 2018; Published 14 November 2018

Academic Editor: Michael Borich

Knowledge on long-term evolution of upper limb function in children with unilateral cerebral palsy (CP) is scarce. The objective was to report the five-year evolution in upper limb function and identify factors influencing time trends. Eighty-one children (mean age 9 y and 11 mo, SD 3 y and 3 mo) were assessed at baseline with follow-up after 6 months, 1, and 5 years. Passive range of motion (PROM), tone, muscle, and grip strength were assessed. Activity measurements included Melbourne Assessment, Jebsen-Taylor test, Assisting Hand Assessment (AHA), and ABILHAND-Kids. At 5-year follow-up, PROM ($p < 0.001$) and AHA scores ($p < 0.001$) decreased, whereas an improvement was seen for grip strength ($p < 0.001$), Melbourne Assessment ($p = 0.003$), Jebsen-Taylor test ($p < 0.001$), and ABILHAND-Kids ($p < 0.001$). Age influenced the evolution of AHA scores ($p = 0.003$), with younger children being stable over time, but from 9 years onward, children experienced a decrease in bimanual performance. Manual Ability Classification System (MACS) levels also affected the evolution of AHA scores ($p = 0.02$), with stable scores in MACS I and deterioration in MACS II and III. In conclusion, over 5 years, children with unilateral CP develop more limitations in PROM, and although capacity measures improve, the spontaneous use of the impaired limb in bimanual tasks becomes less effective after the age of 9 years.

1. Introduction

Becoming independent in activities of daily living requires—amongst others—a smooth coordination between both hands. In children with unilateral cerebral palsy (CP), the occurrence of an early brain lesion elicits sensorimotor impairments in the contralateral upper limb. Such impairments compromise the development of upper limb function, which in turn restrains bimanual coordination [1]. Insights into the long-term evolution of upper limb function in these children are indispensable to inform parents about these restraints and to steer goal setting and treatment selection. Additionally, it may aid in distinguishing whether changes in upper limb function following an intervention program are attributable to therapy response or to natural change over time.

Thus far, four studies focused on long-term development of upper limb function in children with unilateral CP [2–5]. Holmefur et al. and Nordstrand et al. demonstrated improvements in the spontaneous use of the impaired hand during bimanual tasks in children aged between 18 months and 8 years or 12 years, respectively, who were followed over a period of 4.5 or 6 years, respectively [2, 3]. In contrast, two other studies did not find changes in bimanual performance

nor grip efficiency in children with unilateral CP, assessed between 2 to 4 years up to 11 to 17 years of age [4, 5]. Clearly, contradicting results exist regarding the long-term developmental trajectory of bimanual performance while knowledge on the long-term evolution of motor impairments and unimanual capacity is scarce.

Moreover, the identification of characteristics to predict the longitudinal development of upper limb function in children with unilateral CP is crucial for improving prognoses and treatment planning. However, only limited information is available regarding which characteristics determine the long-term outcome of upper limb function in these children. Only one study previously reported the influence of age on spontaneous hand use demonstrating a rapid development at a young age, reaching a plateau between 2.5 and 8 years [3]. The age at which this plateau is reached depends on the initial manual ability of the child. Children with higher manual abilities develop at a faster rate, reaching their limits at a younger age, compared to children with lower manual abilities [2, 3]. Another factor that may influence the long-term evolution of upper limb function in children with CP is timing of the underlying brain lesion, broadly classified as congenital or acquired lesions. Acquired lesions are generally associated with more severe upper limb impairments compared to congenital brain lesions [6]. Moreover, in a one-year follow-up study of upper limb function, Klingels et al. showed that movement speed improved in children with congenital lesions, whereas children with acquired lesions remained stable [7].

In conclusion, there is a need for a better understanding of the long-term evolution capturing the different qualifiers of UL function as well as the identification of which child's characteristics adequately predict the long-term development of upper limb function assessed on body function and activity level according to the International Classification of Functioning, Disability and Health (ICF). Hence, the objectives of this study were (1) to report the evolution of upper limb function over five years in a large cohort of children with unilateral CP, including both measures at the level of body function and activities, and (2) to identify child's characteristics that influence these long-term time trends.

2. Materials and Methods

2.1. Participants. Children were recruited from the University Hospitals Leuven, special education schools, and one rehabilitation centre in Belgium between June 2007 and January 2008. Inclusion criteria were (1) a diagnosis of congenital or acquired unilateral CP and (2) age between 5 and 15 years. Acquired lesions were defined as lesions occurring in the developing infant brain between 28 days postnatally and three years [8]. Children were excluded if they had (1) insufficient cooperation to perform the assessments, (2) upper limb surgery, and (3) botulinum toxin-A injections in the upper limb within six months prior to baseline. In case a child received botulinum toxin-A injections in the upper limb during the study course, this child was excluded from the analysis of a specific time point if the injection was performed within six months prior to assessment. All children had access to the regular rehabilitation services. Ethical approval was obtained from the Ethics Committee of the University Hospitals Leuven (approval number: S50439), and parents signed a written informed consent form prior to participation.

2.2. Procedure. Children were assessed at baseline, at 6 months, and at 1 and 5 years of follow-up by two trained physiotherapists (KK, JH) routinely involved in the clinical evaluation of children with unilateral CP. All assessments were conducted at the place of recruitment. The results of the first year follow-up have been published in a previous paper [7].

2.3. Assessments. At baseline, age, gender, etiology (congenital or acquired lesion), and the Manual Ability Classification System (MACS) [9] were recorded. At each time point, the physiotherapists treating the children were asked to fill in a questionnaire on the intensity and content of the routine therapy the children received.

At body function level, a standardized test protocol was performed including upper limb passive range of motion (PROM), muscle tone, muscle strength, and grip strength. PROM of shoulder flexion, abduction, external and internal rotation, elbow extension, forearm supination, and wrist extension was measured using a goniometer. PROM values were dichotomized (0: no movement limitation, 1: movement limited by 10° or more compared to standard values). A sum score of these seven dichotomized scores resulted in a PROM total score between 0 and 7, with higher scores indicating more movement limitations. Muscle tone was evaluated in 11 muscle groups using the Modified Ashworth Scale (MAS), ranging from 0 to 4 [10]. A total score was calculated (0–44) including the muscle groups of the shoulder (adductors/abductors, extensors, and internal/external rotators), elbow (flexors/extensors), wrist (pronators and extensors/flexors), and fingers (flexors). To assess muscle strength, manual muscle testing (MMT) was administered in nine muscle groups with a score ranging from 0 to 5 [11]. A total sum score was calculated (0–45) for the muscle groups of the shoulder (flexors and abductors/adductors), elbow (extensors/flexors), forearm (supinators/pronators), and wrist (extensors/flexors). Grip strength was assessed with a Jamar® Inc. AUS dynamometer. The average of three consecutive maximum contractions was recorded for both hands. Also, the ratio of grip strength of the affected to the unaffected hand was calculated, expressed as a percentage, to eliminate the correlation with age [12]. Interrater and test-retest reliability of this protocol has been established [13].

At activity level, the *capacity* of the affected hand was assessed with the Melbourne Assessment of Unilateral Upper Limb function (Melbourne Assessment) and the Jebsen-Taylor hand function test. The Melbourne Assessment evaluates quality of movement in 16 functional unimanual tasks [14]. The total raw score (0–122) was converted to a percentage score, with higher scores indicating better capacity. The reported smallest detectable difference (SDD) for the Melbourne Assessment is 7.4% [15]. The Jebsen-Taylor hand function test measures manual dexterity in six unimanual

tasks, by means of movement time expressed in seconds, with lower scores indicating better capacity [16]. Finally, *bimanual performance* was evaluated with the Assisting Hand Assessment (AHA) and ABILHAND-Kids questionnaire. The AHA, a Rasch-based performance scale, measures how effectively the affected hand is spontaneously used during performance of bimanual tasks [17]. Different test items, describing various object-related hand actions are scored on a 4-point scale rating the quality of performance. The raw scores from AHA version 4.4 (baseline, 6 months, and 1-year follow-up) and 5.0 (5-year follow-up) were converted through the Rasch analysis to logit scores varying between 0 and 100, with higher scores indicating higher ability levels. The SDD for the AHA is 5 AHA logits [18]. ABILHAND-Kids questionnaire is a Rasch-based inventory of 21 mostly bimanual activities that the parents were asked to judge as 0 (impossible), 1 (difficult), and 2 (easy) [19]. The raw scores were converted to logit scores. The reported SDD for the ABILHAND-Kids is 1.82 logits [20]. For all activity level assessments, high levels of reliability and validity have been established [19–23]. Videotapes of the Melbourne Assessment and AHA were scored by four experienced physiotherapists, all certified for AHA scoring. Prior to scoring, interrater reliability was verified in 10 children. Intraclass correlation coefficients between raters were 0.91 and 0.93 for the Melbourne Assessment and the AHA, respectively.

2.4. Statistical Analysis. Children's clinical and demographic characteristics were displayed as frequencies with percentages, means with standard deviations (SD), and medians with interquartile ranges (IQR), whichever appropriate. Linear mixed models (LMMs) were used to study longitudinal trends. Such models correct for the correlation amongst repeated observations within subjects using random effects. Also, when some observations are missing, LMMs still provide valid inferences, provided that missingness does not depend on unobserved outcomes (i.e., assuming missingness at random) [24]. To meet the distributional assumptions, an exponential transformation was used for the Melbourne Assessment and a natural logarithmic transformation for the Jebsen-Taylor test. Significant categorical time trends were further investigated with pairwise post hoc tests between baseline and 1-year follow-up and between 1- and 5-year follow-up. To identify factors that influence time trends, interaction terms between the factor time and the following factors were included in the models: age, gender, etiology, MACS, and botulinum toxin injections or participation in a modified CIMT intervention during the study course. To study the influence of age, three age groups were created: 5 to 7, 8 to 11, and 12 to 15 years old. To correct for multiple testing, pairwise post hoc time effects were tested at the 1% level of significance. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Participants. Eighty-one children (43 boys and 38 girls) with congenital ($N = 69$, 85%) or acquired ($N = 12$, 15%) brain lesions were included. Mean age at first assessment was 9 years 11 months (SD 3 y and 3 m). Unilateral CP was left sided in 36 (44%) and right sided in 45 (56%) children. Forty-four (54%) children attended mainstream schools and 37 (46%) special education schools. According to the MACS, 29 (36%) children were classified as level I, 36 (44%) as level II, and 16 (20%) as level III. All children received regular physical therapy throughout the duration of the study, varying from one to five sessions weekly, with a median duration of 90 minutes per week (range 30–240 minutes). Of this time, therapists spent a mean time of 35% per session on upper limb treatment. Of the time spent on upper limb treatment, a mean of 41% of the time was dedicated to functional activities, 32% to stretching, 20% to strength training, and 7% to other aspects such as sensory training or electrical stimulation. The time spent on functional activities was almost equally divided between unimanual (48%) and bimanual activities (52%). Only three children ceased physiotherapy when reaching adulthood. Twenty-one children also received occupational therapy during the study course with a median duration of 45 minutes per week (range 20–90 minutes).

Figure 1 displays a flow chart detailing the number of participating children at the four assessments. During the study course, 10 children received botulinum toxin-A injections, of whom two received it twice. These children were excluded from the analysis of the next assessment if the injection was less than six months prior to the assessment. Between 1- and 5-year follow-up, 15 children participated in an intensive therapy study, including a home program of modified CIMT [25]. After this intensive training period, the children continued their regular physiotherapy sessions.

3.2. Time Course of Upper Limb Function over Five Years. Table 1 shows the results of the LMM analysis. A significant deterioration over five years was noted for PROM ($p = 0.008$) and AHA scores ($p < 0.001$), whereas a significant improvement was seen for grip strength in both hands ($p < 0.001$), Melbourne Assessment ($p = 0.002$), Jebsen-Taylor test in both hands ($p < 0.001$), and ABILHAND-Kids ($p < 0.001$). Post hoc tests showed improvements between baseline and one-year follow-up for grip strength of the nonaffected hand ($p < 0.001$) and for the Jebsen-Taylor test in both hands ($p < 0.001$). Further, between one and five years, improvements were observed in grip strength at both sides ($p < 0.001$), Melbourne Assessment ($p < 0.001$), Jebsen-Taylor test (affected hand $p < 0.001$, nonaffected hand $p = 0.002$), and ABILHAND-Kids ($p < 0.001$). PROM and AHA scores, on the contrary, showed a significant deterioration between 1- and 5-year follow-up (PROM $p = 0.028$ and AHA $p < 0.001$). No significant time effects were found after five years for muscle tone ($p = 0.17$), muscle strength ($p = 0.86$), and the ratio between grip strength of the affected versus nonaffected hand ($p = 0.92$). Figures 2(a)–2(d) show the time trends of the activity outcome measures.

For the outcome measures with reported SDDs, we explored whether individual change scores between baseline and 5-year follow-up exceeded the SDD threshold (7.4%). For the Melbourne Assessment, 13 (19%) children improved

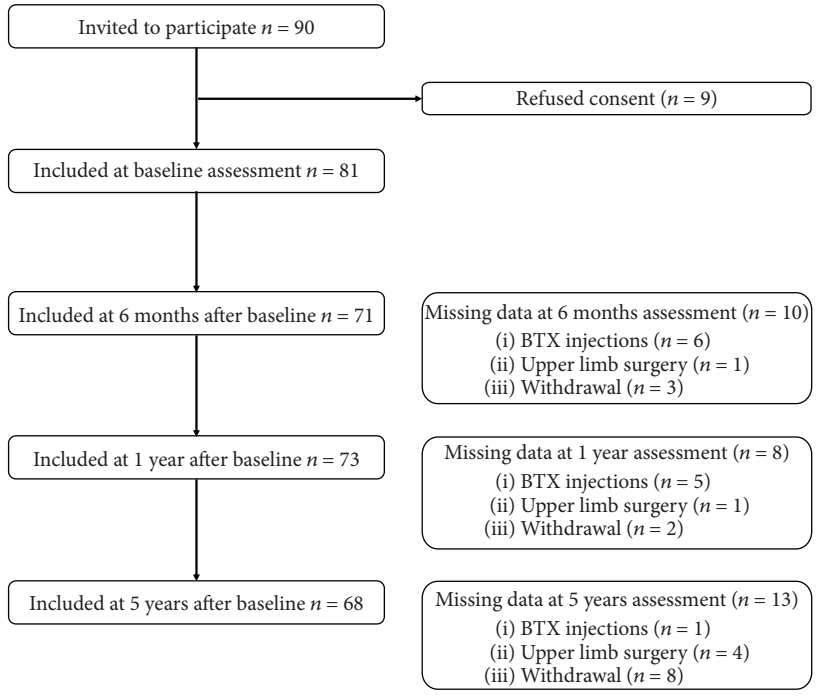

Figure 1: Number of children and details of missing data at all measurement points.

Table 1: Results of the linear mixed models analysis: mean (SE) estimates of outcome measures at baseline, 6 and 12 months, and 5 years.

	Baseline	6 months	1 year	5 years	p value[a]
PROM (0–7)	1.30 (0.2)	1.47 (0.2)	1.46 (0.2)	1.76 (0.2)	**0.008**
Muscle tone (0–44)	7.75 (0.53)	8.06 (0.54)	8.37 (0.53)	8.3 (0.54)	0.17
Muscle strength (0–45)	31.91 (0.54)	32.00 (0.55)	31.85 (0.55)	31.73 (0.55)	0.85
Grip strength					
Absolute scores AS (kg)	6.39 (0.72)	6.87 (0.74)	7.23 (0.73)	10.87 (0.75)	**<0.0001**
NAS (kg)	15.88 (1.03)	17.31 (1.05)	18.12 (1.05)	25.86 (1.06)	**<0.0001**
Ratio (%)	40.0 (3)	39.0 (3)	40.0 (3)	40 (3)	0.92
Melbourne Assessment (%)	67.92 (2.15)	67.82 (2.16)	67.24 (2.16)	70.09 (2.17)	**0.002**
AHA (logits 0–100)	62.12 (2.33)	62.74 (2.35)	62.29 (2.34)	56.58 (2.36)	**<0.0001**
Jebsen-Taylor test (s)					
AS	341.29 (28.74)	331.53 (28.86)	302.1 (28.87)	289.09 (29.93)	**<0.0001**
NAS	53.3 (3.34)	50.39 (3.42)	44.96 (4.43)	37.52 (3.46)	**<0.0001**
ABILHAND-Kids (logits)	1.83 (0.24)	2.11 (0.25)	2.12 (0.25)	2.95 (0.26)	**<0.0001**

PROM: passive range of motion; AHA: Assisting Hand Assessment; AS: affected side; NAS: nonaffected side; SE: standard error; [a]: linear mixed models

more than 7.4%, 51 children (75%) remained stable, and four children (6%) deteriorated more than 7.4%. In contrast, on the AHA, 13 (20%) children improved more than 5 AHA logits, 17 (27%) remained stable, and 34 (50%) deteriorated with at least 5 AHA logits. Finally, for the ABILHAND-Kids, 15 (31%) children improved above the SDD threshold of 1.8 logits, 33 (67%) children remained stable, and only one (2%) child deteriorated over five years.

3.3. Influencing Factors. Age had a significant influence on the time evolution of the PROM ($p < 0.001$), with children between 8 and 11 years old at baseline acquiring more movement limitations between 1- and 5-year follow-up (Figure 3(a)). Age also significantly influenced the evolution of AHA scores ($p = 0.003$), with younger children being stable over time but older children from the age of 9 years, showing a decrease in AHA scores (Figure 3(b)). Secondly, gender influenced the evolution of grip strength, which improved significantly more in boys ($p < 0.001$). Etiology also influenced evolution of grip strength and Jebsen-Taylor scores (both $p < 0.001$), which improved significantly more in children with congenital lesions compared to acquired lesions (Figures 3(c) and 3(d)). Furthermore, MACS levels influenced the evolution of grip strength (Figure 3(e)) and

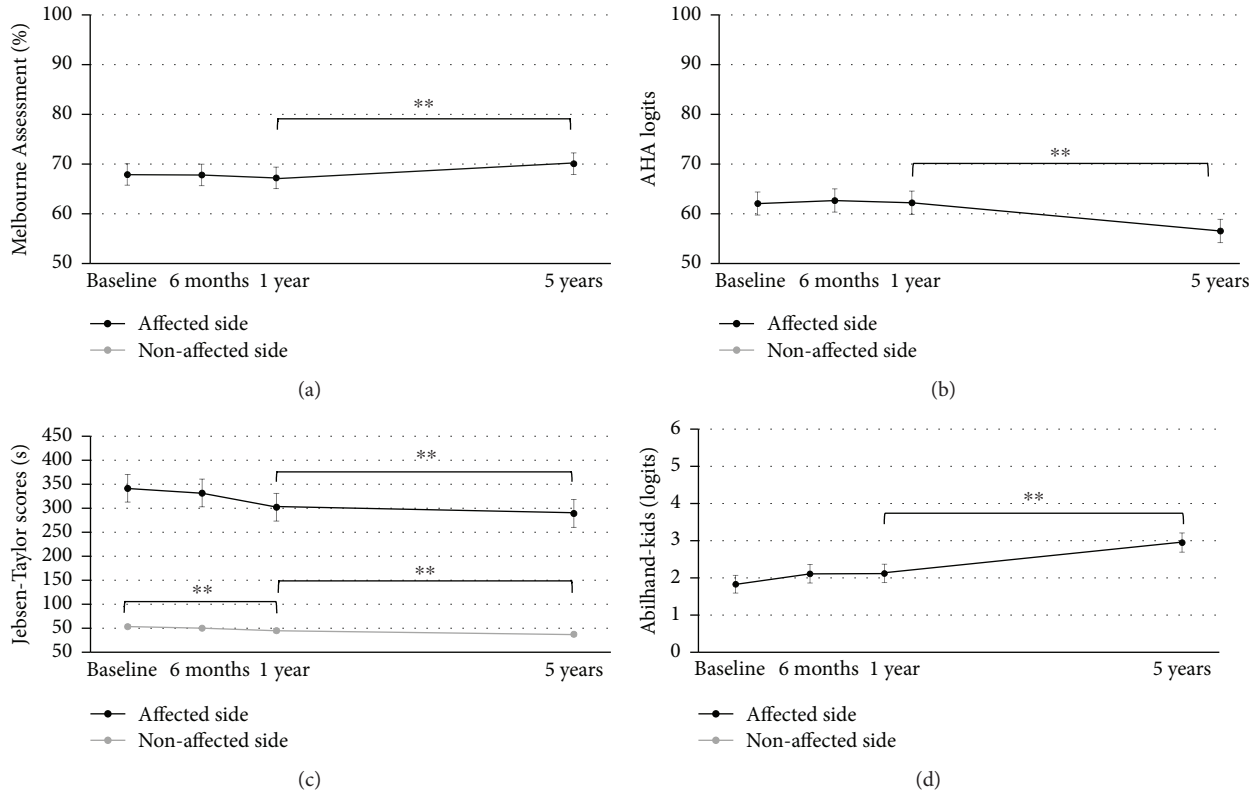

Figure 2: Mean and standard error estimates at baseline, 6 and 12 months, and 5 years for (a) Melbourne Assessment, (b) Assisting Hand Assessment (AHA), (c) Jebsen-Taylor test, and (d). ABIILHAND-Kids.

Jebsen-Taylor scores (Figure 3(f)), with better improvements in grip strength ($p < 0.001$) and Jebsen-Taylor scores ($p < 0.001$) in children with MACS level I.

Children who received botulinum toxin injections during the study course showed significantly more increase in muscle tone ($p = 0.01$), less increase in grip strength at the affected side ($p = 0.0006$), and more pronounced decline in AHA scores compared to children who did not receive injections ($p < 0.0001$) (Figures 4(a)–4(c)). Finally, the participation in a modified CIMT program did not influence the evolution of any of the activity measures ($p > 0.08$).

4. Discussion

This study aimed to map the 5-year time course of upper limb function and the influencing factors in children with unilateral CP according to the ICF body function and activity level. Results showed increased limitations in PROM mainly from the age of 9 years onwards. Furthermore, grip strength and unimanual capacity improved over time, mostly in mildly affected children. On the contrary, the spontaneous use of the affected upper limb in bimanual activities became less effective, again from the age of 9 years onwards.

Results at body function level showed more PROM limitations over time, mainly developing in children aged 9 years and older, while this process stabilizes around 14-15 years of age. Visual inspection showed most pronounced limitations for wrist extension. This confirmed the results of a recent study that reported a twofold increase in skeletal muscle stiffness of the wrist and finger flexors in children with unilateral and bilateral CP compared to typically developing children [26]. The cause of the increased stiffness is however yet unknown, though it can be hypothesized that it is attributed to an increased content of intramuscular collagen [27], together with an increased amount of connective tissue around fiber bundles, i.e., a thickening of the perimysial extracellular matrix [28]. These results imply that current methods to lengthen wrist and finger flexor muscles are of utmost importance to be applied in this age group. This may include stretching, use of splints, and botulinum toxin injections followed by intensive therapy and surgical interventions, e.g., tendon transfer surgery.

Additionally, grip strength increased over time both in the affected and nonaffected hand. Improvements were mainly seen in children with MACS level I and congenital lesions. It seems that children with better hand function are more likely to improve over time [2, 3, 7, 29]. The grip strength ratio between the affected and nonaffected hand remained stable around 40%, implying that grip strength increased at the same rate in both hands.

At activity level, significant improvements were found in unimanual capacity, based on the Melbourne Assessment and Jebsen-Taylor scores. Again, most improvements were seen in children with MACS level I and with congenital lesions. For typically developing children with comparable ages, Taylor et al. reported an age-related 10% reduction in time to perform the Jebsen-Taylor test (i.e., from 31.5 seconds at 10-11 years to 28.4 seconds at 15–19 years) [16].

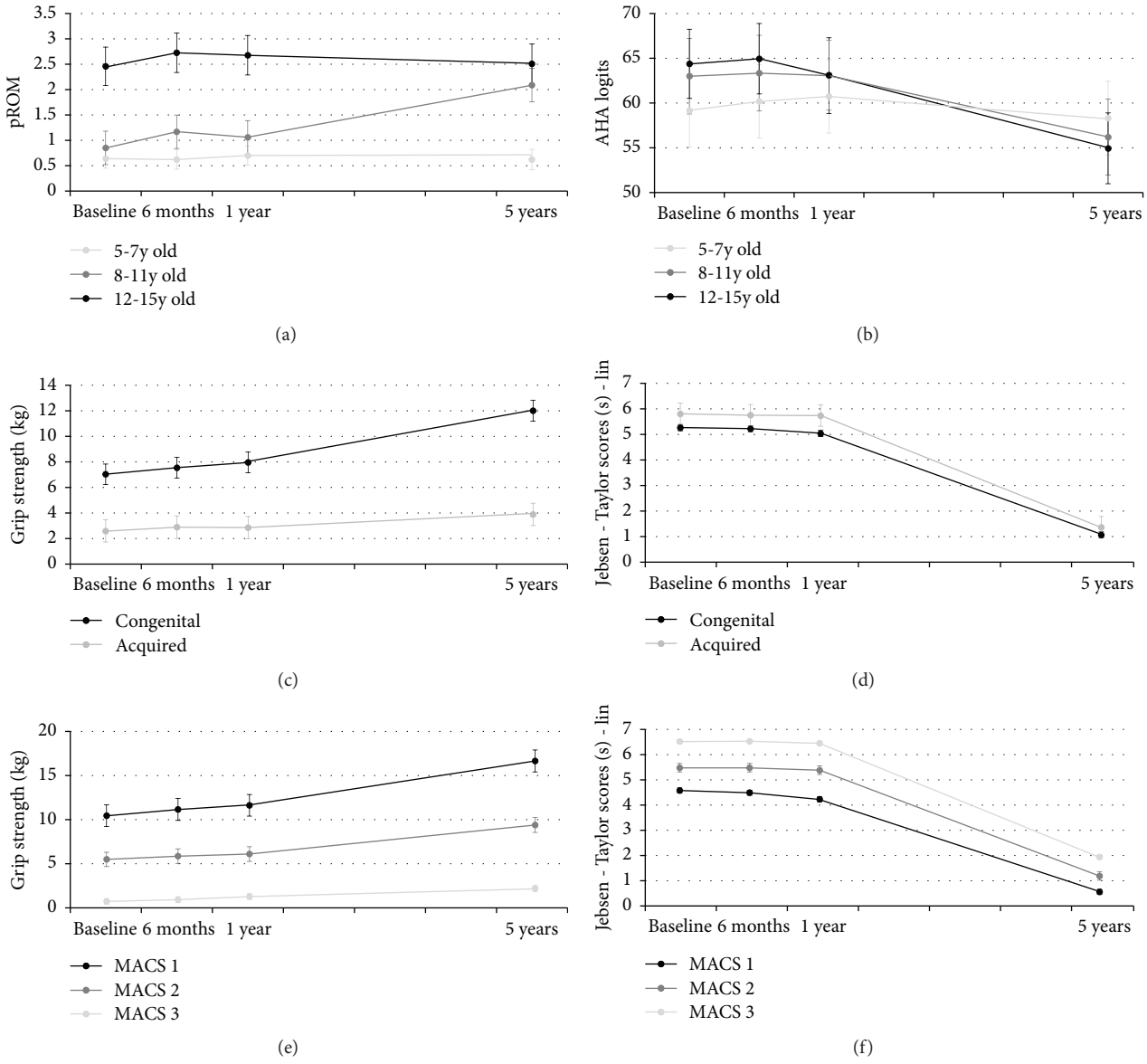

Figure 3: Means and standard error estimates at baseline, 6 and 12 months, and 5 years for the three age groups for (a) passive range of motion (PROM) and (b) Assisting Hand Assessment (AHA); for the two etiology groups for (c) grip strength on the affected side (AS) and (d) Jebsen-Taylor scores on AS; and for the three Manual Ability Classification System levels (MACS) for (e) grip strength on AS and (d) Jebsen-Taylor scores on AS.

The mean time to perform the test in our sample of children with unilateral CP decreased with 15% over five years, which may likely be of clinical significance.

Surprisingly, despite improvements in unimanual capacity, deterioration was seen in bimanual performance. From the age of 9 years onwards, children seem to use the affected arm less and less efficiently in bimanual activities, which is also a common complaint of parents. This finding is in accordance with the study of Fedrizzi et al. reporting less improvement in spontaneous hand use than in grip assessment between the age of 4 and 11 years [4]. We hypothesize that several factors may contribute to this deterioration in bimanual performance such as the presence of sensory deficits [1], mirror movements [30], and developmental disregard [31]. In the study of Nordstrand et al. children with unilateral CP showed a rapid development of bimanual performance at a young age and reached 90% of their estimated limit between 30 months and 8 years [3]. These authors attempted to investigate whether there was a decline in hand function, as the children approached 12 years of age. However, results were inconclusive because of too few data in this age group [3]. The novel finding of decline in bimanual function in our study has important clinical implications. To improve bimanual performance, a wide range of evidence-based therapy models can be applied such as CIMT, bimanual therapy, or combined models [32]. These models involve intensive blocks of goal-directed, skills-based practice. High-level evidence has shown that CIMT is effective for improving unimanual capacity brought about by implicit learning [33]. However, CIMT is not the most optimal modality to target

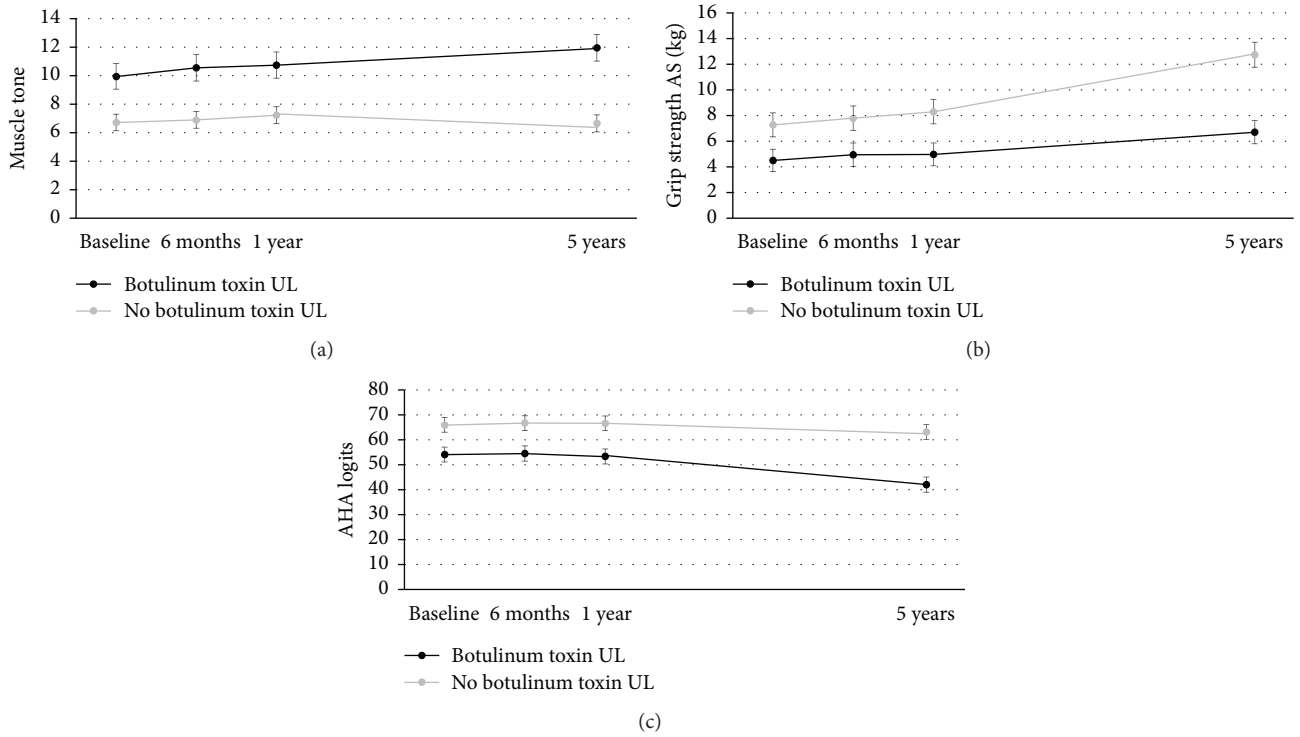

Figure 4: Means and standard error estimates at baseline, 6 and 12 months, and 5 years for the groups of children who did or did not receive botulinum toxin injections during the study course for (a) muscle tone, (b) grip strength on the affected side (AS), and (c) Assisting Hand Assessment (AHA).

explicit learning required for learning how to use both hands together in daily skills. Therefore, from the age of 9 years onwards, it may be more effective to organize intensive training focusing on bimanual performance. According to the motor learning principle of training specificity implying that "you progress to what you actually practice," learning bimanual skills may be best achieved through practice of bimanual tasks [33].

Despite the decrease in bimanual performance as tested with the AHA, a significant improvement was found in ABILHAND-Kids scores over five years. We assume that these differences may be related to the nature of the tests. The AHA is a structured play session of bimanual activities in which the use of the assisting hand is scored, for example, how well the child moves his upper arm or forearm, whether he varies his type of grasp, or how he regulates his grip force. The ABILHAND-Kids on the other hand rates the perception of the parent on the ease or difficulty of the child in performing daily life activities. This does not take into account how the task is performed, whether this is one handed or with the help of other body parts such as their teeth to open a bag of chips or their arm to fixate a bottle to unscrew it. We assume that with maturation, children improve their motor learning and planning and adopt compensation strategies to perform ADL activities with more ease. This results in higher independency in daily life activities.

Children in our study had access to local services. This access includes regular check-ups and a wide range of physiotherapy and occupational therapy interventions. A subsample of 15 children also followed a home-based modified CIMT program between the period of one and five-year follow-up. Significant improvements in bimanual performance were reported immediately after CIMT and were retained at 10-week follow-up [25]. However, follow-up results showed that around three years later, the time course of bimanual performance did not differ between the group that did or did not receive the CIMT program. This may imply that repetitive boosts of therapy are needed to attain long-term improvements.

This study excluded children who received botulinum toxin injections within 6 months prior to the time point testing to rule out immediate effects of the injections. After six months, these children were enrolled again in the study, although we acknowledge that long-term effects of botulinum toxin injections might exist [32]. We did not exclude these children from further follow-up as (1) botulinum toxin can be considered as common care in our settings and (2) excluding these children would have induced selection bias and would result in a nonrepresentative sample of children with unilateral CP. Indeed, further data inspection showed that the children who received botulinum toxin injections during the study course were mostly classified as MACS levels II and III and showed pronounced deficits in muscle tone, grip strength, and bimanual performance at baseline. This may explain why these children also showed more deterioration in function compared to children who did not receive injections. This confirms our statistical assumption for linear mixed models that the missingness of these data points does not depend on unobserved outcomes but on

observed outcomes, namely, MACS levels and assessments of muscle tone, grip strength, and bimanual performance.

This study included a large cohort of children with unilateral CP and a standardized set of reliable outcome measures at body function and activity level. Results were based on robust statistical modelling taking inevitable drop-outs into account. However, some limitations need to be recognized. First, for body function measures of spasticity and strength, ordinal rating scales were used, which are dependent on subjective interpretation. Therefore, great efforts were pursued to maximize standardization. Ordinal scales might also be less sensitive to subtle changes in muscle tone. As an alternative, future study should include quantitative measures such as dynamometers or instrumented spasticity measures [34] that might be more sensitive to change and will improve our understanding of upper limb function evolution in this population. Secondly, this study was based on a convenience sample, recruited in different centres. During the study course, all children received routine therapy and a subset received CIMT or botulinum toxin injections. In our health care system in Belgium, routine physiotherapy is commonly organized in distributed practice with one to five individual physiotherapy sessions per week. Our results, therefore, cannot be generalized to children receiving other service conditions, such as short boosts of intensive therapy. Finally, we acknowledge that also other neurological biomarkers, such as corticospinal tract reorganization, may influence longitudinal development of upper limb function, which warrants further investigation.

5. Conclusions

The novel findings from this large longitudinal study are that although different capacity measures improve over time, the spontaneous use of the affected upper limb in bimanual tasks decreases and becomes less effective from the age of 9 years onwards. Additionally, children with unilateral CP develop more limitations in PROM in the upper limb, more specifically for wrist extension, over a 5-year time period. These novel insights in the spontaneous evolution of upper limb function in children with unilateral CP and the factors that influence these time trends can provide guidance in delineating treatment priorities.

Data Availability

The underlying data related to this submission is available by request to the first author.

Disclosure

An earlier version of the paper was presented as an abstract in the 71st Annual Meeting of the American Academy for Cerebral Palsy and Developmental Medicine (AACPDM) 2017, Montreal, Canada.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

This study received financial support from the Research Foundation Flanders (FWO project, grant G087213N) and the KU Leuven (Special Research Fund, BOF grant OT/14/127).

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Hindawi
Neural Plasticity
Volume 2018, Article ID 9610812, 14 pages
https://doi.org/10.1155/2018/9610812

Clinical Study

Transcranial Direct Current Stimulation (tDCS) Paired with Occupation-Centered Bimanual Training in Children with Unilateral Cerebral Palsy: A Preliminary Study

Tonya L. Rich,[1] **Samuel Nemanich**,[2] **Mo Chen**,[3,4] **Kathleen Friel**,[5,6,7] **Timothy Feyma**,[8] **Linda Krach**,[9] **Tanjila Nawshin**,[1] **Gregg Meekins**,[10] **and Bernadette T. Gillick**[1,2]

[1]*Department of Rehabilitation Medicine, Program in Rehabilitation Science, University of Minnesota, Minneapolis, MN 55455, USA*
[2]*Department Rehabilitation Medicine, Program in Physical Therapy, University of Minnesota, Minneapolis, MN 55455, USA*
[3]*Non-Invasive Neuromodulation Laboratory, MnDRIVE Initiative, University of Minnesota, Minneapolis, MN 55455, USA*
[4]*Department of Psychiatry, University of Minnesota, Minneapolis, MN 55455, USA*
[5]*Burke Neurological Institute, White Plains, NY 10605, USA*
[6]*Weill Cornell Medicine, New York, NY 10065, USA*
[7]*Blythedale Children's Hospital, Valhalla, NY 10595, USA*
[8]*Gillette Children's Specialty Healthcare, St. Paul, MN 55101, USA*
[9]*Courage Kenny Rehabilitation Institute-Minneapolis, Minneapolis, MN 55407, USA*
[10]*Department of Neurology, University of Minnesota, Minneapolis, MN 55455, USA*

Correspondence should be addressed to Tonya L. Rich; rich1038@umn.edu

Received 23 March 2018; Revised 18 July 2018; Accepted 27 August 2018; Published 5 November 2018

Academic Editor: Simona Fiori

Objective. We investigated the preliminary efficacy of cathodal transcranial direct current stimulation (tDCS) combined with bimanual training in children and young adults with unilateral cerebral palsy based on the principle of exaggerated interhemispheric inhibition (IHI). *Methods*. Eight participants with corticospinal tract (CST) connectivity from the lesioned hemisphere participated in an open-label study of 10 sessions of cathodal tDCS to the nonlesioned hemisphere (20 minutes) concurrently with bimanual, goal-directed training (120 minutes). We measured the frequency of adverse events and intervention efficacy with performance (bimanual—Assisting Hand Assessment (AHA)—and unimanual—Box and Blocks), self-report (Canadian Occupational Performance Measure (COPM), ABILHAND), and neurophysiologic (motor-evoked potential amplitude, cortical silent period (CSP) duration, and motor mapping) assessments. *Results*. All participants completed the study with no serious adverse events. Three of 8 participants showed gains on the AHA, and 4 of 8 participants showed gains in Box and Blocks (more affected hand). Nonlesioned CSP duration decreased in 6 of 6 participants with analyzable data. Cortical representation of the first dorsal interosseous expanded in the nonlesioned hemisphere in 4 of 6 participants and decreased in the lesioned hemisphere in 3 of 4 participants with analyzable data. *Conclusions*. While goal achievement was observed, objective measures of hand function showed inconsistent gains. Neurophysiologic data suggests nonlinear responses to cathodal stimulation of the nonlesioned hemisphere. Future studies examining the contributions of activity-dependent competition and cortical excitability imbalances are indicated.

1. Introduction

Children with unilateral cerebral palsy (UCP) due to perinatal stroke or periventricular leukomalacia exhibit great variability in clinical presentation. This heterogeneity may be partially attributed to neuroplastic influences, both developmental and maladaptive, on the corticospinal tract (CST). Developmentally, the CST is established through competitive withdrawal of bilateral CST projection fibers early in infancy driven in part by activity-dependent influences [1]. In children with UCP, bilateral CST projection fibers do not withdraw, as would be observed in children

with typical development [2]. A lack of competitive withdrawal is compounded by decreased activity of the weaker, or more affected, hand during early development [3].

In addition to activity-dependent influences on the CST during development, a potential maladaptive influence is an imbalance in interhemispheric inhibition (IHI) observed in adults with stroke, which may limit motor recovery [4]. Similarly, for some children with UCP, greater inhibition from the nonlesioned hemisphere is observed as compared to the lesioned hemisphere [5, 6]. Interventions targeting inhibition of the nonlesioned hemisphere have resulted in improvements in hand function and goal attainment [7–9]. One factor that may influence the response to novel intervention, such as combined noninvasive brain stimulation (NIBS) and rehabilitation protocols, is altered patterns of underlying brain circuitry of the CST in pediatric populations with neurologic deficits [10].

Assessments of cortical excitability using NIBS, such as transcranial magnetic stimulation (TMS), can be used to examine CST circuitry, a key biomarker in children with UCP related to hand function [11, 12]. Depending on responses obtained from each brain hemisphere, CST circuitry patterns can be described as contralateral, bilateral, and ipsilateral. Contralateral CST circuitry describes when a motor-evoked potential (MEP) is elicited from a muscle contralateral to stimulation (e.g., stimulation to the lesioned hemisphere elicits a MEP from the more affected hand and stimulation to the nonlesioned hemisphere elicits a MEP from the less affected hand) [11, 12]. Bilateral CST circuitry describes responses following stimulation to (1) the lesioned hemisphere with a MEP elicited from the more affected hand and (2) the nonlesioned hemisphere with a MEP elicited from both hands. Ipsilateral circuitry describes when children display a MEP from both hands when stimulating the nonlesioned hemisphere and no MEP from the more affected hand following stimulation to the lesioned hemisphere. Children on the contralateral CST circuitry continuum (e.g., contralateral and bilateral CST circuitry) show greater baseline function of the more affected hand [13]. However, both children with contralateral and ipsilateral circuitry patterns respond to upper limb rehabilitation [5, 8, 14, 15].

Bimanual training is one type of upper limb rehabilitation intervention used for children with all types of CST circuitry [16]. This form of rehabilitation is designed to activate the more affected (e.g., weaker) and less affected (e.g., stronger) limbs during daily living skills and goal-directed training [17]. Furthermore, bimanual intervention focused on child-identified goals may facilitate progress on functional activities within a contextually relevant framework that includes dual roles of the hands to stabilize and manipulate objects depending on the task requirements [18]. Prior investigations suggest that children with UCP demonstrate functional gains following bimanual intervention which are comparable to other upper limb rehabilitation approaches such as constraint-induced movement therapy [19, 20].

To optimize the efficacy of rehabilitation, training may be paired with transcranial direct current stimulation (tDCS), a form of interventional NIBS. TDCS has polarity-specific effects on cortical excitability: anodal is associated with excitatory after-effects, and cathodal is associated with inhibitory after-effects [21]. The pairing of rehabilitation with NIBS has the potential to mitigate maladaptive neuroplasticity and promote the optimal neurophysiologic state for recovery in individuals with stroke [22]. For instance, cathodal tDCS targeting the nonlesioned primary motor cortex paired with constraint-induced movement therapy can rebalance the excitability of both hemispheres and the changes in cortical excitability seen were associated with changes in function [23]. However, a recent study suggested that children with contralateral CST circuitry demonstrated greater benefit from combined intervention than did children with ipsilateral CST circuitry [15].

The purpose of this study was to explore the influence of bimanual intervention paired with cathodal tDCS to the nonlesioned hemisphere on behavioral and neurophysiologic outcomes in children with UCP. We hypothesized that cathodal tDCS will decrease excitability of the nonlesioned hemisphere and pairing with bimanual training will promote the synergistic plasticity between the hemispheres through enhanced sensorimotor integration of information, leading to increased excitability of the lesioned hemisphere. To further examine the effect of underlying CST circuitry patterns on the response to intervention [10, 15], and to minimize heterogeneity in our sample, we focused on children within the contralateral CST circuitry continuum.

2. Materials and Methods

2.1. Design. A single-subject, multiple-baseline, open-label study in children and young adults with UCP was conducted. Changes in behavioral and neurophysiologic outcomes after a 10-day active tDCS + bimanual intervention were compared to their individual baseline performance in the absence of a control group. Each child completed 4 baseline sessions including behavioral and self-reported measures. To meet the needs of participants throughout a wide geographical region, pretesting sessions #1–3 were conducted with real-time video conference calling. Pretesting #4, all intervention sessions, and the posttest were completed in person at a university laboratory (Figure 1). All behavioral testing was completed prior to TMS testing.

2.2. Participants. Children and young adults ages 7–21 (mean 13 years, 3 months ± 3.7 years) with imaging-confirmed perinatal stroke were recruited for this study using a laboratory database of past study participants and recruitment of new participants through physician referrals. Inclusion criteria required the presence of a MEP from both hemispheres as assessed by TMS (i.e., children with contralateral CST or bilateral CST circuitry). Exclusion criteria included seizures within the past two years, implanted metal or medical devices contraindicated for NIBS testing or interventions, co-occurring disorders or medical condition (e.g., brain injury, neoplasm, and pregnancy), communication deficits preventing the answering of safety questions, or a history of phenol or botulinum toxin injections within the past 6 months [24, 25].

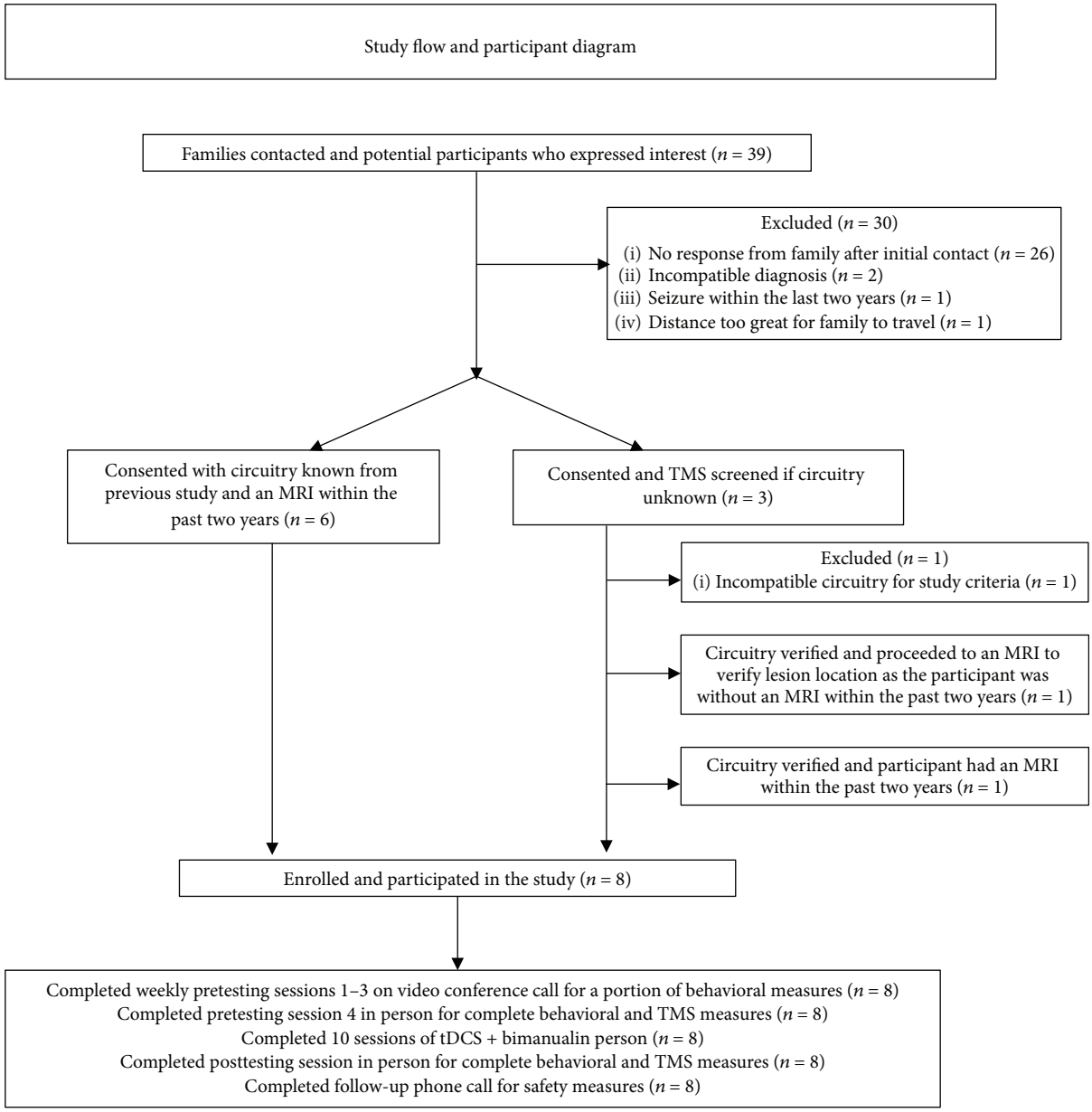

Figure 1: Study flow and participant diagram. MRI: magnetic resonance imaging; tDCS: transcranial direct current stimulation; TMS: transcranial magnetic stimulation.

This study was approved by the University of Minnesota's Institutional Review Board. All participants ages 18 years and older and caregivers of children ages 7–17 provided consent after informed consent discussion. All children ages 7–17 provided assent. This study was registered on clinicaltrials.gov (NCT02250092).

2.3. Outcome Measures

2.3.1. Safety Measures. Safety was monitored with surveys, caregiver input, vital sign assessment, and grip strength tests. Assessment of safety occurred before and after all brain stimulation (e.g., TMS testing and tDCS sessions) [26]. An independent medical monitor reviewed all safety data.

2.3.2. Hand Function Measures. Hand function was assessed with performance and self-report measures. The primary outcome was the Assisting Hand Assessment (AHA). The AHA is a measure of spontaneous, bimanual hand function [27]. A change of five AHA units is the smallest detectable difference (SDD) [28]. A rater blinded to the testing session (i.e., pretest or posttest) scored the AHA videos.

Secondary behavioral outcomes included the Box and Blocks, Canadian Occupational Performance Measure (COPM), and the ABILHAND-KIDS. Self-reported measures incorporated participant and caregiver feedback. The Box and Blocks is an assessment of gross unimanual dexterity, and the average of three trials is reported to the nearest integer (Performance Health, Warrenville, IL, USA). The

COPM is an occupation-centered, child-rated goal-setting measure with a scale of 1–10 (1—lowest, 10—highest) of activities of daily living skills [29]. A change of two points represents a clinically important difference [30]. Prior investigations have established reliability of the COPM with parent-proxies; however, the reliability of repeated COPM ratings in children is unknown [31]. In this study, previous ratings were not reviewed prior to the child self-assessed rating at each testing time point. The ABILHAND-KIDS is a 21-item caregiver-reported measure of perceived manual abilities in children with CP [32]. The ABILHAND-chronic stroke version was used for participants over age 16 [33]. The total ABILHAND score was converted to a linear measure of manual ability using logits. The least measurable difference for the ABILHAND is 0.19 logits [32, 33].

2.3.3. TMS Measures. Neurophysiological changes were assessed using single-pulse TMS including motor threshold, MEP amplitude, cortical motor mapping, and cortical silent period (CSP) as indices of cortical excitability. TMS testing was completed within 1 week prior to intervention and within one week following the completion of the tDCS + bimanual intervention. Neurophysiologic responses were assessed with TMS using a 70 mm coil using a Magstim 200 stimulator (Magstim Company Ltd., Dyfed, United Kingdom). TMS methods are previously described in other publications [34, 35]. Briefly, bilateral electromyography data was monitored in real time and stored in a laptop computer using a customized LabVIEW program (v2012, National Instruments, Austin, Texas, USA) for offline analysis using a custom Matlab program (MathWorks, Natick, Massachusetts, USA). The primary muscle of interest was the first dorsal interosseous. Stereotactic neuronavigation (Brainsight, Rogue Research, Quebec, Canada) was used to guide TMS coil placement based on individual neuroanatomy acquired from previous MRI. All participants were positioned in a semireclined chair (Rogue Research, Montreal, Canada) with a custom-made tray (Gillette Lifetime Specialty Healthcare, St. Paul, MN) for consistent positioning of the upper extremities during TMS assessment.

To characterize the clinical population of participants with UCP, comprehensive TMS testing included motor threshold assessment (resting motor threshold (RMT) or, if an RMT was not present, an active motor threshold (AMT)), single-pulse TMS testing (10 analyzable trials) using 120% RMT or 110% AMT testing intensity, and CSP (10 analyzable trials) using 120% RMT. Single-pulse testing verified the presence or absence of a MEP from each hemisphere. The 10 trials of single-pulse testing were assessed with peak-to-peak amplitude.

CSP testing provided a measure of motor cortical inhibition [36]. Variations in CSP duration are observed in adults with stroke and other neurological conditions [36]. The CSP testing protocol involved the participant maintaining a tonic contraction of 20% maximum voluntary contraction of the first dorsal interosseous followed by single-pulse TMS using an intensity of 120% RMT to the hemisphere contralateral to the hand maintaining the contraction. Visual feedback of muscle activity and the 20% maximum voluntary contraction target level was provided. The CSP was measured using methods previously described by Garvey et al. [37]. The onset/offset was calculated with a custom Matlab (MathWorks Inc., Natick, MA) program based on Garvey et al. [37], and all trials were visually inspected.

TMS-derived motor mapping measured the cortical representation of an individual muscle. The motor mapping protocol used an intensity of 110% RMT with 1–5 trials performed at each grid point guided by stereotactic neuronavigation [34]. Grid points and corresponding cortical locations were constructed using four concentric circles (radius 10 cm, 7.9 mm between adjacent points) centered on the motor hotspot (Brainsight, version 2.3.4), producing a map with 81 total grid points. Counts of mapping sites with a MEP response of $\geq 50\,\mu$V are reported.

Motor maps were rendered using a predetermined algorithm within the stereotactic neuronavigation software (Brainsight, version 2.3.4). Specifically, MEP amplitudes measured during the motor mapping assessment were projected onto the individual participant's grid points and transformed to a color map. Each MEP response data point is associated with the position and orientation of the TMS coil. Prior investigations of motor mapping in children with UCP suggest stability of maps between testing sessions in the absence of intervention and indicate that a 20% change in the number of responsive sites following rehabilitation is considered significant [34].

The number of pulses was tracked using stereotactic neuronavigation with a protocol upper limit of 300 pulses per hemisphere. A protocol limit of 85% maximum stimulator output was used for all testing procedures to maximize comfort to the participant. Participants were assessed with a caregiver present.

During the data analysis phase, individual circuitry patterns were identified. Participants displaying contralateral MEPs with no measurable bilateral electromyographic activity after stimulation were described as having *contralateral CST circuitry* [12, 38]. Participants with a MEP from the more affected hand following stimulation to the lesioned hemisphere and bilateral MEPs following stimulation to the nonlesioned hemisphere were classified as having *bilateral CST circuitry*.

2.4. Intervention. Ten sessions of tDCS + bimanual intervention occurred in a group model over two weeks (Figure 1). Each participant was paired 1 : 1 with the same trained volunteer interventionist for each session. Intervention sessions were 120 minutes of motor training with the first 20 minutes including simultaneous tDCS. Motor training focused on the participant's goals. At the conclusion of the study, all participants and families were surveyed for study satisfaction.

The 20 minutes of 1.5 mA cathodal tDCS targeted the primary motor cortex of the nonlesioned hemisphere (Soterix 1×1 Limited Total Energy (LTE), New York, NY). Medical grade electrode sponges of 5×7 cm with a 25 cm^2 rubber electrode were used. The center of the cathode electrode sponge was placed on the TMS-derived motor hotspot of the nonlesioned hemisphere. The reference electrode was placed on the contralateral supraorbital region.

Table 1: Participant characteristics and function.

ID	Age (y)	Sex	Lesion location	Lesion details	Affected side	MACS level	CST circuitry	Lesioned MT	Nonlesioned MT
1	8	M	Cortical and subcortical	Left MCA distribution, frontal and parietal operculum, left PLIC, cerebral peduncle, and ventral pons	R	II	Contralateral	52	46
2	14	F	Subcortical	Left lateral ventricle, centrum semiovale, and internal capsule	R	II	Contralateral	44	41
3	14	F	Cortical and subcortical	Left lateral ventricle with adjacent thinning of cortex and corpus callosum	R	III	Contralateral	64	57
4	10	F	Subcortical	Left lacunar infarct in thalamus	R	II	Bilateral	66	63
5	15	F	Cortical and subcortical	Left MCA distribution, frontoparietal cortex, left thalamus, and basal ganglia	R	I	Bilateral	46	42
6	19	F	Cortical and subcortical	Right lateral ventricle and posterior right frontal lobe	L	II	Bilateral	44	38
7	12	M	Cortical and subcortical	Right thalamus and periventricular white matter	L	II	Bilateral	75	54
8	8	M	Cortical and subcortical	Left frontal lobe and posterior parietal lobe; left subinsular, caudate, and lentiform nuclei; left basal ganglia and hypothalamic region; and left cerebral peduncle	R	III	Bilateral	77	48

CST: corticospinal tract; F: female, L: left; M: male; MACS: Manual Ability Classification System; MCA: middle cerebral artery; MT: motor threshold; PLIC: posterior limb of internal capsule; R: right; y: years. Lesion location was identified by a pediatric neurologist as cortical, subcortical, or both cortical and subcortical. CST circuitry pattern was identified with single-pulse transcranial magnetic stimulation testing.

The location of the TMS-derived motor hotspot was marked daily after tDCS session using a nontoxic marking pen (i.e., Sharpie marker).

2.5. Statistical Analysis. To determine the score used for baseline, we first evaluated the reliability of behavioral measures. The reliability of repeated pretest measures was assessed with a one-way analysis of variance and the appropriate intraclass correlation coefficient (Supplemental Table 1). Intraclass coefficients of ≥0.90 reflect excellent reliability, ≥0.75 demonstrates good reliability, ≥0.50 indicates moderate reliability, and <0.50 indicates poor reliability [39]. Using these established intraclass coefficient ranges, the reliability of repeated baseline measures indicated moderate to excellent reliability in the ABILHAND and COPM-Performance subscale and poor reliability in the COPM-satisfaction subscale and the Box and Blocks (Supplemental Table 1).

To evaluate for change following intervention, single-subject analysis involved review of the participant's magnitude of change relative to clinically meaningful changes for each behavioral outcome. The statistic used to determine the clinically meaningful change varied for each behavioral measure. Previously published SDD, minimal clinically important difference (MCID), least measurable difference, and standard error of the mean (SEM) were used for the AHA [28], COPM [40], ABILHAND [32], and the Box and Blocks, respectively. In the absence of a published meaningful difference for the Box and Blocks, the SEM calculated from the multiple pretests in this study was used. If there were multiple baselines of a behavioral measure, we used the average for pre-/post-comparisons. For a single baseline measure, this testing time point was used for comparisons. Magnitude of change was calculated with net change scores, comparing the average of all pretest measures to the post-test score [41]. We also performed a responder analysis of the behavioral outcome measures, where a responder achieved at least the SDD of 5 points on the AHA. No formal analyses were conducted within groups due to sample size. Statistical analyses were conducted using SPSS (IBM, Armonk, NY) and GraphPad Prism (GraphPad Software, La Jolla, CA).

3. Results

Nine children enrolled in this study (Figure 1). One participant was excluded following preintervention TMS testing due to the absence of bilateral or contralateral circuitry resulting in a final sample of eight undergoing the pretesting, intervention, and posttesting. Baseline characteristics are reported in Table 1. Structural T1 magnetic resonance images displaying the participant's lesion are provided in the supplemental materials (Supplemental Figure 1).

Structural T1 imaging, data on birth history, age at first imaging, and age at research imaging are provided in supplemental materials (Supplemental Table 3).

3.1. Safety Measures. All enrolled participants completed the study without any serious adverse events. In this study, 37.5% (3 participants) reported minor adverse events related to active tDCS in more than one tDCS session, with the most common symptom being unusual feelings on the skin of the

Table 2: Participant function and behavioral change scores.

ID	Circuitry	AHA	AHA Δ	Box and Blocks MA	Box and Blocks MA Δ	Box and Blocks LA	Box and Blocks LA Δ	COPM-Perf	COPM-Perf Δ	ABILHAND	ABILHAND Δ
1	Contra	60	**8**	19.08	**7**	57.58	**9**	3.45	1.55	1.48	**0.28**
2	Contra	83	**10**	27.08	**8**	70.33	**15**	3.70	1.31	3.90	0.00
3	Contra	52	0	15.08	**−7**	31.33	1	2.67	**4.00**	0.52	**0.62**
4	Bilateral	54	−2	3.78	2	61.17	−4	4.69	**3.56**	2.02	0.15
5	Bilateral	53	0	12.58	−2	48.33	**10**	3.83	**2.83**	3.80	0.10
6	Bilateral	75	0	25.67	**7**	79.33	3	1.00	**5.00**	0.72	**0.48**
7	Bilateral	55	4	41.25	3	65.92	**7**	5.00	**3.00**	1.79	−0.03
8	Bilateral	34	**8**	0	**−10**	34.92	−2	3.35	0.85	0.25	−0.08

AHA: Assisting Hand Assessment; Contra: contralateral; COPM: Canadian Occupational Performance Measure; LA: less affected hand, MA: more affected hand; Δ: change. AHA is reported in 0–100 AHA units, Box and Blocks is reported as a mean, COPM is reported as a mean, and ABILHAND is reported in logits. Baseline testing for Box and Blocks, COPM, and ABILHAND reflects the average of 4 pretests. The change score is calculated with posttest-average baseline score. Achievement of smallest detectable difference (AHA), least measurable difference (ABILHAND), and clinically meaningful differences (COPM) are denoted in bold. Given the precision of the measurement, change in Box and Blocks that exceeds the standard error of the measure is denoted in bold (>3 blocks for MA hand and >6 blocks for LA hand).

head (Supplemental Table 2). Three participants experienced spasms in their more affected hand during tDCS + bimanual intervention. All reported symptoms resolved within the same session. Overall, a small proportion (12–37%) of individuals reported tDCS-related minor adverse events suggesting that the tDCS intensity of 1.5 mA was well tolerated by participants.

3.1.1. Individual Analysis of Meaningful Change. All participants achieved clinically meaningful change on at least one measure (AHA, COPM-Performance and/or Satisfaction, and ABILHAND). Individual changes on behavioral measures are reported in Table 2, and individual performance measures over all testing sessions are provided in the supplemental data (Supplemental Figure 2). Changes on each of the behavioral measures based on the responder analysis are reported in Figure 2.

Collectively, changes in behavioral measures varied for participants in this study including bimanual, unimanual, self-report, and general study satisfaction. For the primary outcome measure, the AHA, 3 of 8 participants achieved the SDD. Increases on the Box and Blocks that exceeded the SEM were observed in 2 participants for the more affected hand and 1 participant for the less affected hand. For the self-reported measures, 5 of 8 participants achieved the MCID for the COPM and 3 of 8 participants achieved the least measurable difference on the ABILHAND. Of the 3 participants who achieved the SDD on the AHA, none of them achieved the MCID on the COPM. For this open-label study, families reported an average satisfaction rating of 9.5 out of 10 related to their study experience.

3.1.2. TMS Measures. Four participants completed full TMS testing on all measures. In the remaining 4 participants, data collection was limited by the presence of an AMT only, machine intensity exceeding protocol limit of 85% maximum stimulator output, and limited tolerance for lesioned hemisphere testing at posttest. No participants reported use of centrally acting medications for seizure control that would influence neurophysiologic responses.

Single-pulse amplitude was measured in 8 of 8 participants on the nonlesioned hemisphere with hypothesized decreases observed in 5 of 8 children. The nonlesioned hemisphere CSP duration was measured in 6 of 8 children and all exhibited decreases from pretest to posttest. Lesioned hemisphere single-pulse amplitude was measured in 7 of 8 children with hypothesized increases observed in 3 children. Lesioned hemisphere CSP duration was measured in 3 of 8 children with increases observed in 2 children. No statistical analyses were conducted on cortical excitability data due to small sample. Individual changes in MEP amplitude and CSP duration are shown in Figure 3.

TMS motor map data were collected on 6 participants; 4 of 6 had mapping data for both hemispheres. Hemispheric differences (lesioned vs. nonlesioned) appeared to influence changes in cortical mapping with variations in map features observed. The lesioned hemisphere cortical map sites decreased in 3 participants and increased in 1 participant (Table 3). The nonlesioned hemisphere cortical map sites increased in 4 participants and decreased in 2 participants (Table 4). In the 4 participants who have cortical maps for both hemispheres, the changes in response sites for one hemisphere resulted in an inverse change in the opposite hemisphere (e.g., if the lesioned hemisphere increased in number of responsive sites, the nonlesioned hemisphere decreased in number of responsive sites). Figure 4 displays map changes in one representative participant with mapping data collected from both hemispheres.

Exploratory correlations were conducted between behavioral and neurophysiologic outcomes. There was a strong correlation observed between baseline AHA score and the motor threshold of the lesioned hemisphere at baseline (Spearman's rho correlation coefficient: −0.71, $p = 0.05$) (Supplemental Table 4). All other correlations between baseline neurophysiologic measures and change in behavior were nonsignificant ($p > 0.05$).

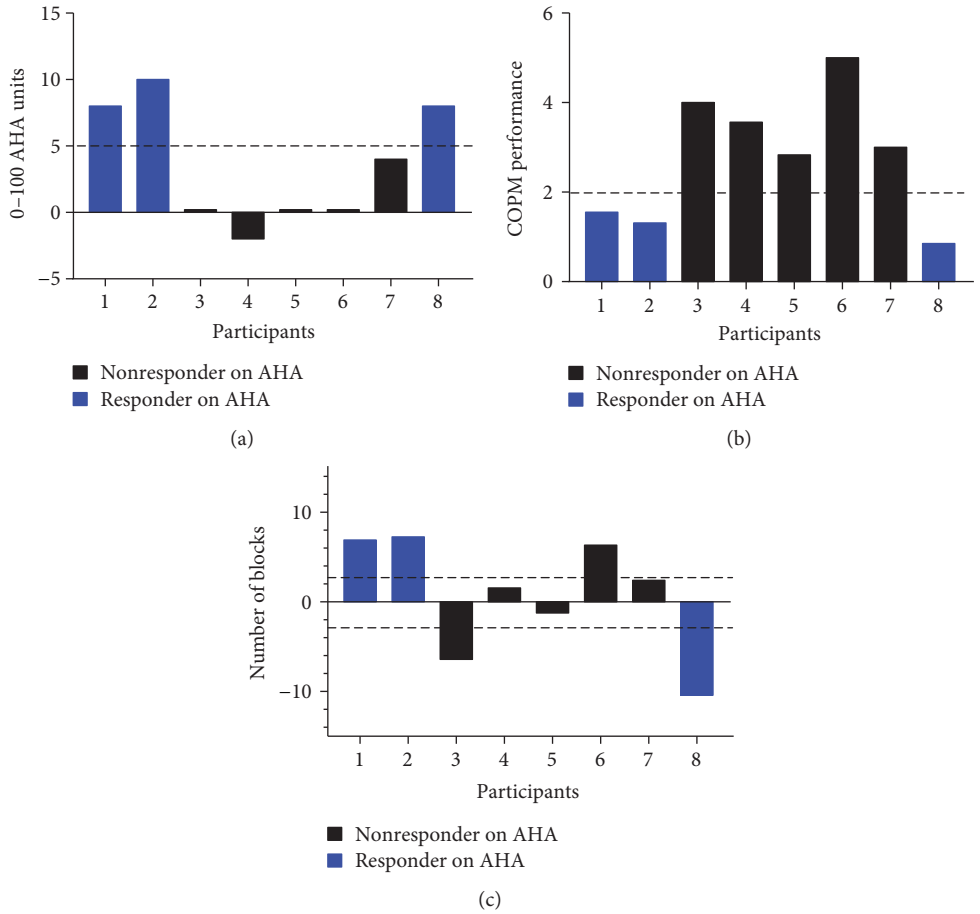

Figure 2: Individual change in behavioral measures (a) AHA with the SDD denoted with a dashed line. (b) COPM-Performance. The MCID of the COPM is denoted with a dashed line. (c) Box and Blocks with more affected hand. The SEM of repeated baseline testing with the Box and Blocks is denoted with a dashed line. AHA: Assisting Hand Assessment; COPM: Canadian Occupational Performance Measure; MCID: minimal clinically important difference; SDD: smallest detectable difference. Note: the *y*-axis representing change differs between the measures. Blue bars represent participants identified as a responder on the primary outcome (AHA), and black bars represent participants identified as a nonresponder on the primary outcome (AHA).

4. Discussion

4.1. Confirming Safety Results. Serial sessions of combined active tDCS + bimanual intervention were safe and feasible in children with UCP. In our study, the most common minor adverse events were unusual feelings on the skin of the head, and symptoms were mitigated using study protocols. For participants who experienced spasms, alternating fine and gross motor activities were found to be beneficial in reducing the occurrence of a spasm during the same session.

4.2. Variable Gains in Hand Function and Self-Reported Measures. For this study, we identified "responders" to tDCS based on changes on the AHA. In this sample, three participants (37.5% of sample) achieved the SDD (5 points) on the AHA following the 20 hours of combined intervention. Others have reported that 30% of participants achieved the SDD on the AHA following 90 hours of bimanual intervention alone [16]. Of the participants identified as a responder, two displayed contralateral circuitry and one displayed bilateral circuitry. In this subgroup of responders, we observed a wide range of baseline AHA scores (34 AHA units to 83 AHA units), age (8 to 14 years old), and both types of circuitry patterns.

The single-case design of our study also allowed for individual analysis which may allow us to generate hypotheses for larger studies and may be preferred over group analyses that mask sensitivity of changes by evaluating group mean response. For example, participants 1 and 2 could be considered strong candidates for tDCS as they both displayed contralateral circuitry and high AHA scores at baseline. Both of these participants demonstrated further improvements following intervention on bimanual (AHA) and unimanual (Box and Blocks) measures. The improvements suggest that these participants had the ability to differentiate roles of the hands for bimanual tasks, and further improvements on the Box and Blocks may reflect a training benefit to each hand. In contrast, participant 8 displayed lower bimanual hand function at baseline and achieved the SDD on the AHA following intervention but did not demonstrate unimanual gains on the Box and Blocks. This suggests that this participant may have significantly benefitted from bimanual

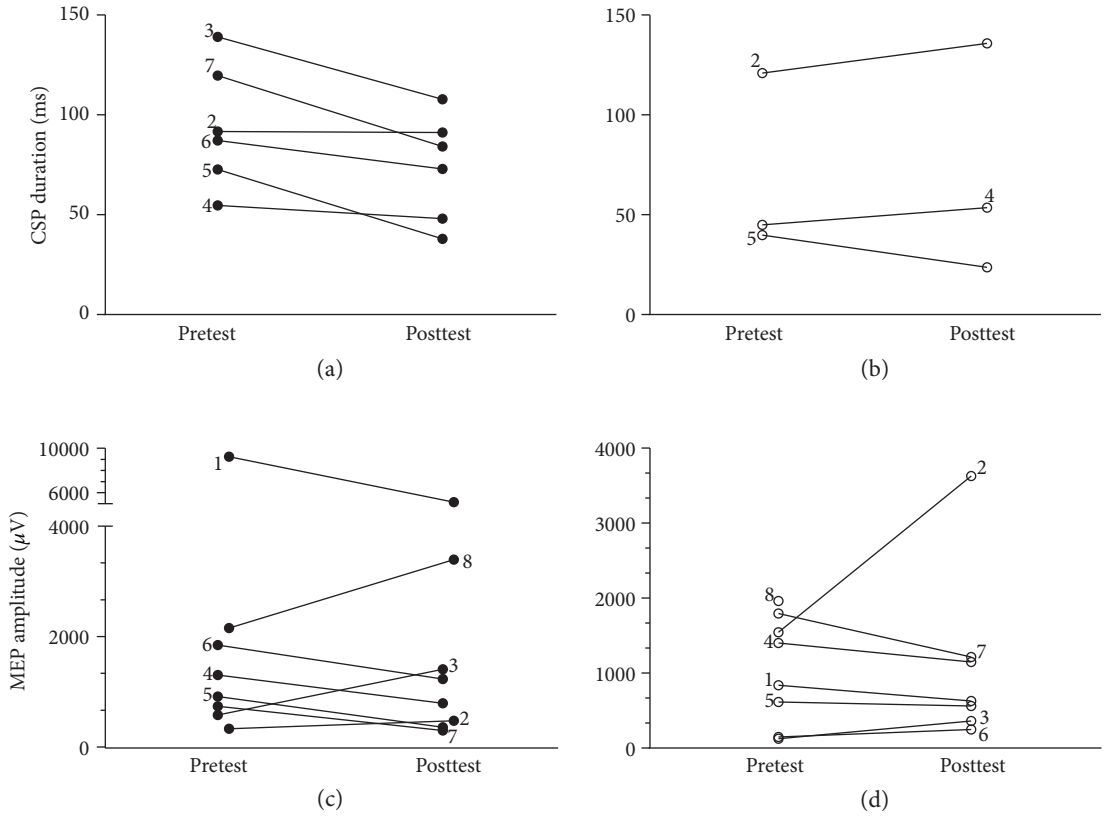

Figure 3: Pre- and posttest neurophysiologic measures. (a) Nonlesioned hemisphere amplitude with single-pulse TMS testing. (b) Lesioned hemisphere amplitude with single-pulse TMS testing. (c) Nonlesioned hemisphere CSP duration. (d) Lesioned hemisphere CSP duration. Nonlesioned data is denoted by a closed circle, and lesioned data is denoted by an open circle. Note: data points are labeled with a superscript participant identifier consistent with Table 1 (participant IDs 1–8). The y-axis representing change differs between the measures. CSP: cortical silent period; ms: milliseconds; SP: single-pulse; TMS: transcranial magnetic stimulation. Amplitudes are measured in μV (microvolts).

Table 3: Lesioned hemisphere cortical excitability mapping measures.

ID	Laterality	Mapping testing intensity (% MSO)	Lesioned hemisphere				
			Pretest FDI sites	Pretest FDI mapping latency (ms)	Posttest FDI sites	Posttest FDI mapping latency (ms)	% Δ in FDI sites
1	Contralateral	†	†	†	†	†	†
2	Contralateral	48	24	22.50	18	20.19	**−25.00**
3	Contralateral	70	24	27.06	23	25.26	−4.17
4	Bilateral	†	†	†	†	†	†
5	Bilateral	51	39	49.95	57	47.51	**46.15**
6	Bilateral	38	38	58.00	27	58.36	**−28.95**
7	Bilateral	†	†	†	†	†	†
8	Bilateral	†	†	†	†	†	†

FDI: first dorsal interosseous; ID: participant identifier; ms: milliseconds; NA: not assessed at baseline; NC: not calculated due to missing baseline data; RMT: resting motor threshold; Δ: change; †: missing data; % MSO: percentage of maximum stimulator output. Motor mapping testing intensity was 110% RMT (resting motor threshold). MEP latency durations are reported using the mean time (ms). Bolded values are considered significant defined as ≥20% mapping response sites.

training alone. These preliminary data contribute to our understanding of who might benefit most from combined interventions or motor training alone which could be assessed in future studies that could guide personalized medicine approaches for stroke rehabilitation.

Our behavioral measures were selected to reflect observed performance of bimanual (AHA) and unimanual (Box and Blocks) hand function, the child's perception of goal attainment (COPM), and the caregiver's perception of hand use (ABILHAND). Each of these assessments contributes to

Table 4: Nonlesioned hemisphere cortical excitability mapping measures.

ID	Laterality	Mapping testing intensity (% MSO)	Nonlesioned hemisphere				
			Pretest FDI sites	Pretest FDI mapping latency (ms)	Posttest FDI sites	Posttest FDI mapping latency (ms)	% Δ in FDI sites
1	Contralateral	†	†	†	†	†	†
2	Contralateral	45	25	20.47	31	20.47	**24.00**
3	Contralateral	63	27	21.41	32	20.20	18.52
4	Bilateral	69	17	18.65	14	16.73	−17.65
5	Bilateral	46	15	19.51	13	19.19	−13.33
6	Bilateral	42	10	18.06	14	19.20	**40.00**
7	Bilateral	59	15	26.50	23	19.30	**53.33**
8	Bilateral	†	†	†	†	†	†

FDI: first dorsal interosseous; ID: participant identifier; ms: milliseconds; NA: not assessed at baseline; NC: not calculated due to missing baseline data; RMT: resting motor threshold; Δ: change; †: missing data; % MSO: percentage of maximum stimulator output. Motor mapping testing intensity was 110% RMT (resting motor threshold). MEP latency durations are reported using the mean time (ms). Bolded values are considered significant defined as ≥20% mapping response sites.

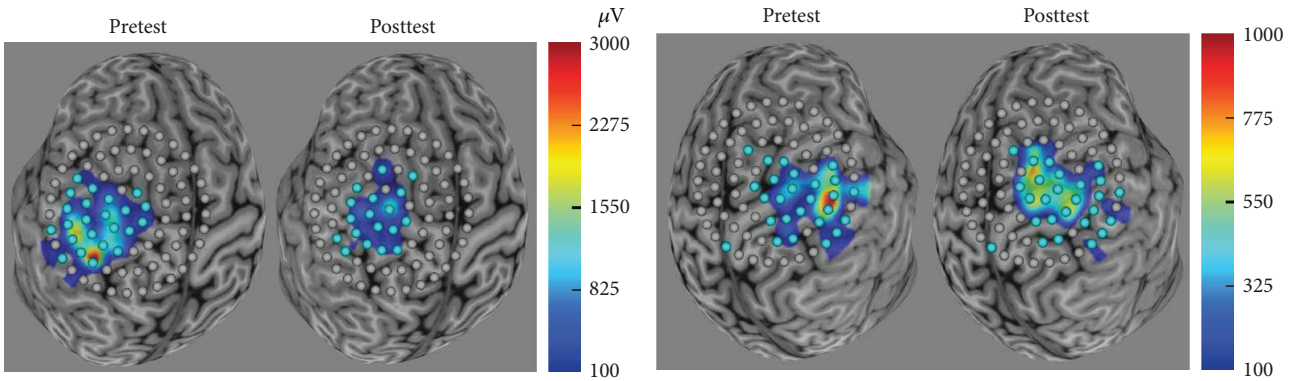

Figure 4: TMS-derived motor map of (a) nonlesioned and (b) lesioned hemispheres of participant 2 with the grid centered on the TMS-derived motor hotspot. The brain reconstruction has been rotated to allow for a direct view of the TMS-derived motor hotspot. Grey grid points signify no MEP responses, and teal grid points signify MEP responses > 50 μV (microvolts). The color bar represents the range of amplitude of MEP responses in μV. The range in the amplitude color bar is dependent on the magnitude of the responses observed. Note: ranges are consistent across pre- and posttesting sessions but may differ across hemispheres. CST: corticospinal tract; MEP: motor-evoked potential; TMS: transcranial magnetic stimulation.

a broader understanding of how combined interventions impacted hand function in this sample of eight children. No one pattern of change was desired as the baseline behavioral function of each child varied.

From a behavioral standpoint, the motor learning resulting from the intervention may have been highly task-specific with changes in motor function not observed in general movements captured on the primary outcome measure, the AHA. The AHA is a measure of how the participant chooses to use his/her hand during a novel task whereas the COPM is a self-reported measure of the participant's perspective of their achievement of goals regardless of how the participant is able to accomplish the goal (e.g., compensatory vs. newly acquired motor movements). Therefore, the construct underlying each of the behavioral measures may have differed providing a comprehensive assessment of the intervention effects on physical abilities for desired activities.

Variability in the Box and Blocks was observed during multiple baseline testing sessions and with average pretest/posttest comparisons. Decreases observed in the more affected hand, measured with the Box and Blocks, could be attributed to sensitivity of the measure or engagement in testing procedures. Others have used the Box and Blocks as a daily measure of safety with results suggesting that individual variability is observed in children with UCP [42]. This evidence warrants continued monitoring for a decrement in unimanual dexterity in combined NIBS and motor training studies with larger samples.

The assessment tools selected for this study when taken together represent components of bimanual and unimanual hand function reporting both on the impairment and activity levels of the World Health Organization's International Classification of Functioning, Disability, and Health framework. The individual variability in gains observed between the

assessment tools reflects differing constructs for each measure as reported by others [43].

4.3. Neurophysiologic Influences. The effects of tDCS can be evaluated through changes in cortical excitability using single-pulse TMS [21]. Varying neurophysiologic effects of the combined tDCS and bimanual intervention were observed in the nonlesioned hemispheres as measured by TMS. We observed changes in the measures of amplitude of the nonlesioned hemisphere in five of eight participants consistent with a hypothesized decrease in excitability after the tDCS + bimanual intervention. In contrast, a shortening of CSP duration in six of six participants and an increase in the number of mapping sites in four of six participants with complete data suggests an increased excitability in the nonlesioned hemisphere. From a neurophysiologic standpoint, the interindividual variability observed in this study could be attributed to known factors (e.g., anatomical differences and lesion locations) and factors unexamined in this study (e.g., the influence of maturation on brain development, genetics, functional organization of circuits, baseline neurophysiologic state, and capacity for change in motor learning) that collectively may influence plasticity [44, 45].

Prior reports have shown that MEP amplitude changes are dependent on tDCS polarity (e.g., anodal stimulation results in increased MEP amplitude) [21]. However, the duration of stimulation and the pairing of stimulation with a cognitive task may result in paradoxical effects, such as cathodal tDCS producing increased MEP amplitude [46, 47]. The combination of bimanual activity and stimulation could have produced similar unexpected changes in cortical excitability and explain the variability observed in MEP amplitude and motor mapping data.

We did not measure IHI directly; however, our CSP measurements suggest decreased intracortical inhibition. Because exaggerated IHI may not be present in all children with injury early in life, a comprehensive understanding of both activity-dependent withdrawal and the balance of IHI on CST development and resultant hand function is needed to understand potential response to rehabilitation interventions [6]. Future longitudinal studies with multimodal outcomes will elucidate the neurophysiologic substrates of change following combined interventions in children and young adults with UCP.

Similar conflicting results between neurophysiologic measures were observed in the lesioned hemisphere. Three participants displayed increased excitability (e.g., a decrease in motor threshold and increase in MEP amplitude) whereas three participants displayed a mixed response to stimulation (e.g., decreased motor thresholds and decreased amplitudes).

Our motor mapping results reflect both expanded and reduced map size on each hemisphere, which differs from prior reports of map expansion in response to motor training in both animal models and human studies. The changes in motor maps suggest that cortical map changes are activity-dependent [34, 48, 49]. These results strengthen the argument for an activity-dependent competition model of neuroplastic change in children with UCP [3]. Recent rehabilitation studies have examined motor map changes after bimanual, occupation-focused intervention reporting bilateral increases in map size and amplitude of responses and no significant change in motor threshold [50] and increases in the number of responsive sites following bimanual intervention in children with UCP [34]. In our study, the changes in the number of responsive sites may reflect a broader intrahemispheric network as our grid encompassed a region beyond primary motor cortical area, but responses outside this region warrant further confirmation in future studies.

4.4. Lesion Location and Clinical Presentation May Influence Variability in Outcomes. In our sample, two participants with a right-sided lesion demonstrated similar patterns of change in neurophysiologic responses in the nonlesioned hemisphere as well as similar change in behavior. The heterogeneity of changes observed in participants with left-sided lesions may be attributed to the potential for hemispheric crowding of function suggesting that reorganization of function to the right hemisphere following a left hemisphere lesion may have a negative impact on typical functions of the right hemisphere [51–53]. Studies with larger samples may allow investigators to discern the association of lesion side to response to intervention in children with UCP.

Lesion location could have influenced the results; however, we are unable to determine this potential with our sample size. Our study represents a clinical sample of individuals with UCP where 6 of the 8 participants displayed combined cortical and subcortical lesions and the remaining 2 participants displayed subcortical lesions only. None of our participants presented with documented bilateral lesions. However, we cannot rule out that the lesion could have influenced the contralateral hemisphere, which may not be noted in the MRI report. We did observe a relationship between single-pulse motor-evoked potential amplitude of the nonlesioned hemisphere and baseline AHA score, but no correlations existed when evaluating changes among neurophysiologic and behavioral measures. One potential explanation is that compensatory descending tracts may be involved in recovery, warranting expanded investigations.

4.5. Limitations. Our conclusions are limited by the sample size, the open-label study design, and the sensitivity of our measures, and as such, the findings should be considered preliminary. Our study was open-label which may have influenced the self-reported measures of change and satisfaction by both participants and caregivers. This study design was selected to explore preliminary findings and provide direction for future studies. The lack of control group is limitation and must be considered when interpreting the findings.

We did not have an immediate measure of cortical excitability following tDCS (e.g., within minutes of completing tDCS). An immediate measure could evaluate not only reliability of single-pulse measures in children with UCP but also the time-effect of observed changes in neurophysiologic responses. For this study, the duration of participation encompassing daily safety measures and motor intervention precluded any additional testing.

In our study, motor training was individualized to the child's goals allowing for impairment-specific intervention (e.g., increasing the distance a child can reach with the more affected limb) in order to improve activity-specific performance (e.g., pushing the more affected limb through a t-shirt sleeve). This form of personalized intervention positively impacted self-reported goal achievement but may have influenced the effects of tDCS on other measures. Additionally, providing the intervention in a group format could have influenced the engagement of the participants, reports of side effects, and the caregiver and/or participant's perspective of change as self-reported on behavioral measures.

4.6. Future Directions. Future personalized medicine will allow for interventions to be optimally paired for children based on biomarkers or clinical practice guidelines given the child's clinical presentation. Critical areas of need in the pediatric investigations include computational modeling to optimize electrode placement, expanded testing (behavioral, neurophysiologic, and neuroimaging), and defined motor training protocols.

Prior investigations suggest that the peak electric fields are higher in children as compared to adults when modeled at the same tDCS intensity [54, 55]. Studies that incorporate computational modeling could allow for individualized stimulation parameters and may assist in controlling for anatomical differences between participants. For example, further comparison of electrode placement based on individual circuitry such as using anodal stimulation to the hemisphere of greatest control of the more affected hand (e.g., participants with contralateral circuitry may benefit from anodal stimulation to the lesioned hemisphere as compared to targeting the nonlesioned hemisphere in participants with ipsilateral circuitry). Electrode placement taken into consideration with comparative effectiveness studies on session frequency and duration of combined intervention in differing CST circuitry subtypes (e.g., greater number of sessions or a longer duration) could provide guidance for treatment protocols in the future. Further, it may be that alternate electrode placements (e.g., premotor area) not previously studied in children with UCP are more efficacious than the primary motor cortex as suggested in the adult literature [56].

Future studies with larger samples and expanded testing protocols will inform our knowledge of the mechanism of tDCS on motor learning and the aspects of motor learning that can be targeted with combined neuromodulatory interventions. Our performance and self-reported behavioral measures may not be sensitive enough to detect changes in neurophysiologic responses. Expanded testing protocols could focus on sensorimotor input including isolated movements and sensory function which may have stronger associations to neurophysiologic changes [57, 58]. Further, daily motor learning curves captured by a kinematic measure of hand function may allow for individualization of intervention [59]. Altogether, these measures may identify the sensorimotor contributions to change in function following intervention [60]. Expanded testing will assist in identifying which pediatric measures have the greatest sensitivity for measuring change following combined neuromodulatory and rehabilitation interventions.

Defining the critical components of the motor training and standardizing the motor training components (e.g., percentage of time spent on unimanual vs. bimanual tasks, types of tasks and motor movements targeted, and home program tasks) will allow for investigators to control for these potential influences when measuring for the effects of tDCS. Prior studies report that tasks with cognitive challenges, isometric contractions, and the duration of noninvasive brain stimulation can influence the observed neurophysiologic changes in response to tDCS [46, 47, 61]. Studies designed with an immediate neurophysiological measure following tDCS can provide insights into pairing activity with stimulation in children with UCP and potential paradoxical effects when pairing tDCS with a motor task.

5. Conclusion

The combined intervention of tDCS with occupation-centered rehabilitation intervention was safe and well-tolerated. Participants identified and rated their performance on goals that were meaningful to them, and customized activities during combined tDCS and bimanual training sessions designed to promote goal achievement proved feasible in a group setting. Our neurophysiologic findings suggest that the combined intervention affects each hemisphere differently, which may underlie variability observed with changes in behavioral hand function measures. A consistent neurophysiologic substrate that influences response to change has not yet been identified in children with UCP. Future clinical trials should consider cortical excitability evaluations based on underlying circuitry to measure changes following interventions, which may elucidate neurophysiologic mechanisms related to recovery.

Data Availability

The data used to support the findings of this study are restricted due to patient privacy. Access to these data will be considered by the author upon request, with permission from the Institutional Review Board.

Conflicts of Interest

No authors reported a conflict of interest.

Acknowledgments

This study was funded by the National Institutes of Health (NIH) Eunice Kennedy Shriver National Institute of Child Health and Development K01 Award (HD078484-01A1), the Cerebral Palsy Foundation, the Foundation for Physical Therapy Magistro Family Grant, Minnesota's Discovery, Research and InnoVation Economy (MnDRIVE), and the University of Minnesota Marie Louise Wales Fellowship. The project described was also supported in part by Award UL1 TR000114 and KL2 TR000113. The authors thank the Center for Neurobehavioral Development and

Center for Magnetic Resonance Imaging (P41 EB015894, 1S10OD017974-01) at the University of Minnesota. The research team also thanks Ben Andre, Andrew Kunz, Isabel Marbaker, Stephanie Palmer, Kayla Stark, Taylor Webster, and April Wheeler for serving as student interventionists; Gillette Lifetime Clinic seating specialist, Wayne Rydberg, for the custom tray fabrication; Dr. Marcie Ward for serving as a study physician; and Karen Chin for video scoring. Most importantly, the research team thank the families, caregivers, and participants involved in this study.

Supplementary Materials

Supplemental Table 1: reliability of pretesting measures. Each participant completed repeated baseline behavioral testing measures (1x/week for four weeks) as a part of this study protocol. This supplemental material includes the reliability data of the repeated pretesting measures and an interpretation of the reliability data. Based on this reliability data, we decided to use an average of the four baseline pretesting scores as the comparator for pre- and postintervention comparisons. Supplemental Table 2: tDCS-related MAE. Supplemental Figure 1: axial images of T1 anatomical magnetic resonance image (MRI) to display lesion location in each participant. Row one of the images reflects participants 1–4, and row two reflects participants 5–8. Verification of diagnosis was a criterion for inclusion for this study. T1 anatomical images were collected either (1) at the time of study participation or (2) within 2 years of study participation. The age range of participants at the time of imaging for this study was 8 years, 1 month to 18 years, and 1 month. A pediatric neurologist verified the lesion location. All MRI data was acquired using a 3 Tesla MRI scanner using a 32-channel head coil (Siemens Prisma scanner). Supplemental Table 3: birth and imaging history of participants. Supplemental Figure 2: change in behavioral measures repeated over time. All participants completed repeated baseline measure of behavioral measures including Canadian Occupational Performance Measure-Performance, ABILHAND, Box and Blocks with each hand, and Assisting Hand Assessment. These data represent individual data for repeated baseline measures and posttesting. Supplemental Table 4: Spearman's correlation coefficient between neurophysiologic and hand function measures at baseline. *(Supplementary Materials)*

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Hindawi
Neural Plasticity
Volume 2018, Article ID 1265143, 11 pages
https://doi.org/10.1155/2018/1265143

Research Article

Impaired Ability to Suppress Excitability of Antagonist Motoneurons at Onset of Dorsiflexion in Adults with Cerebral Palsy

Svend Sparre Geertsen[1,2] **Henrik Kirk**,[1,3] **and Jens Bo Nielsen**[2,3]

[1]*Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark*
[2]*Department of Neuroscience, University of Copenhagen, Copenhagen, Denmark*
[3]*Helene Elsass Center, Charlottenlund, Denmark*

Correspondence should be addressed to Svend Sparre Geertsen; ssgeertsen@nexs.ku.dk

Received 23 March 2018; Revised 14 September 2018; Accepted 19 September 2018; Published 9 October 2018

Guest Editor: Simona Fiori

We recently showed that impaired gait function in adults with cerebral palsy (CP) is associated with reduced rate of force development in ankle dorsiflexors. Here, we explore potential mechanisms. We investigated the suppression of antagonist excitability, calculated as the amount of soleus H-reflex depression at the onset of ankle dorsiflexion compared to rest, in 24 adults with CP (34.3 years, range 18–57; GMFCS 1.95, range 1–3) and 15 healthy, age-matched controls. Furthermore, the central common drive to dorsiflexor motoneurons during a static contraction in the two groups was examined by coherence analyses. The H-reflex was significantly reduced by 37% at the onset of dorsiflexion compared to rest in healthy adults ($P < 0.001$) but unchanged in adults with CP ($P = 0.91$). Also, the adults with CP had significantly less coherence. These findings suggest that the ability to suppress antagonist motoneuronal excitability at movement onset is impaired and that the central common drive during static contractions is reduced in adults with CP.

1. Introduction

When we move, our nervous system ensures that our muscles are activated to the appropriate extent and at the right time in relation to each other so that the movement may progress according to our intentions and with little or no conscious attention required. This is not something that comes easily and quickly. It takes children 10–12 years to attain the mature characteristics of bipedal gait seen in adults [1–4]. Step-to-step variability of gait is significantly larger than in adults and involves significantly more coactivation of antagonistic muscles [1, 3, 4]. Reaching and grasping follow a similar developmental trajectory, and an adult-like movement pattern is not achieved until around 12–14 years of age [5].

People with early brain lesion (cerebral palsy (CP)) in contrast continue to show very significant coactivation of muscles and high step-to-step variability of gait into adulthood [6–8]. They also lack the normal maturation of gating of sensory feedback at rest [9] and during gait [10, 11]. This may possibly be linked to an impaired development of the ability to predict and therefore suppress sensory feedback, which is linked to adequate prediction of the sensory consequences of the movement [12]. However, little is known about the underlying neural mechanisms that are responsible for the maintained coactivation pattern in adults with CP.

One of the mechanisms known to be important for the coordination of antagonist muscles is Ia reciprocal inhibition. Ia reciprocal inhibition involves a group of interneurons, which are activated through collaterals from descending pathways in parallel with agonist motoneurons and project to antagonist motoneurons [13]. In contrast to what is usually observed in other people with lesion of descending motor pathways, such as stroke and multiple sclerosis [14, 15], Ia reciprocal inhibition appears to be similar at rest in adults with CP as in healthy, age-matched controls [9]. However, pathophysiological changes in transmission in spinal motor

circuitries observed at rest may have little relevance for how those circuitries are controlled and modulated during motor activities [16–18]. Indeed, Leonard et al. [19] found that Ia reciprocal inhibition was similar in adults with CP and in healthy adults when measured at rest, but during static agonist contraction, the inhibition was increased in healthy subjects and reduced in adults with CP [19]. Morita et al. have also shown impaired regulation of Ia inhibition at the onset of agonist contraction in adults with multiple sclerosis and suggested that this could explain increased coactivation of antagonists in these subjects [20]. We recently showed that impaired gait function in adults with CP is associated with the ability to perform fast ankle movements [21], but it is not known how reciprocal inhibition is modulated at the onset of contraction in adults with CP.

Here, we consequently hypothesized that impaired descending control of spinal inhibitory circuits is responsible for the inability of adults with CP to adequately suppress antagonist muscle activity in relation to voluntary movement and that this may explain their continued coactivation during functional motor tasks. To assess modulation of spinal reciprocal inhibition, we measured the suppression of the soleus H-reflex at the onset of dorsiflexion, and to assess the central drive to the agonist motor pool (dorsiflexors), we measured the size of coupled oscillations in tibialis anterior motor units.

2. Material and Methods

2.1. Participants. Twenty-four adults diagnosed with CP (age 34.3 years, range 18–57; 15 men, 9 women; GMFCS 1.95, range 1–3) were recruited through the Danish Cerebral Palsy Organization. Fifteen subjects were diplegic, eight hemiplegic, and one quadriplegic. All subjects were described as spastic, and most of the subjects had received antispastic medication for shorter or longer periods. Many subjects had a history of multiple surgeries. See [21] for a detailed description of the participants. Furthermore, 15 age-matched (age 32.9 years, range 23–47; 9 men, 6 women) neurologically healthy adults were recruited to serve as a healthy control group.

The study was approved by the local ethics committee (H-4-2012-107), and all procedures were conducted within the standards of the Helsinki declaration. Prior to the experiments, all the participants received written and verbal information, and a consent form for participation was obtained.

2.2. Testing Procedures. Functional reciprocal inhibition (experiment 1) and central common drive (experiment 2) were assessed on the same day following the application of electromyography (EMG) electrodes and tests of the maximal voluntary contraction strength (MVC) and the rate of force development (RFD).

2.2.1. EMG Recordings. EMG activity was recorded using bipolar electrodes (Ambu Blue sensor N-10-A/25, Ambu A/S Ballerup; recording area 0.5 cm^2, interelectrode distance 2 cm) placed over the soleus muscle and the proximal and distal parts of the tibialis anterior muscle (TA_{prox} and TA_{dist}, respectively). The skin was gently abraded with sandpaper (3M red dot; 3M, Glostrup, Denmark). A ground electrode was placed on the distal part of the tibia. EMG signals were filtered (band-pass, 5 Hz–1 kHz), amplified (500-2000x), sampled at 2 kHz, and stored on a PC for offline analysis.

All EMG and H-reflex measurements (see below) were normalized to the maximal M-response (M_{max}) evoked in either the TA or soleus muscle by supramaximal stimulation (1 ms rectangular pulses; model DS7A, Digitimer, Hertfordshire, UK) of the common peroneal nerve or the tibial nerve, respectively. In these measurements, the intensity of stimulation of the respective nerves was increased from a subliminal level until there was no further increase in the peak-to-peak amplitude of the M-response with increasing stimulation intensity [22].

2.2.2. Measurement of MVC, RFD, and Cocontraction. The MVC and RFD procedures have been comprehensively described by Geertsen et al. [21]. Briefly, subjects were seated in a chair with their leg fastened to a stationary dynamometer and were carefully instructed to contract "as fast and forcefully as possible" and to hold the contraction for about 3 seconds. During each trial, the subject was verbally encouraged by the experimenter to produce maximal torque. Each subject performed 3 dorsiflexions with maximal effort. If an initial countermovement (identified by a visible drop in the torque trace) was observed, a new trial was performed. Data was recorded with Spike 2.611 software (CED 1401+; Cambridge Electronics Design, Cambridge, UK). Offline, the trial that produced the highest dorsiflexion peak torque (MVC_{DF}) was determined. The MVC_{DF} trial was then used to calculate the RFD at 200 ms following the onset of contraction (RFD_{200}) as a measure of explosive muscle force. The level of cocontraction in the MVC_{DF} trial was calculated as the area of rectified, smoothed soleus EMG (in percent of soleus M_{max}) divided by the area of rectified, smoothed TA_{dist} EMG (in percent of M_{max} in TA_{dist}) for the first 1000 ms following the onset of TA_{dist} EMG.

2.2.3. Experiment 1: Functional Reciprocal Inhibition. Functional reciprocal inhibition was evaluated by comparing the size of soleus H-reflexes at rest with H-reflexes elicited at the onset of explosive dorsiflexion contractions. H-reflexes were elicited by stimulation (1 ms rectangular pulses; model DS7A, Digitimer, Hertfordshire, UK) of the tibial nerve using a ball-shaped monopolar electrode (Simon electrode) placed in the popliteal fossa and the anode placed proximal to the patella. All H-reflex measurements were normalized to M_{max}.

To produce comparable afferent input to the soleus motoneuron pool at rest and at the onset of dorsiflexion contraction, the tibial nerve stimulation intensity was adjusted, if necessary, to elicit an M-response of approximately 10% of M_{max} in all trials. However, the actual intensities used were similar at rest (15.78 ± 4.86 mA) and at the onset of dorsiflexion (15.64 ± 4.83 mA). At rest, 15 H-reflexes were elicited with an interstimulus interval of 10 s. The subject was then asked to dorsiflex the ankle as fast as possible every 10 s

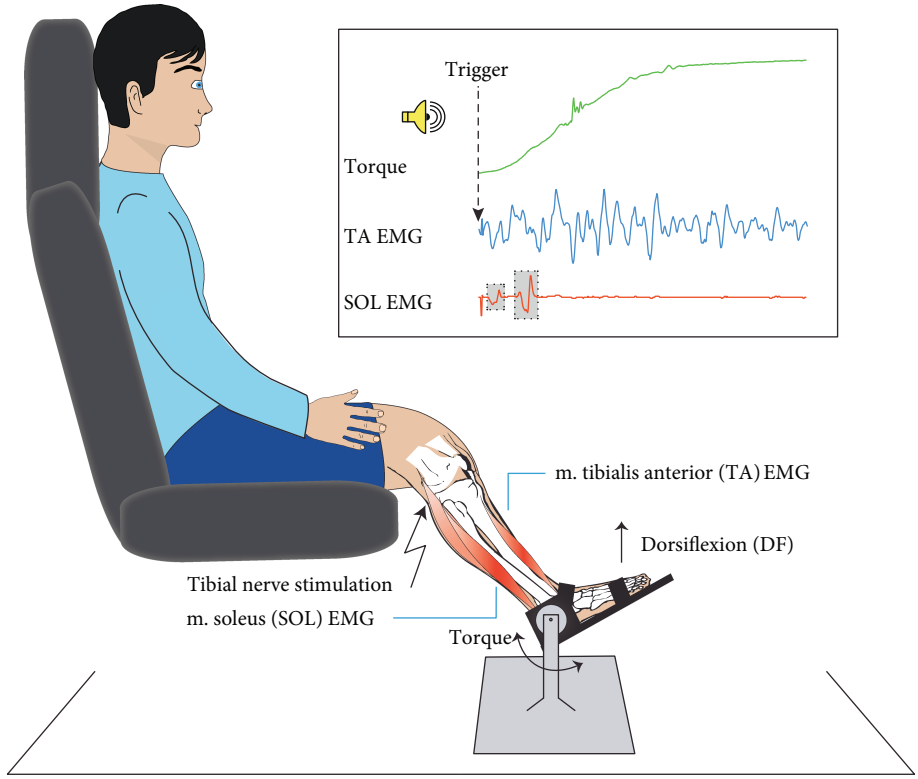

Figure 1: Experimental setup. Subjects were seated with their examined leg fastened to a stationary dynamometer. For experiment 1, subjects were instructed to dorsiflex their foot as fast as possible to 50% of MVC in response to an auditory cue. A window discriminator made it possible to time the tibial nerve stimulation to the onset of tibialis anterior (TA) EMG activity. This elicited an M-response (first grey shaded box) and an H-reflex (second grey shaded box) in the soleus (SOL) EMG. At least 45 trials, 15 tibial nerve stimulation and 30 no stimulation trials randomly interspersed, were obtained during dorsiflexion contraction. In experiment 2, subjects were asked to keep a steady dorsiflexion contraction at 10% of MVC for 2 min while given visual feedback of the target torque.

(to 50% of MVC_{DF} to avoid fatigue) following an auditory cue (see Figure 1). A window discriminator made it possible to time the tibial nerve stimulation to the onset of TA EMG activity.

At least 45 trials, 15 tibial nerve stimulation and 30 no stimulation trials randomly interspersed, were obtained during dorsiflexion contraction. Offline, the peak-to-peak amplitude of the H-reflex at the onset of dorsiflexion was then compared to rest (see Figure 2).

In six participants with CP and one participant from the healthy control group, it was not possible to obtain an H-reflex at rest while keeping the M-response at 10% of M_{max}. Also, two participants with CP could not produce a voluntary dorsiflexion contraction. These subjects were therefore excluded from this part of the analyses.

2.2.4. Experiment 2: Central Common Drive. The common drive to the dorsiflexor motoneuron pool was evaluated by coherence analysis of the surface EMG activity from TA_{prox} and TA_{dist} obtained while subjects performed a static dorsiflexion contraction to a torque level of 10% MVC_{DF} for two minutes while given visual feedback. Coherence in the beta band (15–35 Hz) has been shown to be dependent on intact corticospinal activity [23–25] and is therefore thought to reflect central common drive.

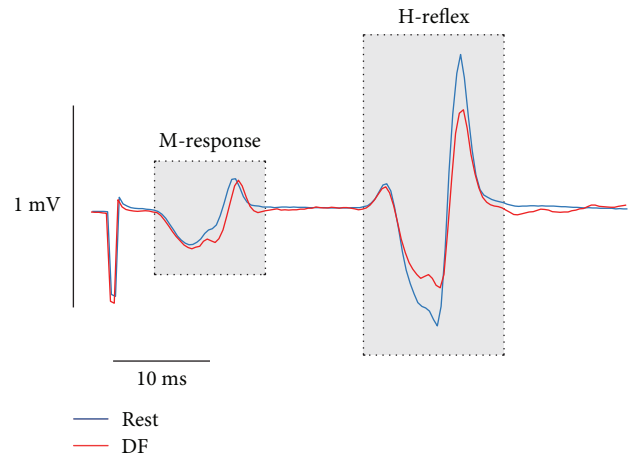

Figure 2: Evaluation of functional reciprocal inhibition in a healthy control. To produce a comparable afferent input to the soleus motoneuron pool at rest and during contraction, the tibial nerve stimulation intensity was adjusted to keep the M-response (first shaded box) at approximately 10% of M_{max} both at rest and at the onset of dorsiflexion (DF). The peak-to-peak amplitude of the H-reflex (second shaded box) could then be compared in the two situations, as a measure of the ability to suppress excitability of antagonist motoneurons at the onset of dorsiflexion (i.e., functional reciprocal inhibition).

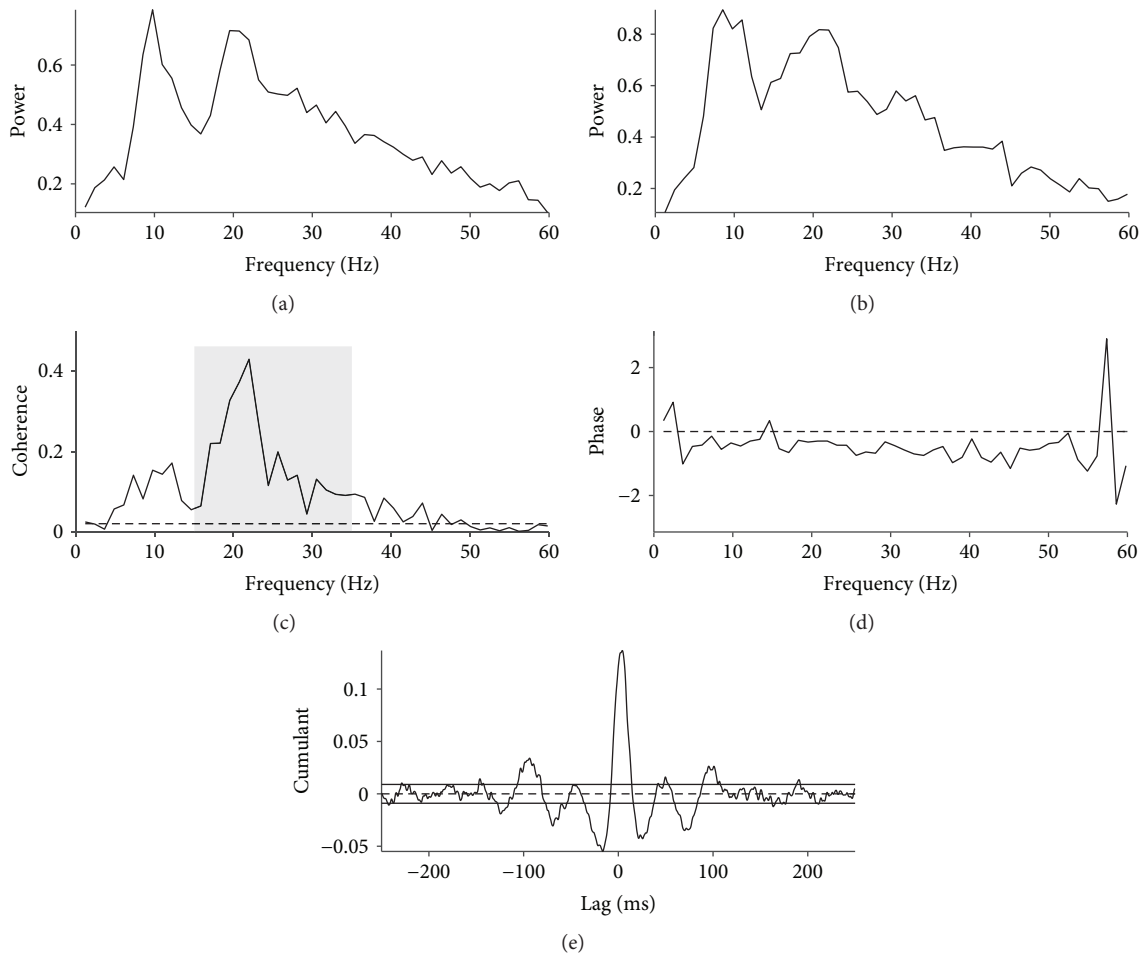

Figure 3: Example of intramuscular coherence analyses from a healthy control. (a, b) Autospectra from the proximal (TA_{prox}) and the distal (TA_{dist}) parts of the tibialis anterior during static dorsiflexion. (c) Coherence at frequencies from 1 to 60 Hz between TA_{prox} and TA_{dist} rectified EMG signals. The dashed horizontal line denotes the upper 95% confidence level, and the grey shaded area highlights the 15–35 Hz frequency band referred to as beta coherence. (d) The phase between the TA_{prox} and TA_{dist} rectified EMG signals indicating the synchronization between coherent EMG frequencies. (e) Cumulant density (range ± 250 ms) associated with the coherence.

Time and frequency domain analysis of the data was performed in MATLAB (version R2016b, MathWorks, MA, USA) using the methods described by Halliday et al. [26] and Farmer et al. [27]. Full-wave rectification of surface EMG signals was performed in order to maximize the information regarding timing of motor unit action potentials while suppressing information regarding waveform shape [28, 29]. The two rectified TA EMG signals were then normalized to have unit variance [30]. Rectified and normalized EMG signals are assumed to be realizations of stationary zero mean time series, denoted by x and y. The analysis of individual records generated estimates of the autospectra of the two EMGs [$fxx(\lambda)$, $fyy(\lambda)$], and their cross-spectra [$fxy(\lambda)$]. Frequency domain analyses were performed with a frequency resolution of 1 Hz. We estimated three functions that characterize the signals' correlation structure: coherence, $R_{xy}(\lambda)^2$; phase, $\Phi_{xy}(\lambda)$; and cumulant density, $q_{xy}(u)$. Coherence describes the linear association between two signals at each frequency of interest and reflects the consistency of phase differences and amplitude ratios between signals across trials. Coherence estimates are bounded measures of association defined over the range of $[0, 1]$ where 0 indicates no association between signals, and 1 indicates a strong association; cumulant density estimates are not bounded, and phase is defined over the range $[-\pi, +\pi]$. For the present data, coherence estimates provide a measure of the fraction of the activity in one surface EMG signal (TA_{prox}) that is correlated with the activity in the second surface EMG signal (TA_{dist}). In this way, coherence estimates quantify the strength and range of frequencies of common rhythmic synaptic inputs distributed across the motoneuron pool [27, 31–33]. The timing relations between the EMG signals are estimated from the phase. The cumulant density provides a time-domain representation of the correlation structure analogous to the cross-correlogram. The significance of the individual coherence and cumulant density estimates are assessed by inclusion of an upper 95% confidence limit in coherence plots and upper and lower 95% confidence limits in cumulant density plots (see example in Figure 3), based on the assumption of statistical independence. For details, see [26].

All individual coherence plots were visually inspected for signs of cross-talk, i.e., high coherence across a wide range of frequencies and close to zero lag synchronization in the time domain as evidenced in cumulant density plots [27]. None of the coherence plots displayed these characteristics, so all data from each group were pooled resulting in single group estimates at each frequency of interest for the adults with CP and the healthy controls, respectively. Pooled coherence estimates, like individual coherence estimates, provide a normative measure of linear association on a scale from 0 to 1 [30]. The interpretation of pooled estimates is similar to those for individual records, except that any interference relates to the population as a whole [27]. Group differences were investigated using the χ^2 extended difference of coherence test [34], a nonparametric test that provides the amount of pooled coherence differences between groups at each frequency in relation to an upper 95% confidence interval limit.

As described previously, two participants with CP could not produce a voluntary dorsiflexion contraction. These subjects were therefore excluded from this part of the analyses.

2.2.5. Statistics. Sigma Plot statistical software version 12.5 was used for statistical analysis. A one-way ANOVA was used to investigate differences in the amount of cocontraction between adults with CP and healthy controls. A two-way repeated measure ANOVA with group (CP or CON) and state (rest or onset of dorsiflexion) was applied for H-reflex and M-response analyses. To investigate possible associations between H-reflex modulation and muscle strength in the adults with CP, we used the Pearson product-moment correlations. For experiment 2, the extended χ^2 test was used to calculate the difference of coherence between adults with CP and healthy controls. Coherence was also quantified as the sum (i.e., area) of alpha (5–15 Hz) and beta (15–35 Hz) coherence. These values were transformed logarithmically to symmetrize distributions for statistical analyses [35] and compared using Student's t-test. Associations between H-reflex modulation and coherence area within and across groups were assessed by means of the Pearson product-moment correlations. Statistical significance was given for P values smaller than 0.05. Data are presented as the means ± standard error unless reported otherwise.

3. Results

Data from the test of the dorsiflexion strength has already been reported by Geertsen et al., where we showed that for adults with CP, MVC_{DF} was 42% of healthy controls ($P < 0.001$) and RFD_{200} only 21% healthy controls ($P < 0.001$) [21]. Further analyses performed here showed that during MVC_{DF}, adults with CP exhibited significantly more cocontraction (10.6 ± 1.5%) than healthy controls (5.9 ± 1.2%; $P = 0.003$).

3.1. Functional Reciprocal Inhibition. We found a significant group-state interaction when comparing the H-reflex amplitude at rest with the amplitude at the onset of dorsiflexion for the adults with CP and healthy controls ($F_{1,27} = 22.35$, $P < 0.001$). Post hoc analysis revealed that the healthy control group significantly reduced the H-reflex amplitude by 37% from 40.7 ± 4.1% of M_{max} at rest to 25.6 ± 5.0% at the onset of contraction ($P < 0.001$). This functional reciprocal inhibition was not evident in the participants with CP (rest: 37.3 ± 5.1%, dorsiflexion: 37.5 ± 4.5%, $P = 0.91$; Figure 4). There was no significant group-state interaction when comparing the amplitude of the M-response at rest with the amplitude at the onset of dorsiflexion for the adults with CP and healthy controls ($F_{1,27} = 1.32$, $P = 0.26$), indicating a comparable afferent input to the soleus motoneuron pool in the two states for both groups (Figure 4).

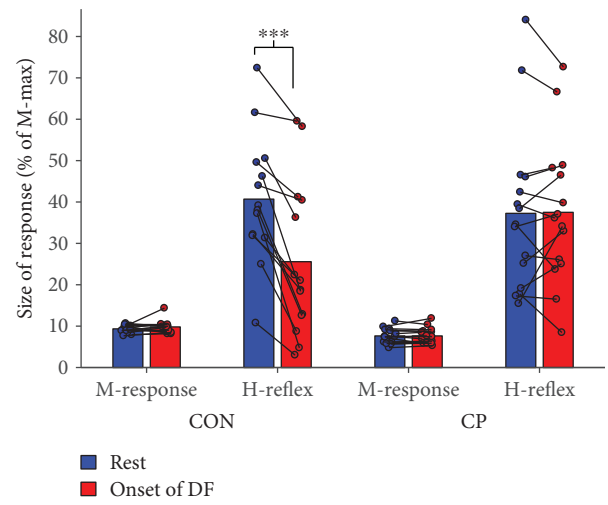

Figure 4: Functional reciprocal inhibition in healthy controls and adults with CP. Mean and individual M-response and H-reflex amplitudes in % of the maximal M-responses (M-max) at rest and at the onset of dorsiflexion (DF). The M-response was comparable at rest and at the onset of DF for both healthy controls (CON) and adults with CP. At the onset of DF, the H-reflex was significantly reduced in the healthy controls, whereas it was unchanged in adults with CP. Significant differences between rest and onset of DF are indicated by $^{***}P < 0.001$.

In adults with CP, the amount of H-reflex suppression was significantly correlated with both MVC_{DF} ($r = 0.58$, $P = 0.02$) and RFD_{200} ($r = 0.56$, $P = 0.03$).

3.2. Central Common Drive. Figure 3 shows individual coherence data from a healthy control during static dorsiflexion. The autospectra for TA_{prox} and TA_{dist} (Figures 3(a) and 3(b)) illustrate the origin of the elements used for time and frequency domain analysis. Coherence estimates calculated from the autospectra and cross-spectra are shown in Figure 3(c). Here, a clear peak can be seen in the beta (15–35 Hz) frequency band, as well as a small peak in the alpha (5–15 Hz) frequency band. Figure 3(d) shows the phase difference between the two rectified EMGs. The cumulant density constructed from the rectified EMG data is shown in Figure 3(e). Note the clear central peak around 0 ms indicating synchronization between the rectified EMG data from TA_{prox} and TA_{dist}.

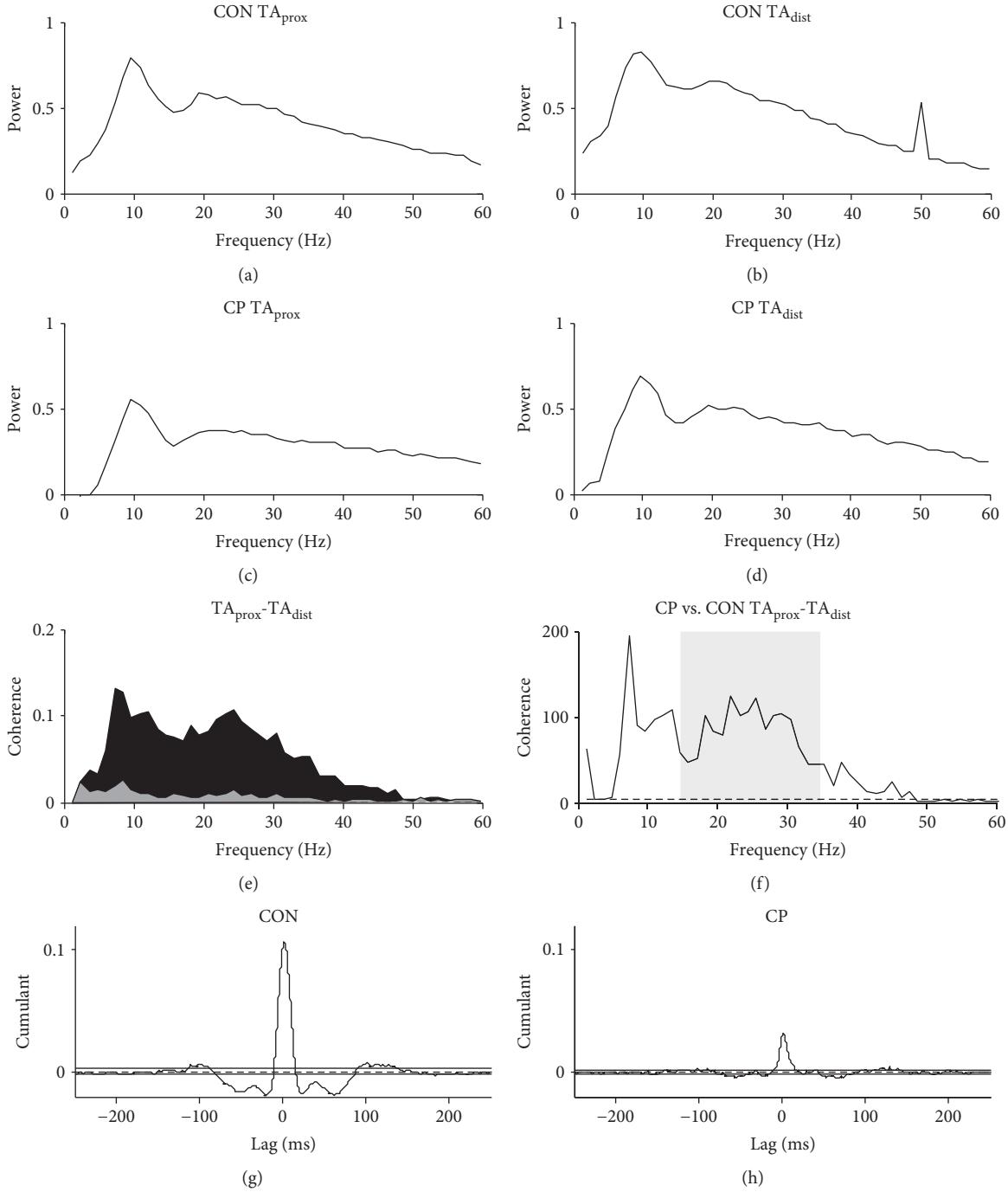

Figure 5: Pooled coherence plots and χ^2 analyses. (a–d) Pooled power in the proximal (TA_{prox}) and distal (TA_{dist}) parts of the tibialis anterior during static dorsiflexion for the healthy control group (CON) and adults with cerebral palsy (CP). (e) Pooled coherence between TA_{prox} and TA_{dist} for adults with CP (grey) and CON (black). (f) χ^2 analyses of the difference between adults with CP and the CON group. (g–h) Pooled cumulant density associated with the coherence for the CON and the CP groups.

Pooled TA-TA EMG coherence estimates from adults with CP and healthy controls are presented in Figure 5(a). For both groups, pooled alpha and beta coherence estimates exceeded significance levels, but adults with CP displayed considerably less coherence across all frequencies compared with the healthy adults. This observation was confirmed by the results from the extended χ^2 test of the group coherence estimates displayed in Figure 5(b), which showed a statistical difference at both alpha (5–15 Hz) and beta (15–35 Hz) frequencies.

Reduced TA-TA coherence in adults with CP was also confirmed when comparing the coherence areas at alpha and beta frequencies across individuals (Figure 6). Compared to healthy controls, adults with CP had significantly less

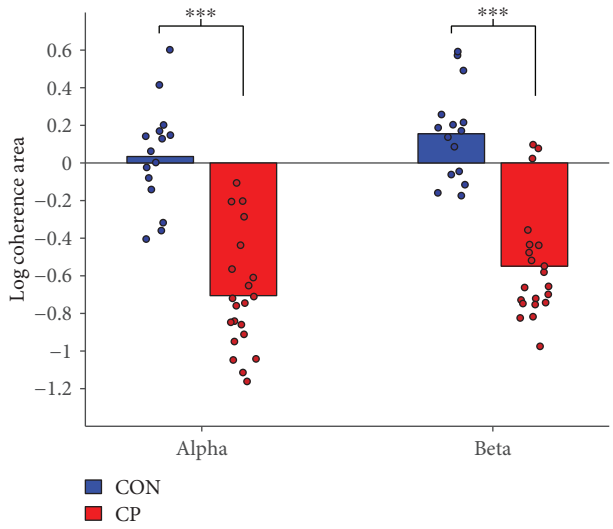

Figure 6: Coherence area estimates. Logarithmic coherence area between the proximal (TA_{prox}) and distal (TA_{dist}) part of the tibialis anterior in alpha (5–15 Hz) and beta (15–35 Hz) frequencies during static dorsiflexion for healthy controls (CON; blue) and adults with CP (red). Significant differences between the groups are indicated by $^{***}P < 0.001$.

alpha (-0.705 ± 0.083 vs. 0.034 ± 0.071; $P < 0.001$) and beta (-0.549 ± 0.079 vs. 0.155 ± 0.064; $P < 0.001$) coherence.

We also investigated possible associations between the central common drive to the TA motoneuron pool (log TA-TA coherence area) and the ability to suppress the antagonist motoneuron pool (reduction in soleus H-reflex amplitude at the onset of DF compared to rest). We observed a negative correlation (i.e., more coherence and larger H-reflex reduction) that approached significance in both healthy controls ($r = -0.53$, $P = 0.05$) and adults with CP ($r = -0.43$, $P = 0.13$), and the correlation was significant across groups ($r = -0.74$, $P < 0.001$).

4. Discussion

The main findings of this study are that the central common drive to ankle dorsiflexors and functional reciprocal inhibition of ankle plantar flexors are impaired in adults with CP. This may contribute to the reduced coordination of antagonistic muscles and impaired gait function observed in adults with CP.

4.1. Impaired Functional Reciprocal Inhibition in Adults with CP. The inability of adults with CP to suppress the soleus H-reflex to the same extent as healthy adults at the onset of dorsiflexion is similar to what has been observed in adults who have acquired lesion of descending motor pathways as adults because of multiple sclerosis [20], stroke [36], or spinal cord injury [37, 38]. Our findings indicate that a similar impaired control in adults may also be seen as the result of a lesion early in life and that the intervening years of motor practice and experience apparently do little to change this. We were not able to address the mechanisms responsible for the reduced suppression of the H-reflex further, but based on previous experiments in healthy subjects [13] and adults with lesion of central motor pathways [20, 39], it appears likely that impaired regulation of spinal interneurons responsible for conveying reciprocal Ia inhibition and/or presynaptic inhibition of Ia afferents is involved. These two spinal interneuronal populations have been shown to be responsible for suppressing stretch reflex activity in antagonist muscles by reducing antagonist motoneuronal excitability and limiting the input from antagonist stretch-sensitive receptors to the motoneurons at the onset of movement [39, 40]. Suppressing transmission in the stretch reflex circuitry through two different populations of interneurons and at two different points may be an efficient safeguard to ensure that stretch of the antagonists does not elicit unwanted stretch reflex activity.

It follows from this that the impaired functional reciprocal inhibition that we have found in adults with CP here could provide an explanation of the inability of the subjects to generate force quickly and efficiently [21]. Previous studies have indicated that people with central motor lesions may move slowly in order to avoid eliciting stretch reflex activity in antagonists when they are stretched at the onset of (fast) movements [15, 16, 41, 42]. However, some caution is required. The adults with CP in our study did not as a group have larger stretch reflexes than healthy subjects at rest (see Figure 4) and the subjects who were the least able to suppress the H-reflex were not those who had the largest stretch reflexes. It should also be kept in mind that the opposite causal relationship is equally likely and that H-reflexes were only slightly suppressed in the adults with CP because they were unable to generate an efficient descending drive to the agonist motoneurons and thereby activate reciprocal inhibitory mechanism efficiently. Our observations of strongly reduced common synaptic drive to ankle dorsiflexors support this interpretation.

4.2. Reduced Central Common Drive in Adults with CP. We used coherence between surface EMG recordings obtained from two different sites over the tibialis anterior muscle as a measure of the central common drive to populations of motoneurons within the same motor pool. This approach requires that the EMG recordings reflected the activity of different populations of motoneurons and that the recordings were not contaminated by cross-talk. Although cross-talk is difficult to rule out definitively, we are confident that we were able to minimize cross-talk to the extent that it cannot explain the findings in the present study: First, we made sure always to position electrodes at least 10 cm apart since muscle fibers in the tibialis anterior muscle have been shown not to exceed 6 cm [43, 44]. Second, cross-talk is easily identified from coherence between the recordings at all frequencies and a large, narrow peak at zero time lag in the cumulant density function [45, 46]. The recordings in the present study only showed coherence within restricted frequency bands (Figures 3 and 5) and peaks of synchronization in the cumulant density function were always found to have a distinct lag with respect to zero. We may therefore safely conclude that the observed coherence and synchronization peaks in the cumulant density function reflect a common central drive

to the tibialis anterior motoneurons in the spinal cord [47–49]. The narrow central peak in the cumulant density function and the coherence dominantly in the alpha and beta bands are similar to what has been observed in numerous studies during static contraction in healthy adults previously [31, 47, 49]. There are strong arguments supporting the notion that the narrow central synchronization reflects input to the motoneurons from collaterals of common last order neurons [31, 47, 49], and there is also strong evidence to suggest that the coherence in the beta band reflects activity in corticospinal neurons and that the central drive responsible for these two phenomena therefore originates in the motor cortex and may possibly be explained by activity in the direct monosynaptic corticospinal pathway to the spinal motoneurons [31, 47, 49]. If so, our findings would be consistent with impaired transmission in the corticomotoneuronal pathway in adults with CP, since both coherence and the central short-term synchronization peak in the cumulant density function were reduced in this group. Similar findings with similar interpretation have been reported previously for children with CP [3, 7] and for adults with spinal cord injury [45], stroke [23, 25], and multiple sclerosis [50], but we believe that our findings are the first to demonstrate this for adults with CP. Although the coherence measurements were performed during static contraction rather at the onset of dorsiflexion where reciprocal inhibition was evaluated, the two measures were correlated, and it makes sense from a physiological perspective that the reduced common drive to the dorsiflexors and the impaired reciprocal inhibition at the onset of dorsiflexion are related. The corticomotoneuronal pathway has been shown to be important for the initiation of fast, ballistic movements such as the dorsiflexion we used when testing reciprocal inhibition [51–53]. Corticomotoneuronal cells have also been shown to have collaterals to the Ia inhibitory interneurons responsible for reciprocal inhibition [54–56]. It is therefore, in our opinion, very likely that the reduced coherence between tibialis anterior motor units during static dorsiflexion and the reduced reciprocal inhibition at the onset of dorsiflexion are both linked to impaired transmission in the corticomotoneuronal pathway in the adults with CP.

Petersen et al. [3] found that coherence between tibialis anterior motor units during both static dorsiflexion and gait reached adult levels when children are 10–12 years old in parallel with reduced step-to-step variability of gait and suggested that this was related to the development of the corticospinal tract. In children with CP, this development of coherence was not observed and Petersen et al. [7] therefore suggested that the development of corticospinal drive was impaired. We may now extend these findings to conclude that adults with CP continue to show impaired corticospinal drive to the dorsiflexors and that this also impacts the coordination of antagonistic muscles. It follows that the intervening years of motor practice have not been sufficient to change this.

In children younger than 10 years, 4 weeks of daily treadmill training may increase coherence between tibialis anterior motor units in parallel with improved ability to lift the toes and make ground contact with the heel during gait [57]. This suggests that transmission in the corticospinal pathway is sufficiently plastic in this age group to induce important functional improvements through relatively short-lasting training. However, Willerslev-Olsen et al. [57] also found that such improvements were not found in children older than 10 years and it may therefore be anticipated that this is also the case in adults, although we have at present no knowledge about this. This may be put into the context of current ideas in computational neuroscience, which suggests that motor abilities are the result of a continuous updating of a predictive model that monitors the discrepancy between predicted and actual sensory consequences of movement [58–60]. With 10–12 years of gait experience, a relatively precise predictive model is likely to have been developed and it may therefore be more difficult to alter and require more training than earlier in life. This is consistent with the findings showing that an adult-like gait pattern with little variability (and little cocontraction) is attained around 10–12 years of age [1–4]. It is of interest in this relation that impedance control (i.e., cocontraction of antagonists) and slow movements (i.e., low RFD) have been found to be an optimal control strategy under dynamic conditions that are difficult to predict [61–63]. The characteristics of gait and other movements in adults with CP thus may reflect the most optimal strategy that their nervous system could find under the restrictions imposed by weak muscles and noisy and relatively unpredictable sensory feedback signals. It follows from this that efficient interventions in this group will have to involve "de-learning" of the unwanted movement pattern (cocontraction). This may be followed by learning of a more adequate movement pattern once the prerequisites for this have been established by strengthening muscles, reducing noise in the motor and sensory systems and facilitating relevant sensory signals.

5. Conclusion

We have shown in this study that the central common drive to ankle dorsiflexors and functional reciprocal inhibition of ankle plantar flexors are impaired in adults with CP. This likely reflects the most optimal control strategy under the constraints imposed by an early brain lesion. We suggest that the development of efficient functional interventions in adults with CP will have to take into account that all movements—including "abnormal" movements—may have to be seen as the result of a long learning process involving predictive coding of the sensory consequences of movement.

Data Availability

All data files will be available from the https://zenodo.org/database.

Disclosure

A preliminary account of these results has been presented as a poster at the 10th FENS Forum of Neuroscience in Copenhagen, Denmark, 2016.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

The study was supported by a grant from the Elsass Fonden.

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Hindawi
Neural Plasticity
Volume 2018, Article ID 2671613, 13 pages
https://doi.org/10.1155/2018/2671613

Research Article

Corticospinal Tract Wiring and Brain Lesion Characteristics in Unilateral Cerebral Palsy: Determinants of Upper Limb Motor and Sensory Function

Cristina Simon-Martinez[1], **Ellen Jaspers**[1,2], **Lisa Mailleux**[1], **Els Ortibus**[3], **Katrijn Klingels**[1,4], **Nicole Wenderoth**[2] and **Hilde Feys**[1]

[1]*Department of Rehabilitation Sciences, KU Leuven-University of Leuven, Leuven, Belgium*
[2]*Neural Control of Movement Lab, Department of Health Sciences and Technology, ETH Zurich, Zurich, Switzerland*
[3]*Department of Development and Regeneration, KU Leuven-University of Leuven, Leuven, Belgium*
[4]*Rehabilitation Research Centre, BIOMED, Hasselt University, Diepenbeek, Belgium*

Correspondence should be addressed to Cristina Simon-Martinez; cristina.simon.martinez@gmail.com

Received 23 March 2018; Revised 18 July 2018; Accepted 5 August 2018; Published 13 September 2018

Academic Editor: Michael Borich

Brain lesion characteristics (timing, location, and extent) and the type of corticospinal tract (CST) wiring have been proposed as determinants of upper limb (UL) motor function in unilateral cerebral palsy (uCP), yet an investigation of the relative combined impact of these factors on both motor and sensory functions is still lacking. Here, we first investigated whether structural brain lesion characteristics could predict the underlying CST wiring and we explored the role of CST wiring and brain lesion characteristics to predict UL motor and sensory functions in uCP. Fifty-two participants with uCP (mean age (SD): 11 y and 3 m (3 y and 10 m)) underwent a single-pulse Transcranial Magnetic Stimulation session to determine CST wiring between the motor cortex and the more affected hand ($n = 17$ contralateral, $n = 19$ ipsilateral, and $n = 16$ bilateral) and an MRI to determine lesion timing ($n = 34$ periventricular (PV) lesion, $n = 18$ corticosubcortical (CSC) lesion), location, and extent. Lesion location and extent were evaluated with a semiquantitative scale. A standardized protocol included UL motor (grip strength, unimanual capacity, and bimanual performance) and sensory measures. A combination of lesion locations (damage to the PLIC and frontal lobe) significantly contributed to differentiate between the CST wiring groups, reclassifying the participants in their original group with 57% of accuracy. Motor and sensory functions were influenced by each of the investigated neurological factors. However, multiple regression analyses showed that motor function was predicted by the CST wiring (more preserved in individuals with contralateral CST ($p < 0.01$)), lesion extent, and damage to the basal ganglia and thalamus. Sensory function was predicted by the combination of a large and later lesion and an ipsilateral or bilateral CST wiring, which led to increased sensory deficits ($p < 0.05$). These novel insights contribute to a better understanding of the underlying pathophysiology of UL function and may be useful to delineate individualized treatment strategies.

1. Introduction

Upper limb (UL) function is commonly impaired in individuals with unilateral cerebral palsy (uCP), negatively impacting on daily life activities [1]. The large variability in the clinical presentation of UL function, but also in treatment response, has resulted in increasing interest in understanding the underlying neural mechanisms that determine UL function and its contribution to further optimize therapy planning for the individual with uCP. A number of neurological factors have been put forward as potential predictors of UL function, i.e., the structural brain lesion characteristics (i.e., lesion timing, location, and extent), and the type of corticospinal tract (CST) wiring [2–6].

The timing of the lesion during gestation is closely related to the type of the damaged tissue and can be classified into

three categories: malformations (1st and 2nd trimesters of pregnancy), periventricular lesion (PV, early 3rd trimester), and corticosubcortical lesions (CSC, late 3rd trimester and around birth) [7]. Previous studies investigating the impact of lesion timing on UL function have shown that individuals with a later lesion (i.e., CSC lesions) present with poorer UL motor and sensory functions [2, 3, 5]. Besides lesion timing, lesion location and extent have shown to play an important role in determining UL function, whereby damage to the posterior limb of the internal capsule (PLIC) and the basal ganglia, and a larger lesion extent is related to worse UL motor and sensory functions [2, 3]. However, there is still large variability in UL function that remains unexplained based on these factors.

The unilateral brain damage in individuals with uCP can also result in a partial or complete reorganization of the CST toward the nonlesioned hemisphere [8]. This reorganization of the CST wiring is unique in uCP and refers to the efferent motor input to the affected hand. Researchers have identified three types of CST wiring, i.e., contralateral (CST_{contra}, the affected hand receives input from the crossed CST, originating in the lesioned hemisphere), ipsilateral (CST_{ipsi}, the affected hand receives input from the uncrossed CST, originating in the nonlesioned hemisphere), and bilateral (CST_{bilat}, the affected hand receives input from both the crossed and uncrossed CSTs, originating in the lesioned and nonlesioned hemispheres, respectively) [8, 9]. It has been suggested that the type of CST wiring is the main factor influencing UL function, whereby individuals with CST_{contra} present with more preserved UL function compared to the other groups [6, 10–13]. Nevertheless, assessing the underlying CST wiring with Transcranial Magnetic Stimulation (TMS) in young children might become challenging. Therefore, the identification of either behavioural or brain lesion features that relate to the underlying CST wiring could be useful to define tailor-made interventions in a clinical setting.

Whilst the role of lesion timing, location, and extent has been well investigated [2, 3, 14], only a few studies examined the impact of the CST wiring on UL function and they often have several limitations (i.e., small sample sizes, ordinal scoring of impairments, and limited to motor deficits) [5, 10, 15]. Moreover, studies thus far focused on each factor independently, whereas only one study described the impact of the CST wiring and lesion timing on UL function in uCP [10], and only one study reports the impact of CST wiring and lesion extent in children with PV lesions [4]. Although the authors suggested the relevance of both lesion timing and type of CST wiring in predicting UL function, the small sample size, the lack of a standardized evaluation of motor function, and the merely descriptive nature of the study hampered the possibility of drawing strong conclusions.Furthermore, it has been shown that an intact sensory function is essential to develop an adequate motor function in other neurological disorders (such as adult stroke) [16, 17]. Also in individuals with uCP, sensory and motor functions are highly related [1], although the impact of the CST wiring on this relationship remains unknown.

In this study, we investigated the impact of CST wiring and structural brain lesion characteristics on UL motor and sensory functions in a large group of individuals with uCP, using a systematic and comprehensive evaluation. Our first hypothesis is that the type of the CST wiring pattern in unilateral CP can be predicted based on a linear combination of measures of lesion timing, location, and extent. Second, we hypothesize that the combination of these predictors together with the CST wiring has a stronger predicting value for UL motor and sensory functions than any of these factors alone. Last, we speculate that the relation between motor and sensory functions is disrupted by the type of CST wiring.

2. Materials and Methods

2.1. Participants. Children and adolescents with uCP aged between 5 and 21 years old were recruited via the CP reference center of the University Hospitals Leuven between 2014 and 2017. They were excluded if they (1) received UL botulinum toxin injections six months prior to the assessment, (2) had UL surgery two years prior to the assessment, and/or (3) had other neurological or genetic disorders. All individuals assented to participate; all parents signed the informed consent (participants younger than 18 years old), and participants older than 12 years also signed the informed consent, in accordance with the Declaration of Helsinki. This study was approved by the Medical Ethical Committee of the University Hospital Leuven (S55555 and S56513).

Participants with contraindications for the MRI (e.g., metal implants) or the Transcranial Magnetic Stimulation (TMS; ventricular-peritoneal (VP) shunt, seizure two years prior to the study) did not undergo the respective assessment. All TMS measurements were conducted by two experienced physiotherapists (CSM and EJ), and UL function was evaluated by four experienced physiotherapists (LM, CSM, JH, and EJ) at the Clinical Motion Analysis Laboratory of the University Hospitals Leuven (campus Pellenberg, Belgium).

2.2. Upper Limb Evaluation

2.2.1. Motor Function. Grip strength, unimanual capacity, and bimanual performance composed the motor evaluation. Maximum *grip strength* was assessed using the Jamar® hydraulic hand dynamometer (Sammons Preston, Rolyan, Bolingbrook, IL, USA). The less-affected hand was measured first, and the mean of three maximum contractions was calculated per hand. The ratio between hands was used for further analyses to cancel out the effect of age (grip strength ratio = grip strength less − affected hand/grip strength affected hand, whereby a lower score (closer to 1) indicates a grip strength in the affected hand similar to that of the less-affected hand). *Unimanual capacity* was assessed with the Jebsen-Taylor hand function test (JTHFT). The JTHFT reliably measures movement speed during six unimanual tasks [18, 19]. Similar to other studies, we used a modified version for children and adolescents with uCP in which the writing task was removed and the time to carry out each task was reduced from 3 to 2 minutes to avoid frustration [19, 20]. The time to perform every task was summed up, and the ratio

between hands was used for further analyses to cancel out the effect of age (JTHFT ratio = JTHFT affected hand/JTHFT less-affected hand, whereby a lower score (closer to 1) indicates movement speed in the affected hand similar to that of the less-affected hand). *Bimanual performance* was evaluated with the Assisting Hand Assessment (AHA), which assesses how effectively the affected hand is used in bimanual activities [21–23]. The spontaneous use is evaluated during a semistructured play session with standardized toys requiring bimanual handling. Given the age range of the participants of this study, the School Kids AHA and the Ad-AHA were administered [22, 24]. The AHA was scored by certified raters (LM and CSM), using the 5.0 version which includes 20 items that are scored from 0 ("does not do") to 4 ("effective use"), resulting in a final score between 0 and 100 AHA units.

2.2.2. Sensory Function. Sensory assessments comprised measures of exteroception (tactile sense), proprioception (movement sense), two-point discrimination (2PD, Aesthesiometer®), and stereognosis (tactile object identification), which have been shown to be reliable in this population [25]. Tactile and movement senses were classified as normal (score 2), impaired (score 1), or absent (score 0). 2PD was classified according to the width between the two points that the participants could discriminate: normal (0–4 mm, score 2) or impaired (>4 mm, score 1) [26]. Tactile object identification was used as the number of objects that the children could recognize (0–6). In addition, a kit of 20 nylon monofilaments (0.04 g–300 g) (Jamar Monofilaments, Sammons Preston, Rolyan, Bolingbrook, IL, USA) was used to reliably determine threshold values for touch sensation [27, 28]. Touch sensation was categorized as normal (0.008–0.07 g), diminished light touch (0.16–0.4 g), diminished protective sensation (0.6–2 g), loss of protective sensation (4.19–180 g), and untestable (300 g), according to the manual (Jamar Monofilaments, Sammons Preston, Rolyan, Bolingbrook, IL, USA).

2.3. Structural MRI. Structural images were acquired using three-dimensional fluid-attenuated inversion recovery (3D FLAIR) (321 slices, slice thickness = 1.2 mm, slice gap = 0.6 mm, repetition time = 4800 ms, echo time = 353 ms, field of view (FOV) = $250 \times 250\,\text{mm}^2$, $1.1 \times 1.1 \times 0.56\,\text{mm}^3$ voxel size, acquisition time = 5 minutes). In addition, magnetization prepared rapid gradient echo (MPRAGE) was acquired (182 slices, slice thickness = 1.2 mm, slice gap = 0 mm, TR = 9.7 ms, TE = 4.6 ms, FOV = $250 \times 250\,\text{mm}^2$, voxel size = $0.98 \times 0.98 \times 1.2$, acquisition time = 6 minutes). The structural MRI was used to provide a detailed description of the lesion location and extent and to classify the timing of the lesion, which was conducted by a paediatric neurologist (EO).

Timing of the brain lesion was classified according to the predominant pattern of damage as described by Krägeloh-Mann and Horber [7]: malformations (1st and 2nd trimesters of pregnancy), periventricular lesion (PV, early 3rd trimester), corticosubcortical lesions (CSC, late 3rd trimester and term), or acquired brain lesions (between 28 days and two years postnatally).

Lesion location and extent were determined using a semiquantitative scale recently developed by Fiori et al. [29]. The scale consists of a graphical template with six axial slices of the brain and an extra template for the basal ganglia (lenticular and caudate), thalamus, posterior limb of the internal capsule (PLIC), brainstem, corpus callosum, and cerebellum. Firstly, the slices corresponding to the template slices are to be found and the lesion is drawn onto the template. Next, the damage to the periventricular, middle, and corticosubcortical layers of each lobe is scored for both hemispheres separately. The sum of the damage to each lobe results in the lobar score, ranging from 0 to 3 for each lobe. Damage to the basal ganglia (lenticular and caudate), thalamus, PLIC, and brainstem directly is binarily scored from the MRI (affected or nonaffected). Damage to the corpus callosum is scored from 0 to 3, based on the involvement of the anterior, middle, and posterior thirds of the corpus callosum on a sagittal view. Last, the involvement of the cerebellum is based on damage to the vermis (0–1) and each of the hemispheres (0–2), resulting in a total score ranging from 0 to 3. A total ipsilesional score is calculated based on the damage to the lobes (0–3 for each lobe, i.e., total of 0–12) and damage to the subcortical structures (0–5; ranging from 0 to 17). More detailed information about the scale and its scoring procedure can be found in the respective study [29]. This semiquantitative scale has been shown valid and reliable in children with uCP [29, 30].

In the present study, lesion location was indicated by the damage to the frontal and parietal lobes (0–4), damage to the basal ganglia and thalamus (0–3), and damage to the PLIC (0–1). These locations were chosen based on their relation to the sensorimotor system [31]. Lesion extent was indicated by the total ipsilesional score (0–17).

2.4. Transcranial Magnetic Stimulation. Single-pulse TMS was conducted to assess CST wiring. TMS was applied using a Magstim 200 stimulator (Magstim Ltd., Whitland, Wales, UK) equipped with a focal 70 mm figure-eight coil and a Bagnoli electromyography (EMG) system with two single differential surface electrodes (Delsys Inc., Natick, MA, USA). A Micro1401-3 acquisition unit and Spike software version 4.11 (Cambridge Electronic Design Limited, Cambridge, UK) were used to synchronize the TMS stimuli and the EMG data acquisition. Motor evoked potentials (MEPs) were bilaterally recorded from the muscles opponens pollicis brevis. During the TMS assessment, participants wore a cap that allows creating a grip with a coordinate system to identify the optimal point to stimulate (hotspot) in a standardized and systematic way. The hotspot and the resting motor threshold (RMT, defined as the minimum intensity required to obtain 5/10 MEP of at least 50 μV in the corresponding muscle) were identified by starting the stimulation intensity at 30% with an incremental increase of 5% [4]. For each hemisphere, stimulation started from the assumed "motor hotspot," which is located 5 cm lateral and 1 cm anterior from the scalp middle point (Cz), at 30%. After approximately 2–3 pulses, the stimulation intensity was increased 5% for another 2–3 pulses, until MEPs were found. If no MEP can be elicited after increasing up to 60 to 80%, the coil would be moved to a

different location on the scalp grid and the procedure would be repeated until an MEP was elicited. Stimulation up to 100% of the maximum stimulator output was continued until an MEP was elicited. The nonlesioned hemisphere was always stimulated first and allowed to identify contralateral CST projections to the less-affected hand. Stimulation in the nonlesioned hemisphere was continued up to 100% of the maximum stimulator output to search for possible ipsilateral CST projections to the affected hand. Next, the lesioned hemisphere was stimulated to identify possible contralateral CST projections to the affected hand. If only contralateral MEPs from each hemisphere were found, the child was categorized as having a CST_{contra} wiring. If MEPs in the affected hand were evoked from both hemispheres, the child was categorized as having a CST_{bilat} wiring. Lastly, if MEPs in the impaired hand were only evoked when stimulating the nonaffected hemisphere, the child was categorized as having a CST_{ipsi} wiring. TMS measures have been shown to be reliable in adults [32, 33] and in children [34]. In this study, the TMS assessment was used for diagnostic purposes. In cases when high intensities were not tolerated, the stimulation intensity was increased up to at least 80% of the maximum stimulator output and children were asked to hold a pen to ensure precontraction of the evaluated muscle and thereby facilitate the CST and MEP detection. This allowed us to rule out the possibility of miscategorizing the child regarding their CST wiring pattern.

2.5. Statistical Analyses. First, descriptive statistics were used to document the distribution of brain lesion characteristics according to the CST wiring. Next, we investigated the differences in occurrence of lesion timing, location, and extent between the CST wiring groups by using analysis of contingency tables (chi-square and Fisher's exact tests), Kruskal-Wallis test (ordinal data), and ANOVA (lesion extent). Lastly, we used discriminant analysis to explore whether the type of CST wiring would differ depending on the linear combination of lesion timing, location, and extent, in a multivariate way. Cross-validation procedure was included to investigate the accuracy of the model in reclassifying the participants in the original CST wiring groups. Variables related to lesion timing, lesion location (damage to the frontal lobe, parietal lobe, PLIC, basal ganglia, and thalamus), and extent (ipsilesional extent of the lesion) were included in the model, which was fitted using the stepwise selection method.

To investigate the impact of the type of CST wiring and brain lesion characteristics on UL function, we first used linear simple regression and then multiple regression analysis to investigate the combined impact of these factors on UL motor and sensory functions. For the continuous variables related to motor function, normality was first verified by inspecting the histograms and with the Shapiro-Wilk test, showing a normal distribution only for the AHA. For the JTHFT ratio and the grip strength ratio, a logarithmic transformation was applied ($y' = \log 10\,(y)$). To investigate the impact of the type of CST wiring and brain lesion characteristics on UL motor function, we computed a multiple regression analysis. Similarly, for UL sensory function, we conducted a simple ordinal logistic regression for stereognosis and thresholds for touch sensation and a simple logistic regression for 2PD to investigate the impact of each individual neurological factor on the sensory function. Next, we performed multiple regression analyses (ordinal and logistic) to investigate the combined impact of the neurological predictors on the sensory deficits. The predictors included in the multiple regression model were the type of CST wiring, lesion timing, location (damage to the frontal lobe, parietal lobe, PLIC, basal ganglia, and thalamus), and ipsilesional extent of the lesion. To predict both motor and sensory functions, interaction terms were built between the CST wiring and (i) lesion timing and (ii) lesion extent and included in the model. The multiple regression models were fitted with the backward elimination method until a set of variables significantly contributing to the model was identified.

Lastly, to investigate the relation between sensory and motor functions for the whole group and within CST wiring groups, Spearman rank correlation coefficients were used between each of the motor function variables and deficits in stereognosis. Correlation coefficients were considered as little or no correlation (<0.30), low (0.30–0.50), moderate (0.50–0.70), high (0.70–0.90), and very high correlation (>0.90) [35].

In addition, effects sizes were calculated for the comparisons and interpreted according to Cohen, depending on the computed test: η^2 (partial eta squared) for the prediction models (small 0.01, medium 0.06, and large 0.14) [36, 37]. Statistical significance was set at $\alpha < 0.05$ for main tests with Bonferroni correction for post hoc tests. All statistical analyses were computed with SPSS Statistics for Windows version 24.0 (IBM Corp., Armonk, NY).

3. Results

3.1. Participants. Seventy-five children and adolescents with uCP participated in this study (mean age (SD): 11 y and 1 m (3 y and 6 m); 33 girls; 39 left uCP). According to the Manual Ability Classification System (MACS), 25 individuals were classified as MACS I, 25 as MACS II, and 25 as MACS III. Sixteen participants did not have CST wiring data ($n = 1$ panic attack, $n = 2$ hemispherectomy, $n = 3$ VP shunt, $n = 2$ epilepsy, $n = 1$ tumor, $n = 4$ refusals to participate, and $n = 3$ inconclusive TMS results), resulting in a total of 59 participants. The TMS assessment identified 20 individuals with CST_{contra}, 18 with CST_{bilat}, and 21 with CST_{ipsi}. For the analyses in this study, participants with malformations ($n = 1$), acquired lesions ($n = 4$), or no visible lesions ($n = 2$) were excluded due to the very small sample size of these subgroups, resulting in a total group of 52 participants (mean age (SD): 11 y and 4 m (3 y and 10 m); 22 girls; 28 left uCP) with available CST wiring ($n = 17$ contralateral, $n = 19$ ipsilateral, and $n = 16$ bilateral) and data related to the timing, location, and extent of the lesion. A summary of the lesion locations and extent according to the lesion timing is provided in Supplementary Materials (Table 1). Thirty-four individuals had a PV lesion, and 18 had a CSC lesion. Clinical motor and sensory data was missing in one participant (boy, 19 y and 7 m, PV lesion, and CST_{contra} wiring), and sensory data

Table 1: Contingency table (count and percentage, descriptive statistics) of the occurrence of lesion timing, location, and extent according to the CST wiring.

			CST wiring			p value
			Contralateral	Bilateral	Ipsilateral	
Timing						
Lesion timing[¥]	PV	N (%)	15 (88.2%)	8 (50%)	11 (57.9%)	0.04
	CSC		2 (11.8)	8 (50%)	8 (42.1%)	
Location						
PLIC[¥]	Not affected	N (%)	8 (47%)	1 (6%)	0 (0%)	<0.001
	Affected		9 (53%)	15 (94%)	19 (100%)	
Basal ganglia and thalamus[◊]		Me (p25–p75)	0 (0–1)	1.50 (0–2.50)	1 (1–2)	0.006[a,b]
Frontal lobe[◊]		Me (p25–p75)	1 (1–1)	1.50 (1–2.25)	1 (1–1.50)	0.004[a,b]
Parietal lobe[◊]		Me (p25–p75)	2 (1–2)	2 (1.25–3)	2 (2–2.50)	0.09
Extent						
Ipsilesional extent[○]		X (SD)	5.18 (3.07)	8.38 (3.95)	9.05 (3.27)	0.004[a,b]

CST: corticospinal tract; PV: periventricular; CSC: corticosubcortical; PLIC: posterior limb of the internal capsule. [¥]Chi-square statistic. [$]Fisher's exact test. [◊]Kruskal-Wallis test. [○]ANOVA. [a]Contralateral vs. ipsilateral. [b]Contralateral vs. bilateral.

was evaluated in a subsample of participants (see Section 3.3.2 for more details).

3.2. CST Wiring and Brain Lesion Characteristics. Table 1 displays the distribution of lesion timing, location, and extent variables according to the three CST wiring groups. Except for the damage to the parietal lobe, all variables were significantly different between the CST wiring groups ($p < 0.05$) (Table 1).

In the discriminant analysis, we found that the combined value of the damage to the PLIC and the damage to the frontal lobe could significantly discriminate between the type of CST wiring (Wilks' $\lambda = 0.611$, chi-square test = 23.88, df = 4, canonical correlation = 0.602, $p < 0.001$). The two functions extracted accounted for nearly 57% of the variance in the type of CST wiring. The standardized discriminant function coefficients of the two extracted functions indicated the contribution of each retained independent variable (damage to the PLIC and damage to the frontal lobe) to each function, showing how strongly the discriminant variables affect the score. These coefficients can be then used for the classification of a single individual (function 1 = 0.81 ∗ damage to the PLIC + 0.50 ∗ damage to the frontal lobe; function 2 = −0.60 ∗ damage to the PLIC + 0.88 ∗ damage to the frontal lobe).

Cross-validated reclassification of cases based on the new canonical variables was successful in 57.7% of the cases: 89.5% were correctly classified in the CST_{ipsi} group, 47.1% in the CST_{contra} group, and only 31.3% in the CST_{bilat} group (Figure 1).

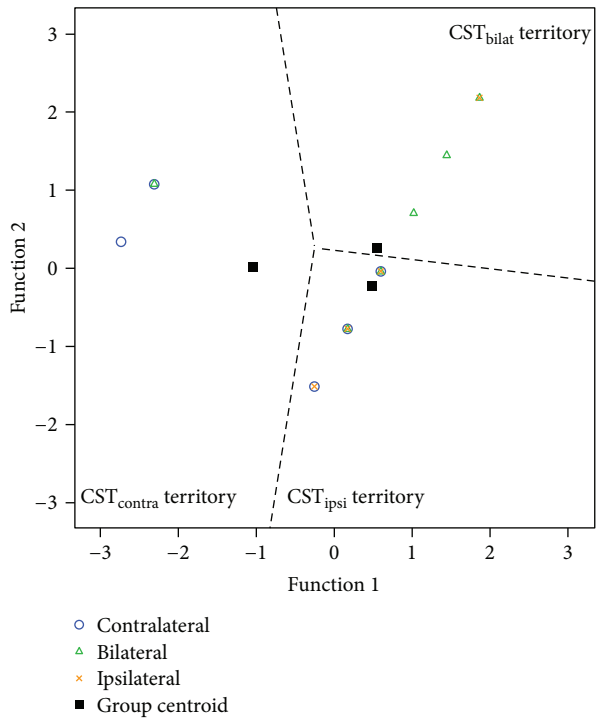

Figure 1: Territorial map showing the relative location of the boundaries of each CST wiring category and the location of each of the participants. The group centroids are indicated with a black-filled square (CST_{contra} (−1.05, 0.01), CST_{ipsi} (0.48, −0.23), and CST_{bilat} (0.54, 0.26)).

3.3. CST Wiring, Brain Lesion Characteristics, and UL Function

3.3.1. Motor Function. Descriptive statistics of the motor function according to the type of CST wiring, lesion timing, location, and extent are presented in Supplementary Materials (Table 2). The simple linear regression analyses to predict motor function based on a single neurological factor showed that every factor had an influence on motor function (grip strength, $p < 0.04$; JTHFT, $p < 0.004$; AHA, $p < 0.01$; see Supplementary Materials Table 2 for detailed information).

When all the neurological factors were included in the same model in a multiple regression analysis, the backward elimination method identified the variables that were significantly contributing to the model. Table 2 documents the estimated marginal means, which represent the mean response

Table 2: Descriptive statistics of the observed and estimated marginal means of upper limb motor function according to the CST wiring groups.

	Estimated marginal means and SD		
	CST_{contra} ($n = 16$)	CST_{ipsi} ($n = 19$)	CST_{bilat} ($n = 16$)
Grip strength ratio (log)[a]	0.14 (0.13)	0.55 (0.20)	0.46 (0.24)
JTHFT ratio (log)[b]	0.30 (0.24)	0.67 (0.23)	0.64 (0.22)
AHA (0–100)[c]	79.66 (10.28)	58.70 (9.81)	61.58 (9.67)

CST: corticospinal tract; JTHFT: Jebsen-Taylor hand function test; AHA: Assisting Hand Assessment; SD: standard deviation. [a]The values coincide with the observed values, as there is no significant covariate in the model. [b]Adjustments based on ipsilesional lesion extent mean = 7.67. [c]Adjustments based on ipsilesional lesion extent mean = 7.67 and damage to the basal ganglia and thalamus mean = 1.12.

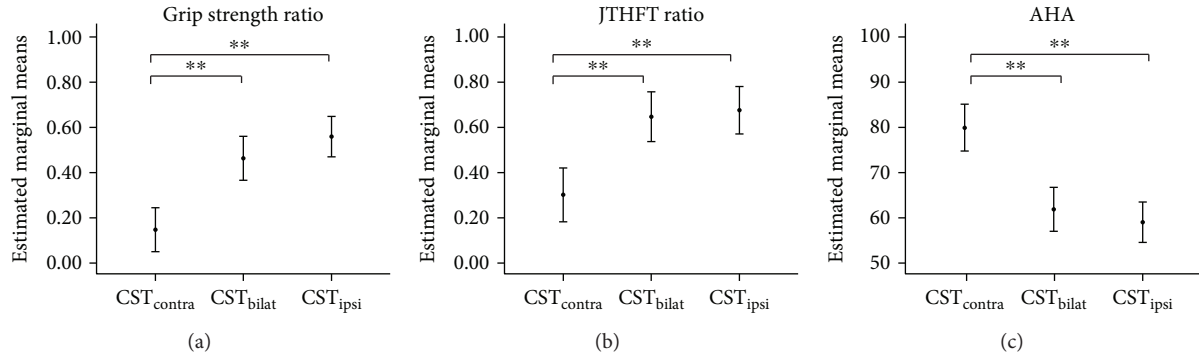

Figure 2: Upper limb motor function differs in individuals with CST_{contra} wiring compared to those with CST_{bilat} or CST_{ipsi} wiring. Estimated marginal means and 95% CI per CST wiring type and lesion timing group for (a) grip strength (log ratio, i.e., closer to zero indicates preserved grip strength), (b) JTHFT (log ratio, i.e., closer to zero indicates preserved manual dexterity, measured by speed), and (c) AHA. AHA: Assisting Hand Assessment; JTHFT: Jebsen-Taylor hand function test; CST: corticospinal tract. $^{*}p < 0.01$; $^{**}p < 0.001$. Estimated marginal means are adjusted according to the significant covariates (see Table 2 for details).

in each CST wiring group adjusted by the covariates that significantly contribute to the model. The multiple regression model to predict grip strength deficits only retained the type of CST wiring, explaining 46% of the variance ($F(2, 51) = 20.90$; $p < 0.001$; $\eta^2 = 0.47$). For the JTHFT, 54% of the variance was explained by the type of CST wiring ($F(2, 51) = 12.20$; $p < 0.0001$; $\eta^2 = 0.34$, $R^2 = 46\%$) and the total extent of the lesion ($F(1, 51) = 8.05$; $p = 0.007$; $\eta^2 = 0.15$, $\Delta R^2 = 8\%$). For bimanual performance (AHA), the regression model explained 61% of the variance, with the type of CST wiring ($F(2, 51) = 19.03$; $p < 0.0001$; $\eta^2 = 0.45$, $\Delta R^2 = 52\%$), the total extent of the lesion ($F(1, 51) = 10.65$; $p < 0.001$; $\eta^2 = 0.19$, $\Delta R^2 = 5\%$), and the damage to the basal ganglia and thalamus ($F(1, 51) = 4.90$; $p = 0.03$; $\eta^2 = 0.10$, $\Delta R^2 = 4\%$) significantly contributing to the model (Figure 2). No interaction effects were identified for any of the motor outcome variables.

3.3.2. Sensory Function. Descriptive information of sensory function according to each neurological factor is summarized in Table 3 of Supplementary Materials. Sensory function data (tactile sense, movement sense, stereognosis, and 2PD) and thresholds for touch sensation, as assessed with the monofilaments, were available in 46 and 35 individuals, respectively. Due to the lack of variation in the tactile sense and movement sense modalities, the predictive model was only applied to the stereognosis, 2PD, and the thresholds for touch sensation.

The simple linear analyses to predict sensory function based on a single neurological predictor indicated that every predictor impacted on stereognosis ($p < 0.032$). In contrast, 2PD was influenced by all neurological predictors ($p < 0.04$) except the damage to the PLIC ($p < 0.17$) and touch sensation could be significantly predicted by all factors ($p < 0.01$) except damage to the PLIC ($p = 0.99$) and type of CST wiring ($p = 0.42$).

When all the neurological factors were included in the same model in a multiple regression analysis, the backward elimination method identified predictors that were significantly contributing to the model. For stereognosis, the retained main effects were the CST wiring (Wald chi-square test (2) = 9.09, $p = 0.011$), lesion timing (Wald chi-square test (1) = 4.34, $p = 0.04$), and ipsilesional extent of the lesion (Wald chi-square test (1) = 7.15, $p = 0.008$) (Table 3(a)). These results show that the odds of having better stereognosis function were 5.56 times higher in the group with PV lesions than in the CSC group ($p = 0.04$). Similarly, individuals with a CST_{contra} wiring show 10.23 and 9.7 times higher probability of having better scores in the stereognosis test compared to those with a CST_{ipsi} or CST_{bilat} wiring, respectively ($p = 0.02$), whilst there was no difference between the last two ($p = 0.34$). Lastly, the odds of having higher stereognosis scores decrease by 0.74 for every unit change in the ipsilesional extent of the lesion ($p = 0.01$). No interactions were found between the CST wiring and the brain lesion characteristics to predict deficits in stereognosis ($p > 0.05$).

Table 3: Descriptive statistics of the sensory function ((a) stereognosis (number of correctly recognized objects), (b) two-point discrimination, and (c) touch sensation) according to each of the variables significantly contributing to each prediction model.

(a)

		Stereognosis (number of correctly guessed objects)						
		0	1	2	3	4	5	6
Lesion timing								
PV	*N* (%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	5 (71%)	6 (67%)	17 (44%)
CSC	*N* (%)	5 (100%)	2 (100%)	3 (75%)	1 (100%)	2 (29%)	3 (33%)	1 (6%)
CST wiring								
Contralateral	*N* (%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	1 (11%)	13 (72%)
Bilateral	*N* (%)	4 (80%)	0 (0%)	2 (50%)	0 (0%)	3 (43%)	3 (33%)	3 (17%)
Ipsilateral	*N* (%)	1 (20%)	2 (100%)	1 (25%)	1 (100%)	4 (57%)	5 (56%)	2 (11%)
Lesion extent								
Ipsilesional	Me (IQR)	13 (2.07)	13 (—)	10 (3.88)	—	6 (3.50)	6 (5.25)	5.25 (3.75)

(b)

		Two-point discrimination	
		Normal (≤4 mm)	Impaired (>5 mm)
Lesion timing			
PV	*N* (%)	26 (93%)	3 (17%)
CSC	*N* (%)	2 (7%)	15 (83%)
Lesion extent			
Ipsilesional	Me (IQR)	5.25 (3.88)	12 (5.25)

(c)

		Threshold of touch sensation				
		Normal	Diminished light touch	Diminished protective sensation	Loss of protective sensation	Untestable
Lesion extent						
Ipsilesional	Me (IQR)	6 (4.50)	—	10.50 (11.25)	13 (2.41)	12.50 (—)

PV: periventricular lesion; CSC: corticosubcortical lesion; CST: corticospinal tract; *N*: number of cases; Me: median; IQR: interquartile range.

The logistic multiple regression to predict 2PD showed lesion timing (Wald chi-square test (1) = 10.62, $p = 0.001$) and ipsilesional extent of the lesion (Wald chi-square test (1) = 3.75, $p = 0.05$) to be significant contributors ($p > 0.05$) (Table 3(b)). The odds of having an impaired 2PD are 31 times higher in the group with CSC lesions than in the PVL group ($p = 0.001$). Secondly, the odds of having impaired 2PD increase by 1.34 for every unit change in the ipsilesional extent of the lesion ($p = 0.05$). No interactions were found between the CST wiring and the brain lesion characteristics to predict deficits in 2PD ($p > 0.05$).

The ordinal logistic multiple regression for touch sensation, as measured by the monofilaments, indicated that only the lesion extent significantly contributed to the deficits in touch sensation (Wald chi-square test (1) = 10.75, $p = 0.001$) (Table 3(c)). The odds of having better touch sensation decrease by 0.66 for every unit change in the ipsilesional extent of the lesion. No interactions were found between the CST wiring and the brain lesion characteristics to predict deficits in touch sensation ($p > 0.05$).

3.3.3. Impact of CST Wiring on the Relation between Motor and Sensory Functions. The correlation analyses between the motor and sensory functions for the whole group indicated a moderate association between the stereognosis score and grip strength ratio ($r_s = -0.60$, $p < 0.001$), JTHFT ratio ($r_s = -0.60$, $p < 0.001$), and AHA ($r_s = 0.61$, $p < 0.001$).

After group division according to CST wiring, there was no low correlation between motor function and stereognosis in the CST_{contra} and CST_{ipsi} groups (r_s (range) = −0.31–0.36, $p > 0.05$). Interestingly, in the CST_{bilat} group, moderate correlations were found with the JTHFT ratio ($r_s = -0.48$, $p = 0.07$) and the AHA ($r_s = 0.65$, $p < 0.01$), despite a low correlation with grip strength ratio ($r_s = -0.31$, $p = 0.2$). An illustration of the individual data points regarding these results can be found in Figure 3.

4. Discussion

In this study, we explored the predictive value of brain lesion characteristics on the type of CST wiring as well as the impact of these factors on UL motor and sensory functions. A comprehensive and standardized evaluation of both motor (grip strength, unimanual capacity, and bimanual performance) and sensory functions was used to predict UL function in a large cohort of individuals with uCP.

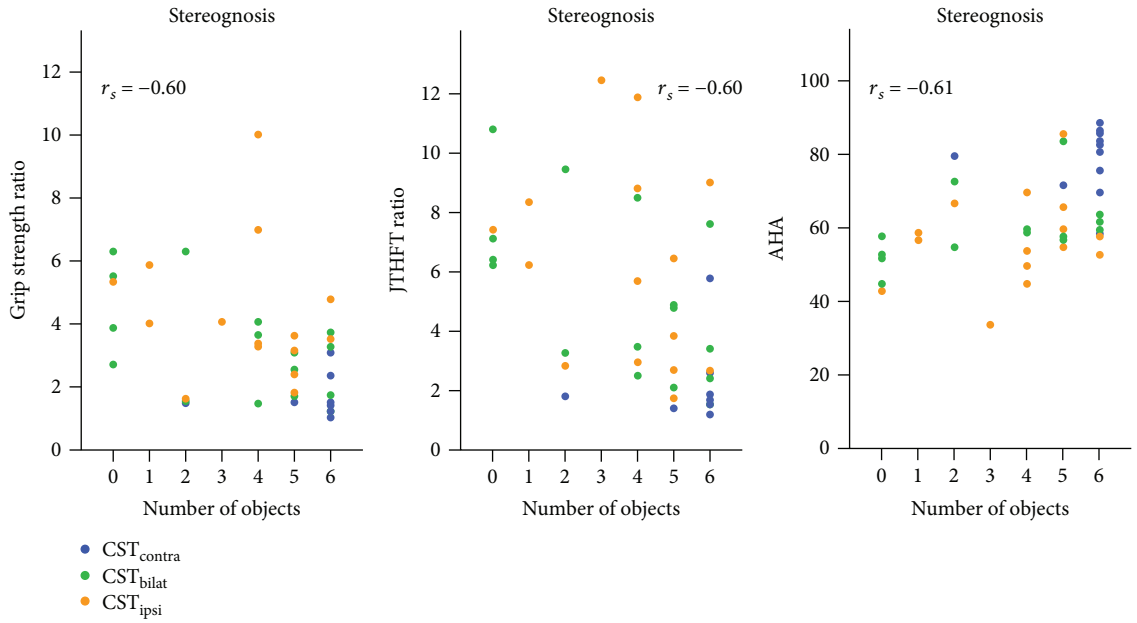

Figure 3: The relation between motor and sensory functions seems to vary depending on the CST wiring. Individuals with a CST_{contra} and CST_{ipsi} wiring showed no low correlations, whereas those with CST_{bilat} showed moderate correlations. Each dot represents an individual child, with CST_{contra} (blue), CST_{bilat} (green), and CST_{ipsi} (orange). Correlations between stereognosis with grip strength ratio (ratio, i.e., closer to one indicates preserved grip strength), JTHFT ratio (ratio, i.e., closer to one indicates preserved grip strength), and AHA. Correlation coefficients correspond to the analysis for the whole group.

Our first research question examined the discriminant ability of lesion timing, location, and extent to predict the type of CST wiring. A simple linear analysis demonstrated that lesion timing, location, and extent were significantly different between the CST wiring groups. Our results showed that a CST_{contra} was only seen in 2 out of 18 children with a CSC lesion, compared to 15 out of 34 children with a PV lesion. Current results suggest that damage to cortical and/or subcortical structures (i.e., CSC lesion) reduces the potential of the CST to develop according to its typical contralateral trajectory. We hypothesize that this is likely driven by the reduced neural activity in the motor cortical areas after a CSC lesion, which are crucial for the development of the CST during the postnatal period [38]. However, a contralateral development of the CST is still possible in CSC lesions, and it may occur differently depending on lesion location and extent.

Once all predictors were simultaneously entered in a multiple linear analysis, we found that the combination of the damage to the PLIC and the frontal lobe significantly discriminated between the CST wiring groups. Half of the children in the CST_{contra} group showed damage to the PLIC, in contrast to the 94% and 100% in the CST_{bilat} and CST_{ipsi} groups who showed damage to this white matter bundle. Furthermore, the frontal lobe was also more damaged in the CST_{bilat} and CST_{ipsi} groups, compared to the CST_{contra} group. Although it is not unexpected that the PLIC and the frontal lobe are the two significant predictors in the model, due to their undoubtable relation with the motor cortex and the performance of actions, this is the first time that this interaction with the type of CST wiring is shown. Contrary to the importance of the location, Staudt et al. [4] postulated that the type of CST wiring depended on the lesion extent. However, as they only included children with a PV lesion, their results cannot be extended to all the uCP populations. Further efforts should be made to underpin whether structural damage of the brain lesion may serve as a biomarker of the underlying CST wiring.

Next to the predictive model, we also investigated how accurate the two functions derived from the discriminant analysis would be to reclassify the individuals in their original categories. Despite the significant contribution of the PLIC and the frontal lobe to the discriminant model, the classification accuracy only reached 57%, suggesting that timing, location, and extent of the lesion (as included in the model) do not provide sufficient accurate information to predict the underlying type of CST wiring. Notwithstanding the validity and reliability of the semiquantitative scale that was used to investigate lesion location and extent, we acknowledge that the semiquantitative character of the scale may have underestimated the predictive value of the structural brain damage. Therefore, these results should be replicated in the future with volumetric measures of the different brain structures. For example, the projections to the PLIC have been shown to be topographically organized with reduced microstructural integrity in children with uCP [39] by using diffusion measures. Investigating the volumetric damage to the frontal lobe and the microstructural integrity of the PLIC may provide with further insights in determining the type of CST wiring in uCP.

For our second research question, we investigated the impact of CST wiring and brain lesion characteristics (timing, location, and extent) on motor and sensory functions. Regarding *motor outcome*, simple linear regression

analyses indicated that the CST wiring and all brain lesion characteristics had an influence on the grip strength, manual dexterity, and bimanual performance, which confirmed what previous studies have shown [5, 6, 10]. However, in the multiple linear regression analysis, we found that the underlying CST wiring plays a major, but not unique, role in determining UL motor function, as lesion location and extent also significantly contributed to increasing the explained variance for the JTHFT and AHA. Specifically, the type of CST wiring explained 46% and 52% of the JTHFT and AHA variances, respectively, which was increased up to 54% and 61% by including lesion extent and damage to the basal ganglia and thalamus into the model. In general, our results show that a CST_{ipsi} or CST_{bilat} leads to poorer UL motor function compared to CST_{contra} for all motor outcomes, even when controlling for the significant contribution of lesion extent and location. The importance of the underlying CST wiring is an expected result, as the CST is the main motor drive and its damage causes vast disturbances on voluntary motor control, drastically reducing motor capabilities [38]. Whilst lesion timing, location, and extent have been put forward as a predictor of UL function [2, 3] and were also confirmed in our linear regression analysis, the huge variability in motor function reported by previous studies seems to be mainly explained by the underlying CST wiring. Staudt et al. [10] were the first to report on the relation between CST reorganization potential at different gestational ages and UL motor function. These authors also found that, along with the CST wiring, UL motor function further worsened in later lesions (CSC lesions) [10]. Linear regression analysis also showed that later lesions led to poor motor outcome, but multiple regression analysis revealed that lesion location and extent were key factors, next to the type of CST wiring. Although later lesions seem to be associated to a larger extent [3], it seems that the lesion extent itself plays a more important role in motor outcome, i.e., children with a PV lesion with large extent will also present with poorer hand function. Interestingly, the damage to the basal ganglia and thalamus explained an extra 4% of the variability in the AHA. In accordance with our results, previous studies have reported the negative impact of these subcortical structures on UL motor outcome [2, 5].

It is important to note that we still found large variability in the three motor outcome measures within both the CST_{ipsi} and CST_{bilat} groups, whereas the variability in the CST_{contra} group was rather small (Figure 2, see also Table 2 Supplementary Materials for observed means). In other words, some individuals with a CST_{ipsi} and CST_{bilat} wiring had good motor function, similar to those with a CST_{contra} wiring. This variability could not be completely explained by the location and extent of the lesion, and other factors may play a role. In the CST_{ipsi} group, this large variability may be explained by the amount of overlap of the hotspot within the nonlesioned hemisphere to evoke MEPs in the affected and less-affected hands. Vandermeeren et al. [40] showed that dexterity indeed varies in individuals with ipsilateral wiring depending on the location of the hotspot of the CST innervating the affected hand and less-affected hand; overlapping hotspots resulted in poorer dexterity, whereas distinct nonoverlapping hotspots resulted in a preserved dexterity. Conversely, in the CST_{bilat} group, the large variability may be explained by a predominant contralateral or ipsilateral projection that controls the affected hand, as Jaspers et al. [9] proposed in their theoretical framework. Altogether, this seems to point toward a distinct underlying pathophysiology of the UL motor impairments in these two CST groups (CST_{ipsi} or CST_{bilat}), suggesting that individuals with either a CST_{bilat} or CST_{ipsi} pattern should be treated as two separate groups for future research. To further unravel the underlying mechanisms of the pathophysiology of motor control and motor capabilities in uCP, additional functional measures should be included such as excitatory and inhibitory intracortical circuits based on TMS (e.g., cortical silent period or paired-pulse paradigms) [15, 41] or functional connectivity of the sensorimotor network based on resting-state functional MRI [42, 43].

We also investigated the impact of the CST wiring and brain lesion characteristics on *sensory function*, based on the fact that CST projections also extend from the primary sensory cortex and mediate several sensory functions at the level of the spinal cord (control of nociceptive, somatosensory, and somatic motor functions) [44, 45]. Although our simple linear regression analyses suggested that all neurological factors individually played a role in determining sensory function, the multiple prediction model showed that a larger lesion extent, a later lesion (i.e., CSC lesion), and a CST_{ipsi} or CST_{bilat} led to higher chances of developing sensory deficits. Our results are in agreement with a recent study by Gupta et al. [6], who showed that more than 80% of the children with larger extent and later lesions (CSC) had disrupted somatosensory anatomy and physiology (lack of ascending sensory tracts and lack of somatosensory evoked potentials), consequently leading to a loss of sensory function [6]. If the sensory tracts are present, there is evidence suggesting that their main compensatory mechanism is an intrahemispheric reorganization, i.e., the sensory system reaches the original cortical destination on the postcentral gyrus, regardless of lesion timing (PV or CSC lesion) or CST wiring [11, 46, 47]. Current study results suggest that lesion extent best predicts the sensory deficits in individuals with uCP, although lesion timing and CST wiring also play an important role. Future research focusing on the pathophysiology of the sensory system based on noninvasive neurophysiological techniques (e.g., short-latency afferent inhibition [48] or sensory evoked potentials [11]), as well as functional connectivity measures, may contribute to increase our understanding of the underlying sensory pathways in uCP.

Lastly, we investigated whether the relationship between motor and sensory functions was disrupted by the type of CST wiring. We first confirmed previous study results indicating a significant relation between the motor and sensory outcomes in the total group [1, 25]. However, this association was disrupted by the type of CST wiring, whereby no little association was shown in the CST_{ipsi} and CST_{contra} groups, but a moderate association was found for the CST_{bilat} group. In the CST_{contra} group, the lack of a significant (or high) correlation seems to be due to the fact that these participants show both adequate motor and sensory functions, with little variation in the sensory scale, due to its ordinal nature. This

scale used to evaluate sensory function may not be sensitive enough to detect subtle sensory deficits, leading to a possible ceiling effect in the CST_{contra} group. By measuring with more quantitative techniques and devices, e.g., KINARM End-Point Lab (BKIN Technologies) [49], we may be able to discern the potential sensory problems that these individuals may present with. Secondly, the sensorimotor dissociation found in the CST_{ipsi} group may be explained at two different levels of the central nervous system. At the level of the spinal cord, the descending CST fibres entering the dorsal horn play an important role in presynaptic inhibition of primary sensory afferent fibres [45, 50], ensuring smooth execution of a movement. A CST_{ipsi} wiring may have consequences in the presynaptic inhibition at the level of the spinal cord and could, consequently, affect the relation between motor and sensory functions. On the other hand, at the level of the brain, the intrahemispheric communication between M1 and S1 has been shown to be very relevant for adequate processing of sensorimotor information [51–53]. As such, the lack of intrahemispheric corticocortical connections may affect the processing of sensory information, having a negative impact on the motor command. On the contrary, the CST_{bilat} group seems to preserve the relation between motor and sensory functions, as shown by the stereognosis modality. This may be potentially explained by the predominant behaviour that those with a CST_{bilat} wiring hypothetically show [9]. A relation between adequate sensory and adequate motor functions, as seen in the CST_{contra} group, may indicate a more "contralateral" behaviour, whilst a disparate relation may be indicative of rather an "ipsilateral" behaviour. However, this needs further confirmation with neurophysiological tools. Although current data do not allow drawing strong conclusions regarding sensorimotor integration, our results highlight the importance of investigating these aspects in the future to better understand the mechanisms of sensorimotor information processing in uCP. By using more advanced techniques to unravel the coupling between the sensory and motor systems, we will be able to determine the impact of such dissociation on motor control and motor performance. For instance, short-latency afferent inhibition has been put forward as a valuable indicator of the process of bilateral sensorimotor integration [48] and may potentially aid in measuring the reorganization of sensorimotor pathways in uCP.

There might be some important clinical implications based on the results of this study. A better understanding of the underlying mechanisms of motor and sensory impairments will surely contribute to developing new treatment approaches, specifically targeting the individual pathophysiological deficits. First, the type of CST wiring has been investigated as a potential biomarker of treatment response. Although motor improvement does not seem to be CST-type dependent after bimanual training [12, 54], there are conflicting results regarding unimanual training [55–57]. Furthermore, our results highlight the importance of considering the sensory system together with the available motor execution paradigms during UL training. Preliminary results of recent studies have shown the effectiveness of bimanual and sensory training on both motor and sensory functions in uCP [58, 59]. To further support interventions targeting sensory deficits, there is evidence in healthy adults suggesting that sensory input can modulate the excitability in both motor cortices simultaneously, as well as the communication between hemispheres [60]. In this line, it seems relevant to combine bimanual and sensory training to enhance the excitability of both motor cortices, which may increase intra- and interhemispheric connections between the sensory and motor systems, potentially resulting in long-lasting neuroplastic changes.

Next to the training approaches, it is also important to identify clinically feasible measures to infer the CST wiring and the sensory system. As these assessments are not always pleasant in young children nor practical in a clinical setting, there is a necessity to find tools that are more applicable to daily practice than neurophysiological techniques. To probe the motor system, mirror movements have been put forward as a valid clinical assessment tool that may reflect the underlying individual CST wiring [9, 61]. On the other hand, it seems very challenging to develop an accessible and simple tool to clinically probe the sensory system in uCP. Further research in this field is required to develop quantitative and valid measures of sensory function (e.g., perceptual threshold of touch with electrical stimulation [62] or robotic measures of proprioception [49, 63]) and to link these measures to the underlying mechanisms of the sensory system in uCP.

There are some limitations to be considered for the current study. First, we used scales for the evaluation of lesion location and extent, as well as for assessing sensory function that was based on an ordinal scoring. Although they have been shown to be reliable in uCP [25, 29], such scales may lack sensitivity. Second, our study lacked a neurophysiological technique to probe the sensory system (i.e., sensory evoked potentials) that may contribute to better understand the underlying mechanisms of sensory function in individuals with uCP. Third, the main limitation of the TMS assessment itself lays in the maximum stimulator output intensity that can be reached. This intensity may not have been sufficient to elicit a MEP from either the lesioned or the nonlesioned hemisphere, as the resting motor thresholds are normally higher in children and may be even higher in individuals with uCP. This limitation might have prevented us from finding a CST projection to eventually diagnose the individual as CST_{bilat} or CST_{ipsi} wiring. Furthermore, the MEP data were not analysed, which may provide with useful insights in future studies. Lastly, although our sample size was large and covers the most common lesion timing groups, our results cannot be completely extended to those children with malformations or postnatally acquired brain injuries, as these were not included in the analyses.

5. Conclusions

CST wiring mainly determines UL motor function, although also lesion extent and damage to the basal ganglia and thalamus significantly contributed to the prediction of UL motor deficits. For sensory function, lesion extent, timing, and the type of CST wiring pattern seem to be important to develop adequate sensory function. The underlying CST

wiring seems to disrupt the association between sensory and motor functions, pointing toward different mechanisms of sensorimotor integration in uCP. The results of our study contribute to a better understanding of the underlying pathophysiology of motor and sensory functions and highlight the importance of investigating sensorimotor integration in future studies. Subsequently, these insights will aid in developing new intervention strategies tailored to the specific deficits of the motor and sensory systems of the individual child with uCP.

Data Availability

All data concerning this study is available within the manuscript. Detailed data is available upon request to the first author.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

We would like to express our deepest gratitude to the children and families who participated in this study. We also specially thank Jasmine Hoskens for her assistance during the clinical assessments. Lastly, we would like to acknowledge the biostatisticians from the Leuven Biostatistics and Statistical Bioinformatics Centre (L-BioStat) of the KU Leuven (Prof. Geert Molenberghs and Dr. Annouschka Laenen) for their advice regarding the statistical analysis. This work is funded by the Fund Scientific Research Flanders (FWO project, grant G087213N) and by the Special Research Fund, KU Leuven (OT/14/127, project grant 3M140230).

Supplementary Materials

Table 1: descriptive information of the distribution of the lesion location and extent according to the lesion timing groups. Table 2: descriptive statistics (X (SD)) and univariate analysis of upper limb motor function according to the CST wiring and the brain lesion characteristics. Table 3: descriptive statistics (Me (IQR)) and univariate analysis of upper limb sensory function (3A, stereognosis and 3B, two-point discrimination and thresholds of touch sensation) according to the CST wiring and the brain lesion characteristics. *(Supplementary Materials)*

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Hindawi
Neural Plasticity
Volume 2018, Article ID 6950547, 15 pages
https://doi.org/10.1155/2018/6950547

Research Article

Reorganization of the Action Observation Network and Sensory-Motor System in Children with Unilateral Cerebral Palsy: An fMRI Study

Giuseppina Sgandurra,[1,2] **Laura Biagi**,[3] **Leonardo Fogassi**,[4] **Elisa Sicola**,[1] **Adriano Ferrari**,[5,6] **Andrea Guzzetta**,[1,2] **Michela Tosetti**,[3] **and Giovanni Cioni**[1,2]

[1]*Department of Developmental Neuroscience, IRCCS Fondazione Stella Maris, Calambrone, Pisa, Italy*
[2]*Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy*
[3]*Laboratory of Medical Physics and Biotechnologies for Magnetic Resonance, IRCCS Fondazione Stella Maris, Calambrone, Pisa, Italy*
[4]*Department of Neuroscience, University of Parma and Istituto Italiano di Tecnologia (RTM), Parma, Italy*
[5]*IRCCS S. Maria Nuova Hospital, Reggio Emilia, Italy*
[6]*University of Modena and Reggio Emilia, Modena, Italy*

Correspondence should be addressed to Laura Biagi; laura.biagi@fsm.unipi.it

Received 23 March 2018; Revised 14 May 2018; Accepted 22 May 2018; Published 25 July 2018

Academic Editor: Sergio Bagnato

Little is known about the action observation network (AON) in children with unilateral cerebral palsy (UCP). Using fMRI, we aimed to explore AON and sensory-motor network (SMN) in UCP children and compare them to typically developed (TD) children and analyse the relationship between AON (re-)organization and several neurophysiological and clinical measures. Twelve UCP children were assessed with clinical scales and transcranial magnetic stimulation (TMS). For the fMRI study, they underwent a paradigm based on observation of complex and simple object-manipulation tasks executed by dominant and nondominant hand. Moreover, UCP and TD children carried out a further fMRI session to explore SMN in both an active motor and passive sensory task. AON in the UCP group showed higher lateralization, negatively related to performances on clinical scales, and had greater activation of unaffected hemisphere as compared to the bilateral representation in the TD group. In addition, a good congruence was found between bilateral or contralateral activation of AON and activation of SMN and TMS data. These findings indicate that our paradigm might be useful in exploring AON and the response to therapy in UCP subjects.

1. Introduction

Functional representation of actions, either observed or performed or even imagined, relies on the human action observation network (AON), constituted by the premotor, inferior frontal, parietal, and temporal regions. Its functionality is crucial for action understanding and for subserving imitation by observation of new motor skills [1, 2].

Some neurophysiological studies exploring the presence and functionality of AON networks in children have suggested that maturation of AON has an age-related course from a more bilateral to a more lateralized representation, indicating physiological plasticity [3–5]. These properties are very meaningful, and it would be important to know if similar mechanisms could also be observed in pathological conditions such as unilateral or asymmetrical early brain injuries in children with unilateral cerebral palsy (UCP). It has been extensively demonstrated that the type of lesion and reorganization, studied through functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS), of the central nervous system have an impact on severity of upper limb deficits [6, 7]. Types of lesion,

underlying UCP, are often categorized into three groups, according to location and timing of insult: type I (prenatal): malformations or 1st and 2nd trimester patterns, presumed to occur in utero such as lissencephaly, focal cortical dysplasia, unilateral schizencephaly; type II (perinatal): periventricular white matter lesions mainly occurring in the early 3rd trimester and often in preterm born infants such as periventricular leukomalacia (PVL); type III (connatal): cortical or deep grey matter lesions that occur towards the end of gestation, that is, around term age, such as infarcts in the territory of the middle cerebral artery (MCA) [7–9].

Previous studies have shown that children with type II lesions demonstrated better upper limb sensorimotor functioning, compared to children with type III lesions [10, 11]. Regarding type of motor reorganization, there are two main types of (re-)organization: ipsilesional and contralesional [12]. In adults with stroke and in some children, the main mechanism for reconnection of the motor cortex to spinal cord consists of (re-)organization within the ipsilesional cortex. This mechanism is based on partial sparing of the primary motor cortex or on the possibility that functions may be taken over by intact nonprimary motor areas within the damaged hemisphere (ipsilesional (re-)organization). However, when lesions occur at an early development stage, either during intrauterine life or soon after birth, a different mechanism can be observed. This is based on the persistence of a significant component of monosynaptic fast-conducting ipsilateral motor projections, normally withdrawing within the first months of life, that may be permanently maintained if brain damage occurs early in life [6, 13, 14]. In this case, the unaffected hemisphere directly controls both upper limbs, giving rise to a pattern of reorganization unknown in adult pathologies (contralesional (re-)organization). It has been extensively demonstrated that ipsilesional motor projection is definitely correlated to better motor outcomes, measured by functional scales (e.g., Melbourne Unilateral Upper Limb Measurement [15] and Assisting Hand Assessment [16]), than contralesional reorganization [11]. The sensory system generally follows an ipsilesional reorganization, but when a dissociation of sensorimotor representation occurs, that is, a contralesional reorganization for motor function and an ipsilesional reorganization for sensory one, quality of motor function is usually more affected [6].

Regarding AON in UCP children, Dinomais et al. [17] have shown, in eighteen UCP patients, aged 7–21 years, that observation at rest of a simple opening-closing hand movement performed by either the left or the right hand of an actor produces large bilateral activations in the occipito-temporo-parieto-frontal network, including most AON nodes. Moreover, a stronger ipsilesional activation of primary motor cortex (M1) was shown when they viewed movement of the hand corresponding to the affected one. Finally, observation of hand movement engaged motor execution networks regardless of degree of motor impairment.

The fMRI paradigm in this latter study was created around the observation of a simple movement without an object. We have developed an fMRI paradigm to explore AON based on the observation of simple and complex object-manipulation tasks executed by both dominant and nondominant hand. This fMRI paradigm has been already tested on healthy adults [18] and in a sample TD children [5].

The aim of this fMRI study was to explore AON and sensory-motor network (SMN) in UCP children in comparison to age-matched TD children and analyse the relationship between AON (re-)organization and several neurophysiological and clinical measures.

2. Methods

2.1. Subjects. Twelve UCP patients (6 with left UCP, age range = 6.2 – 16.3 y, mean age ± standard deviation (SD) = 10.3 ± 2.9 y) were enrolled in this study. The sample included seven males (age range = 7.5 – 14.5 y; mean age ± SD = 10.7 ± 2.6 y) and five females (age range = 6.2 – 16.3 y; mean age ± SD = 10.5 ± 4.0 y). All UCP children had IQ > 70.

Dataset from 12 healthy right-handed children and adolescents (6 M, 6 F; age range = 7.0 – 15.3 y, mean age ± SD (SD) = 10.6 ± 2.1 y) already described in a previous study [5] were used as age-matched controls (TD children).

All subjects and their parents gave written informed consent in accordance with protocol approved by the Ethics Committee of the IRCCS Fondazione Stella Maris.

2.2. Clinical Tests. All children were classified according to the House Functional Classification System (HFCS) for assessing upper limb function. HFCS consists of nine grades ranging from a completely excluded hand (grade 0) to a spontaneous and independent one (grade 8) [19, 20]. In addition, they were clinically assessed with two standardized function tests, Assisting Hand Assessment (AHA, Version 4.4) [16] and Melbourne Assessment of Unilateral Upper Limb Function (MUUL) [15], in order to evaluate assisting hand use during bimanual performance and upper limb movement capacity, respectively.

AHA is a standardized, criterion-referenced test based on observations of affected hand/arm used during a videotaped 15-minute play session with toys from the AHA test kit. Video scoring produces raw values ranging from 22 (low ability) to 88 (high ability) that are converted to scaled scores ranging from 0 to 100. AHA was administered and scored by a certified rater.

MUUL is a standardized tool for measuring quality of upper limb movement capacity during 16 criterion-referenced items representative of reach, grasp, release, and manipulation. Performance is videotaped and scored using criteria for rating qualities of movement range, fluency, and dexterity [15, 21]. Scores vary from 0 to 100%, the latter indicating best performance.

Presence or absence of mirror movements in the unaffected hand during voluntary unimanual movements of the affected hand was evaluated by consensus by two experienced child physical therapists (ES and EB, 30 and 8 years of experience in clinical evaluation of UCP), analyzing the videotapes of the standardized clinical tests.

2.3. Transcranial Magnetic Stimulation. TMS was performed using a mapping procedure as described in Borghetti et al. [22], by using a Magstim 200® device (Magstim Company Ltd., Whitland, Wales, UK) connected to a figure-eight coil (diameter: 11 cm). Both hemispheres were searched

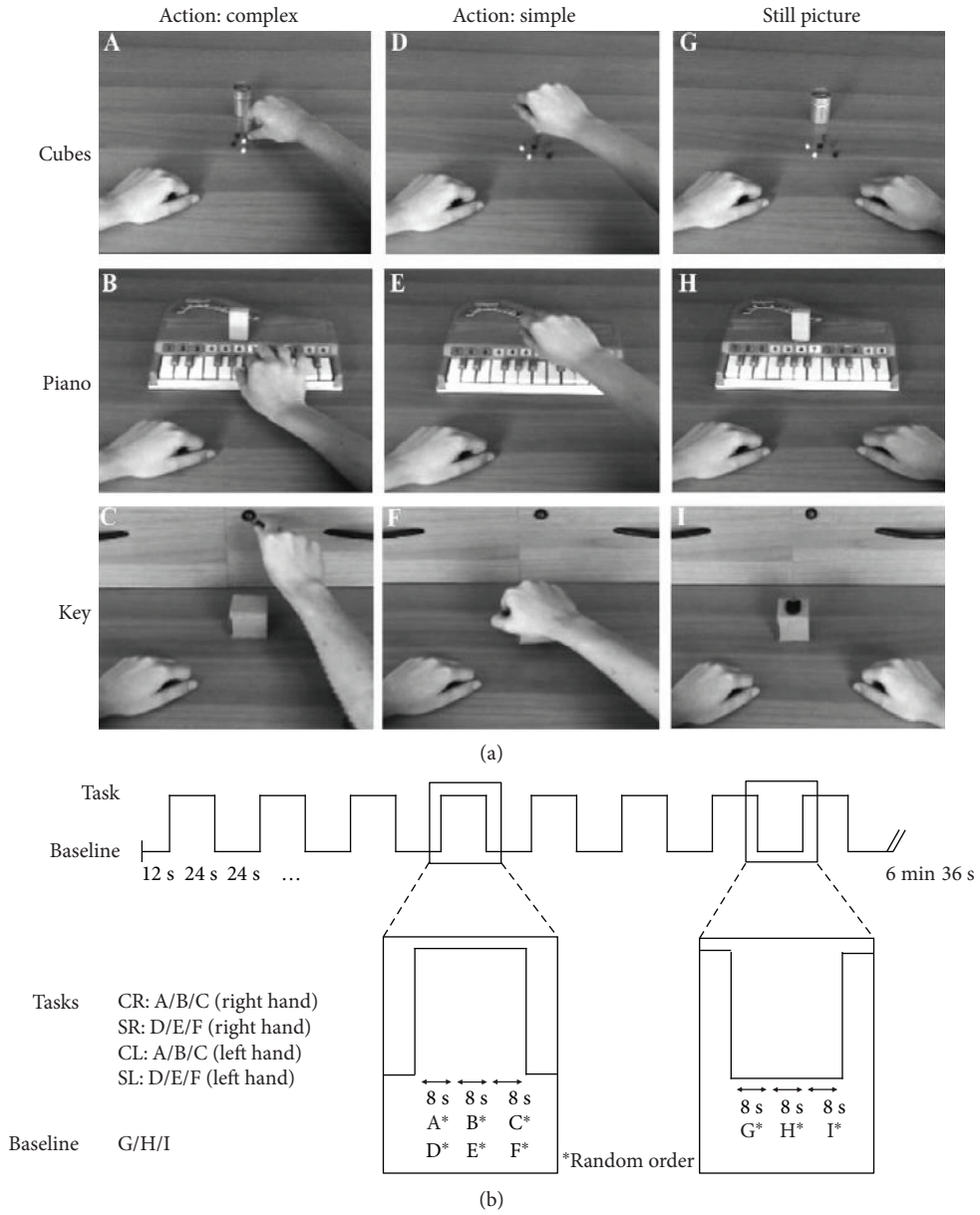

Figure 1: (a) Examples, taken from a single frame, of the six videoclips showing object manipulation performed by the right hand in three different contexts ("cubes," "piano," and "key"): three complex actions (A, B, C) and three simple actions with the same object (D, E, F). (G, H, I) Initial static frames of the corresponding action types, used as BASELINE conditions. (b) Diagram of the functional series presented to children: the block design comprises two TASK blocks for each of the four different conditions (CR, SR, CL, and SL) for complex (C) or simple (S) actions performed by the right (R) or left (L) hand, alternating with the same number of BASELINE blocks. Each block lasts 24 seconds and is composed of the random sequence of the 8-second videoclips of hand actions or still pictures of the resting hands. The presentation of the different conditions in the TASK blocks was completely randomized. Each functional series included four initial extra scans (12 s) to allow the stabilization of signal. Reproduced with permission (Copyright © 2015 John Wiley & Sons Ltd) from Biagi et al. [5].

systematically for ipsilateral or contralateral motor-evoked potentials, during a monitored low-level contraction of abductor digiti minimi (ADM) muscles. TMS results were used globally to establish the type of reorganization of sensorimotor system. In particular, contralesional (CL) reorganization was used to indicate a reorganization involving the unaffected hemisphere, and ipsilesional (IL) for a reorganization involving the lesioned hemisphere. Data acquisition was conducted following the international guidelines for TMS in children for aspects of safety [23].

2.4. Experimental Design. In order to investigate the relation of AON with reorganization of SMN, two different fMRI paradigms were used.

AON was explored as described by Biagi et al. [5]. Eight-second videoclips in a first-person perspective of three simple and three complex hand actions, performed by dominant and nondominant hand, were presented (Figure 1(a)). The three complex actions were grasping little cubes and putting them into a box (cubes), performing a simple scale on a piano keyboard (piano), and grasping

a key, putting it into a lock, and turning it (key). The three simple actions consisted of a whole hand grasping a small box performed in the same visual contexts of the complex actions, in order to match luminance, colour, and visual information. Videoclips of the same type (hand, complexity) in the three contexts (cubes, piano, and key) were randomly combined to create four distinct conditions (TASKS), each lasting 24 seconds, corresponding to the presentation of a simple or a complex action performed by dominant or nondominant hand (simple-dominant (S-D), complex-dominant (C-D), simple-nondominant (S-ND), and complex-nondominant (C-ND)). The 24-second corresponding control condition (BASELINE) was created by combining a sequence of three still pictures of resting hands in the respective contexts. The paradigm of stimulus presentation was built on a block design scheme, with two blocks for each of the previous four TASKS intertwined with the same number of BASELINE blocks (16 blocks total). In each functional series, the order of TASKS blocks was randomly generated and four initial extra scans (dummies, 12 seconds) were added to allow for stabilization of signal, giving a total acquisition time of $6'36''$ (Figure 1(b)). The AON experiment consisted of the acquisition of two functional series. Visual stimuli were presented through LCD goggles (Resonance Technology, USA). Subjects were asked to observe videos, staring at the middle of a screen. Gaze and attention to stimuli were continuously monitored using an eye tracker infrared camera mounted on goggles. Recorded eye movements were analysed to verify gaze and attention during AON stimuli.

For SMN localization, a block design paradigm was designed with both an active movement task (alternated hand opening and closing, MOTOR TASK) and a passive sensory task (palm and fingers passively brushed by an external operator by means of a wooden spatula, at a frequency of about 1 Hz, SENSORY TASK), in the same functional series. The series included eight blocks of 18 seconds each, alternating between motor and sensory tasks, each intertwined by an equivalent number of REST periods (REST-MOTOR TASK-REST-SENSORY TASK…). All subjects received detailed instructions before acquisition. They were asked to keep their eyes closed. During motor task, they were asked to repetitively open and close their hand at a frequency of 1 Hz; commands "move" and "stop" were given at the beginning and end of each block. During the sensory task and rest periods, they were asked simply to stay still. The examiner visually controlled the task performance. Each series included four initial extra scans (dummies, 12 seconds) for a total acquisition time of 5 minutes. Two sessions were performed, the first for the dominant hand and the second for the nondominant one, obtaining four conditions of interest (sensory-dominant (Sens-D), motor-dominant (Mot-D), sensory-nondominant (Sens-ND), and motor-nondominant (Mot-ND)).

During fMRI acquisitions, ambient scanner noise was constant and attenuated by ear plugs.

2.5. Imaging Acquisition and Processing. MR exams were performed on a 1.5 T MR scanner (HDx, GE Healthcare, Milwaukee, WI, USA). Standard MR protocol included FSE T2-weighted, SE T1-weighted, FLAIR, and DWI sequences. A whole brain, 3D high-resolution, T1-weighted series (FSPGR) was collected in an axial plane (TR/TE = 12.3 ms/2.4 ms; TI = 700 ms; voxel size = 1 mm^3 isotropic) for anatomic localization of activated regions and delineation and description of lesions.

MRI anatomical findings were classified retrospectively according to literature [7, 9] into three main forms related to timing of lesion: type I, brain malformations (early malformative); type II, abnormalities of periventricular white matter (prenatal); and type III, cortical-subcortical lesions, mainly due to middle cerebral artery infarction (connatal).

The fMRI session included four series, two functional series for AON task (each lasting $6'36''$) and two series for sensory-motor task ($5'00''$), one for each hand. Blood oxygenation level-dependent (BOLD) responses were registered by using an echo planar imaging gradient-echo sequence (GRE-EPI) with the following parameters: TE/TE = 3000/50 ms, FA = 90°, field of view (FOV) = 240 × 240 mm, matrix = 64 × 64, slice thickness = 5 mm.

Data preprocessing, performed using BrainVoyager QX Software Package (BV, Brain Innovation, Maastricht, the Netherlands), included mean intensity adjustment to compensate for interscan intensity differences, temporal interpolation and resample to compensate for slice-dependent time differences (sinc function), 3D motion correction (sinc interpolation), and high-pass temporal filtering (GLM-Fourier approach, two cycles/time course).

Functional data were coregistered on the three-dimensional anatomical T1-weighted images by using an affine alignment with the standard BV nine parameters (three for translation, three for rotation, and three for FOV scale). Anatomical datasets were in turn transformed into standard Talairach's Space [24].

In order to combine data from UCP children in a group analysis, and considering that TD children were all right-handed, we designated the left hemisphere as the hemisphere contralateral to the dominant hand (unaffected hand in UCP, right hand in TD) and the right hemisphere as the hemisphere contralateral to the nondominant hand (affected hand in UCP, left hand in TD). To do this, we flipped the right-left (x) direction [17] of both functional and structural T1-weighted images of children with left hemisphere lesions (right hemiplegia), in order to have all lesioned hemispheres on the right side of the brain and all unaffected hemispheres on the left side.

2.6. fMRI and Statistical Analysis. BOLD responses were analysed using the general linear model (GLM) approach, using the same number of regressors as conditions of interest. Each regressor was obtained by convolving a box-car function for each stimulation block with the standard Boynton hemodynamic response function [25]. Four regressors were selected both for the AON stimulus (S-D, C-D, S-ND, and C-ND) and for the SMN task (Sens-D, Mot-D, Sens-ND, and Mot-ND). In all analyses, six spurious movement regressors (outputs of the 3D motion correction procedure) were included in GLM.

Table 1: Demographic and clinical data of children with UCP enrolled in fMRI study.

ID	Sex	Age (y)	Type of lesion	Side of hemiplegia	Type of reorganization	HFCS	MUUL	AHA	MM
1	M	7.5	I	RH	CL	5	72.13	65.15	Y
2	M	11.0	I	LH	CL	4	60	47	Y
3	M	11.5	I	LH	CL	5	74.59	60.61	Y
4	F	16.3	I	LH	CL	4	55	42	Y
5	M	8.1	II	RH	IL	7	90.98	86.36	N
6	M	10.6	II	RH	IL	8	93.58	77.27	N
7	F	12.0	II	LH	CL	5	79	53	Y
8	M	13.6	II	LH	CL	5	79.51	56.06	Y
9	F	6.2	II	RH	IL	6	81.15	63.64	Y
10	F	7.2	III	RH	IL	8	81.15	69.7	N
11	M	9.3	III	LH	IL	7	91	80.3	N
12	F	10.6	III	RH	CL	5	80.32	75.76	Y

F: female; M: male; y: years; I: early malformative; II: prenatal; III: connatal; RH: right hemiplegia; LH: left hemiplegia; IL: ipsilesional; CL: contralesional; HFCS: house functional classification system; MUUL: Melbourne Assessment of Unilateral Upper Limb Function; AHA: Assisting Hand Assessment; MM: mirror movements; Y: present; N: absent.

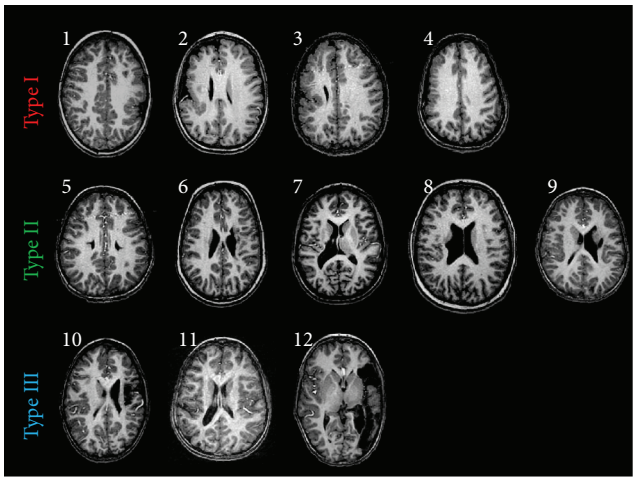

Figure 2: Representative slices of 3D T1-weighted images depicting the brain lesion of each UCP child. Numbers correspond to the ID of UCP children as reported in Table 1.

Multisubject analyses were conducted using random-effects (RFX) GLM-based analysis, for both groups (UCP and TD) in order to identify a group representation of cortical activations and to detect possible differences between groups. Threshold of statistical maps was $p < 0.05$ Bonferroni-corrected, and a minimum cluster size of 150 mm^3 was also applied. Group analyses were also used to reveal and define regions of interest (ROIs), to be selected subsequently in single-subject analysis.

For the AON stimulus, contrasted activity for all observed actions versus control condition was used (all TASKS > BASELINE) in order to identify possible differences in the representation of AON in UCP children with respect to their age-matched controls. Moreover, contrasted activities for observation of complex and simple actions performed by the dominant or nondominant hand versus control condition ((C-D + S-D) > BASELINE, (C-ND + S-ND) > BASELINE)) were also performed, in order to investigate hand identity properties in UCP children.

As in Biagi et al. [5, 18], a ROI analysis was conducted on activated areas in order to investigate possible differential responses to observation of both hands (laterality) and to observation of complex and simple actions (complexity). In particular, a 2×2 factorial design ANOVA was performed in specific areas (anterior intraparietal cortex (AIP), inferior temporal gyrus, MT, as control), considering as factors "hand" laterality (two levels: dominant and nondominant) and the "complexity" of action (two levels: simple and complex).

Finally, probabilistic functional maps were calculated to evaluate spatial consistency of activity patterns across subjects.

For the SMN task, two contrasts were employed, considering together sensory and motor stimuli of each hand, dominant or nondominant ((Sens-D + Mot-D) > REST and (Sens-ND + Mot-ND) > REST).

For the same contrasts, single-subject analyses were performed by using a fixed-effects (FFX) approach, with a lower, uncorrected statistical threshold ($p < 0.001$, minimum cluster size $\geq 150\ mm^3$) in order to extract, from each participant, coordinates of foci and number of activated voxels of areas identified by multisubject group analysis. Average and variability (defined as standard deviation divided by mean: SD/mean $\times 100$) across subjects were also calculated. Moreover, a laterality index (LI) was calculated by comparing the size of homologous areas in both hemispheres. In particular, for the AON task, LI was obtained by computing the ratio $(N_{DS} - N_{nonDS})/(N_{DS} + N_{nonDS})$, where N_{DS} and N_{nonDS} are the number of activated voxels in the dominant side of the brain (DS, hemisphere contralateral to dominant hand) and in the nondominant side (non-DS, hemisphere contralateral to nondominant hand), respectively. For the SMN task, a laterality index was also calculated specifically for the primary sensorimotor cortex (pSMC), using the

Table 2: Areas elicited by observation of all object-related hand actions versus BASELINE condition in UCP children and age-matched TD children [5].

Area name	BA		UCP children					TD children				
			Talairach's coordinates			Cluster size	t-value	Talairach's coordinates			Cluster size	t-value
			x	y	z			x	y	z		
Inferior temporal gyrus	37	DS	−44	−65	0	8307	18.4	−44	−64	0	15,536	20.6
		Non-DS	47	−61	−2	8369	15.6	44	−61	1	18,659	18.7
Superior temporal gyrus	22	DS	−48	−35	12	1357	10.4	−51	−40	11	1531	8.2
		Non-DS	53	−33	9	1534	8.6	50	−40	11	2584	9.2
Inferior parietal lobule	40	DS	−44	−40	39	4278	7.6	−56	−31	30	3751	9.9
		Non-DS	48	−36	36	2281	7.7	57	−31	27	1669	6.9
Anterior IPS	40-7	DS	−36	−45	45	4231	12.7	−32	−44	50	4806	15.4
		Non-DS	37	−50	48	3023	7.3	33	−45	49	2265	10.6
Superior parietal lobule	7	DS	−16	−66	45	5112	11.9	−25	−67	46	11,593	15.7
		Non-DS	22	−62	45	3212	8.0	23	−64	47	8594	11.7
Precentral gyrus	6-4	DS	−35	−11	51	1688	6.2	−33	−16	52	3292	7.9
		Non-DS	35	−9	53	1458	7.2	32	−14	52	1865	5.3
	6-9	DS	−40	4	38	1260	8.3	−47	−2	32	2019	9.5
		Non-DS	43	6	39	1808	7.1	43	2	34	3242	9.7
Middle-superior frontal gyrus	9-10-46	DS	−50	14	24	925	5.0	−39	38	22	1180	5.0
		Non-DS	41	23	17	899	7.2	43	17	21	1154	5.1
Inferior frontal gyrus	45–47	DS						−33	23	2	441	5.2
		Non-DS	45	32	5	982	5.1	41	23	3	538	6.2
Middle occipital gyrus	18	DS	−23	−85	4	1465	10.3	−22	−83	3	3897	11.6
		Non-DS	22	−84	7	1417	7.4	24	−83	2	1369	14.9

BA = Brodmann area; UCP = unilateral cerebral palsy; TD = typically developing; DS = dominant side (controlateral to dominant hand); non-DS = nondominant side (controlateral to nondominant hand); IPS=inferior parietal sulcus. For convention, the dominant hand corresponds to the unaffected hand in UCP and to the right hand in TD children, while the nondominant hand corresponds to the plegic hand in UCP and to the left hand in TD children.

same formula, but considering only the number of activated voxels in the two pSMCs.

For hemispheric dominance [26], we assumed a standard threshold of 0.20 in absolute value: $LI > 0.20$ dominance in the hemisphere contralateral to the dominant hand, $LI < -0.20$ dominance in the hemisphere contralateral to the nondominant hand, and $-0.20 \leq LI \leq 0.20$ bilaterality.

Statistical analysis and data fitting were performed via the software package OriginPro 9.0 (OriginLab Corporation). For linear data fit, Pearson correlation coefficient and equivalent p value were calculated. For data comparison of both groups (UCP and TD) and for data regarding the two hands and respective hemispheres, a nonparametric Mann–Whitney U test was used.

3. Results

Table 1 shows demographic and clinical data of enrolled UCP children. According to type and timing of lesions, the sample was classified into three groups: the first one was composed of four children with early malformative lesions (type I), the second one of five children with white matter damage (type II) and the third group by three children with connatal stroke (type III). In all patients, except one, the lesion was strictly unilateral (see Figure 2). In patient 5, who had bilateral alterations on imaging, the more affected hemisphere was contralateral to the side of motor impairment.

Based on TMS results, all children with type I lesions showed a CL reorganization, while children with the other two types of lesion showed either CL or IL reorganization.

Mirror movements (MM) were present in all children with type I lesion, while they were variably present or not in children with the other two types of lesion.

In the fMRI experiment, all children were able to understand tasks and succeeded to collaborate and to maintain gaze in the middle of the screen for the AON task. For the SMN task, one child (number 4) in the UCP group was discarded from the following analysis because of the presence of excessive movement artefacts during functional acquisition, which were difficult to correct or compensate for.

3.1. AON in UCP and TD Children. As previously observed in TD children [5], also UCP children showed activation of areas belonging to the action observation network such as the inferior temporal gyrus (BA37), superior temporal sulcus (BA 22), anterior intraparietal sulcus (BA40-7), inferior

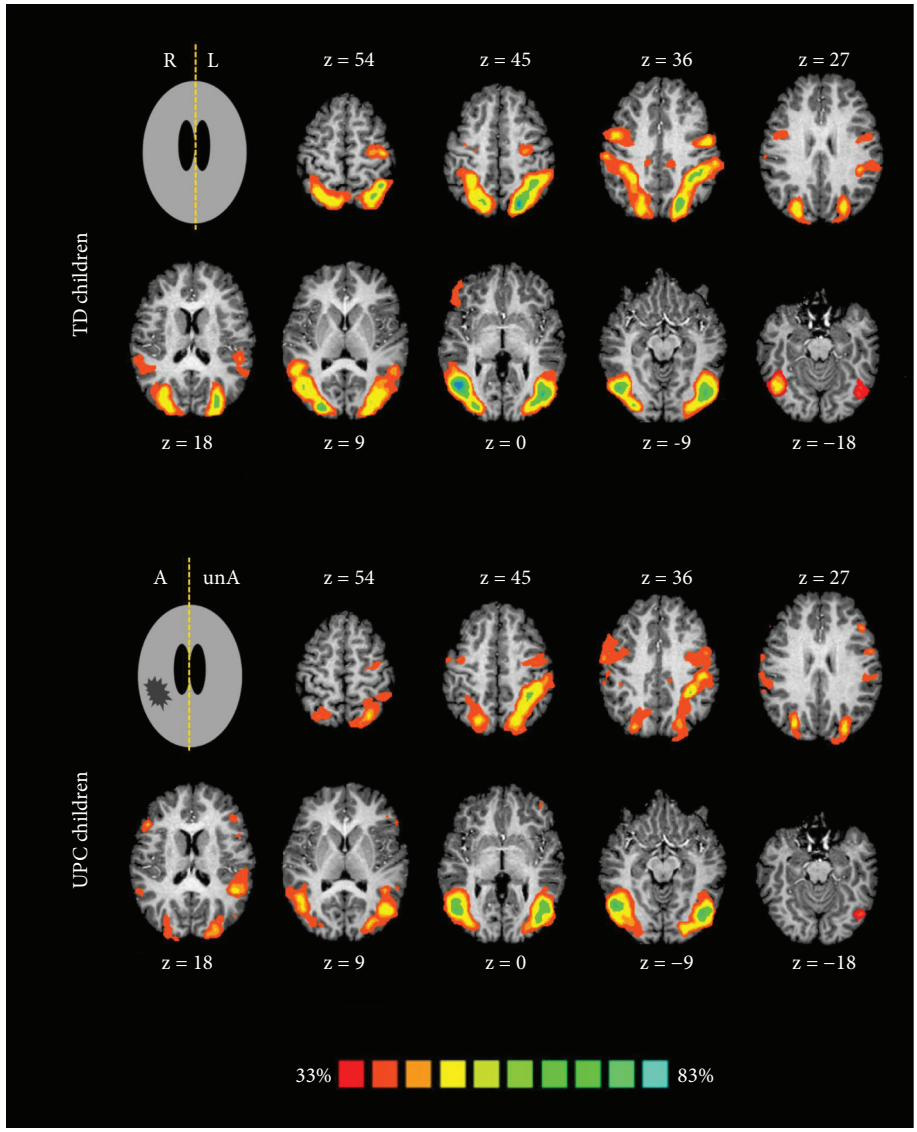

Figure 3: Probabilistic functional maps of the action observation circuit for the ALL TASKS > BASELINE contrast in TD children (as previously published in Biagi et al. 2015, top [5]) and in UCP children (bottom). Colour bar represents different levels of probability of activation for the action observation task from 33% (red, meaning that a brain region appeared in the map only if it was activated in at least 4 subjects) to 83% (cyan, equivalent to areas activated by more than 10 subjects). For each transversal slice, the z Tailarach's coordinate is indicated. On the right, we represent the left hemisphere for TD children (radiological convention; R = right, L = left) and the unaffected hemisphere, that is, the hemisphere contralateral to the unaffected hand, in UCP children (unA = unaffected, A = affected); on the left, the right hemisphere for TD and the hemisphere contralateral to the affected hand in UCP.

parietal lobule (BA40), superior parietal lobule (BA7), precentral gyrus (dorsolateral, BA6-9 and BA6-4), and inferior frontal gyrus (BA45-47). As in TD children, further activations were found in visual and somatosensory areas and in the middle frontal gyrus. Table 2 reports averages of Talairach's coordinates and of cluster sizes of activated areas, calculated across subjects of both groups, for the AON task.

Similarly, Figure 3 shows probabilistic maps about the contrast of all TASKS > BASELINE for UCP and TD children, allowing for a comparison of results between both groups.

Pattern of activations of AON in TD children presented higher levels of probability with respect to the UCP group, suggesting a more reproducible network. This was also confirmed by variability analysis conducted at the level of single-subject data, where the coefficient of variation of number of activated voxels across subjects was overall significantly different between TD children (67%) and UCP children (89%) (Mann Whitney U test, $p = 0.003$).

Regarding features of stimuli ("hand" laterality and "complexity" of action), a 2×2 factorial design ANOVA revealed a significant effect only for hand identity property

Table 3: Areas elicited by sensory-motor task for the dominant hand (DH) and nondominant hand (non-DH) in UCP children and in age-matched TD children.

				UCP children					TD children				
		BA		Talairach's coordinates			Cluster size	t-value	Talairach's coordinates			Cluster size	t-value
				x	y	z			x	y	z		
Motor-sensory task of DH	pSMC	2-3-4	CLH	−36	−27	53	8926	16.4	−40	−33	53	8811	17.9
			ILH	37	−29	48	1794	6.1	42	−30	54	1046	5.0
	IPL	40	CLH	−50	−29	26	1404	6.3	−50	−26	23	1212	5.5
			ILH	51	−24	25	1946	5.0	54	−20	22	931	6.0
	PrC gyrus	6	CLH	−56	−3	29	1430	5.1	−57	−4	36	712	5.0
			ILH										
	Insula	13	CLH	−44	−11	16	663	6.2					
			ILH										
	SMA	6–31	IH	−5	−17	48	1565	7.1	−4	−25	47	1024	6.9
	Cerebellum		CLH										
			ILH	14	−52	−23	1243	5.0	20	−52	−28	562	5.0
Motor-sensory task of non-DH	pSMC	2-3-4	CLH	35	−29	49	5928	10.1	38	−31	52	8645	18.3
			ILH	−41	−22	46	1032	7.5	−40	−25	55	621	5.8
	IPL	40	CLH	50	−20	27	1719	6.6	50	−25	22	1168	7.2
			ILH	−50	−29	25	502	5.1	−54	−30	28	1445	6.5
	PrC gyrus	6	CLH	48	3	31	878	5.3	42	−9	43	839	6.6
			ILH	−54	−5	29	554	5.7					
	Insula	13	CLH	33	−24	17	613	5.6					
			ILH	−46	−13	11	677	5.6					
	SMA	6–31	IH	0	−12	47	988	5.0	1	−18	52	788	6.1
	Cerebellum		CLH										
			ILH	−14	−52	−21	1495	7.3	−13	−61	−22	2288	7.0

BA = Brodmann area; UCP = unilateral cerebral palsy; TD = typically developing; pSMC = primary sensory-motor cortex; IPL = inferior parietal lobule; PrC gyrus = precentral gyrus; SMA = supplementary motor area; DH = dominant hand; non-DH = nondominant hand; CLH = contralateral hemisphere; ILH = ipsilateral hemisphere, IH = interhemispheric. For convention, the dominant hand corresponds to the unaffected hand in UCP and to the right hand in TD children, while the nondominant hand corresponds to the plegic hand in UCP and to the left hand in TD children.

("hand," $p < .001$) in AIP of the hemisphere contralateral to the nondominant hand. However, no significant effects were found for the "complexity" factor ($p = 0.23$) and the interaction effect ("hand" × "complexity," $p = 0.28$). No significant effect was found in either right or left MT, used as a control area.

3.2. Sensory-Motor Task in UCP and TD Children. BOLD responses to both sensory and motor tasks of both hands (contrasts: (Sens-D + Mot-D) > REST and (Sens-ND + Mot-ND) > REST) allowed for the identification of similar activity patterns in a number of cortical areas belonging to SMN in both groups.

In particular, the majority of subjects showed bilateral activations in the primary sensory motor cortex (pSMC, BA 1-2-3-4) and in inferior parietal lobule (BA 40). Other activations were found medially in the supplementary motor area (SMA), in a sector comprised between medial frontal gyrus and cingulate gyrus (BA 6-31), in the precentral gyrus of the hemisphere contralateral to the stimulated hand (BA 6), and in the cerebellar hemisphere ipsilateral to the stimulated hand. Differently from TD children, UCPs also showed activity in the insula (BA 13), contralaterally in the case of stimulation of the dominant hand, and bilaterally for the nondominant one. Table 3 reports averages of coordinates and number of activated voxels, calculated across subjects for both groups.

3.3. Lateralization of AON and of pSMC in UCP and TD Children. Concerning AON, Figure 3 shows bilateral brain activation in TD children, as previously reported in Biagi et al. [5]. On the contrary, UCP children presented a mildly lateralized circuit in the hemisphere contralateral to the dominant hand. This finding was obtained by computing global lateralization indices, using number of voxels in the two hemispheres obtained by multisubject analysis ($LI_{TD} = 0.01$, $LI_{UCP} = 0.23$).

Regarding the two hands separately, Figure 4 reports data on lateralization indices obtained in each subject for both stimuli. For AON, LIs were calculated considering the whole neuronal circuit identified by multisubject analysis, while for SMN, LIs referred more specifically to lateralization of the primary sensorimotor cortex (pSMC).

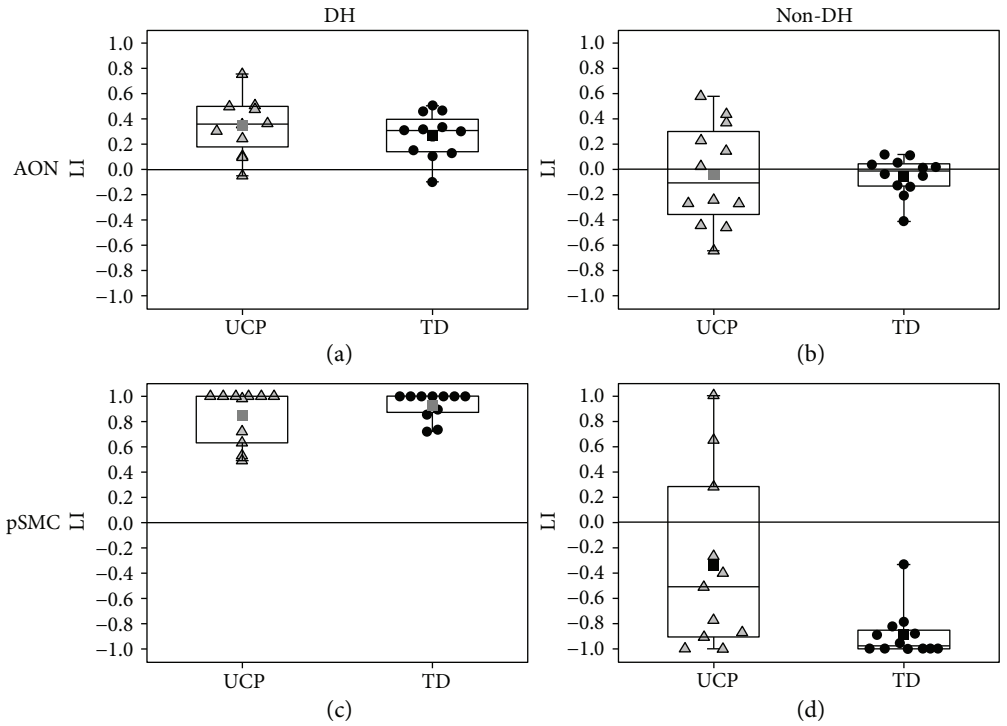

Figure 4: Box plots of the laterality indices of each single subject for AON (top row) of the dominant hand (DH) (a) and of the nondominant hand (non-DH) (b), as well as for the primary sensory-motor cortex, pSMC (bottom row), for the stimulation of the dominant hand (c) and of the nondominant hand (d). UCP children are indicated by light grey triangles, TD children by black circles. Each box is defined by the 25th and 75th percentiles. The whiskers are determined by the minimum and maximum values; the square indicates the mean value, while the line corresponds to the median value.

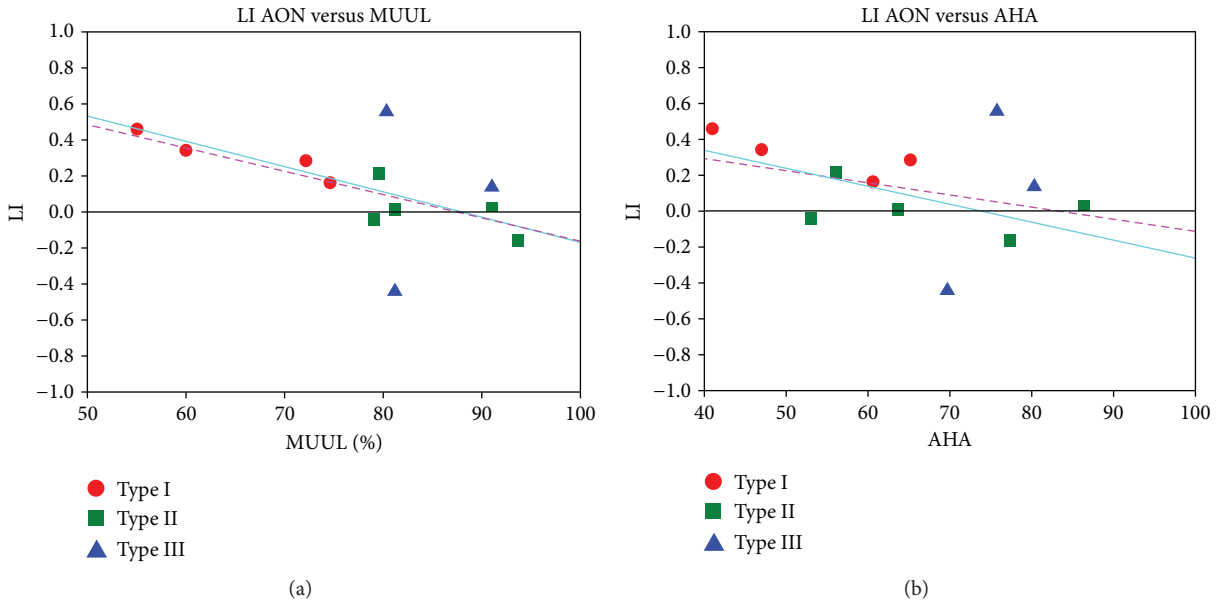

Figure 5: Lateralization index (LI) for the observation of all object-related actions versus BASELINE for each UCP child, plotted against his/her clinical scores (MUUL scale on panel a; AHA scale on b). Children were represented with different colours and symbols according to the classification of their lesions (type I = red circles, type II = green squares, type III = blue triangles). Data were fitted with a standard linear function. Considering all the subjects, the correlations are not significant (Pearson's value $R = -0.55$, $p = 0.06$ for MUUL; $R = -0.34$, $p = 0.28$ for AHA; pink dotted line). They become significant when only children with lesions of type I and type II are used in the fit ($R = -0.90$, $p = 0.0007$ for MUUL; $R = -0.68$, $p = 0.04$, for AHA; cyan solid line), due to big variability of data from children with type III lesion.

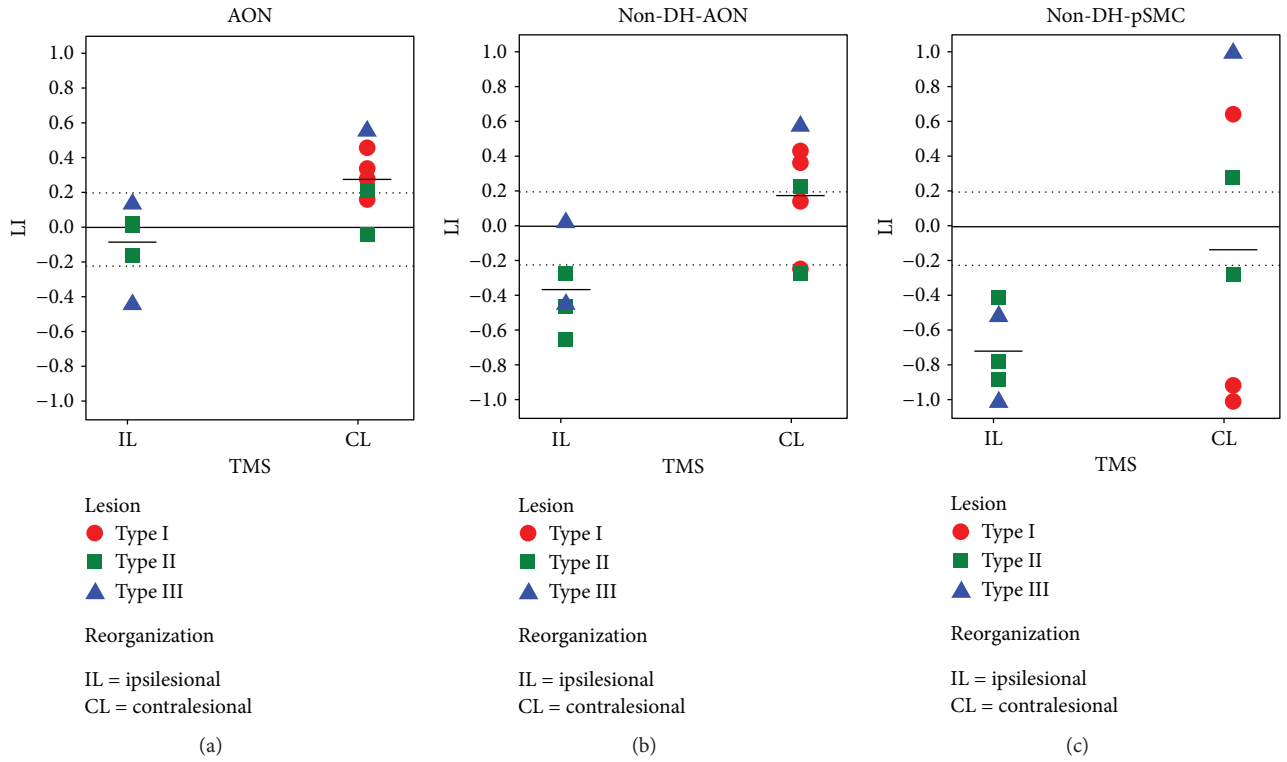

Figure 6: Box plots of LI values obtained by different contrasts (AON task: all TASK>BASELINE, panel a; AON task: (C-ND + S-ND) > BASELINE, panel b; and sensory-motor task: (Sens-ND + Mot-ND) > REST, panel c) in UCP children, grouped according to TMS data (CL = contralesional reorganization, IL = ipsilesional). As in Figure 5, children were represented with different colours and symbols with respect to the classification of their lesions (type I = red circles, type II = green squares, type III = blue triangles). Grey dotted lines represent the threshold value of |0.20| for hemispheric lateralization.

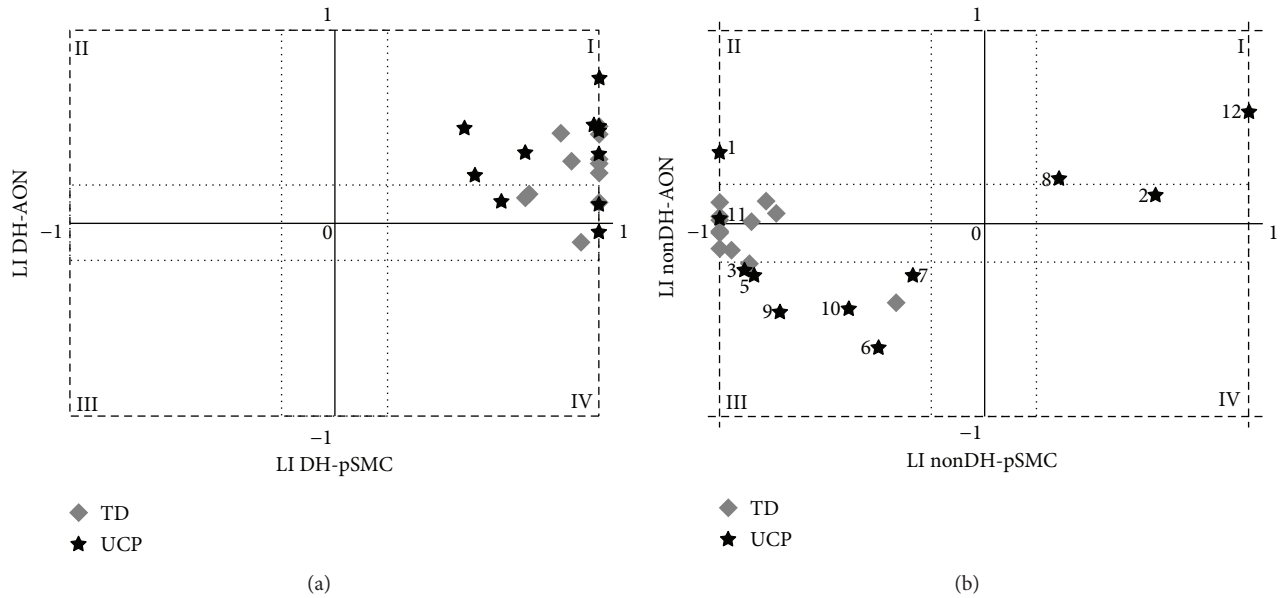

Figure 7: Four-quadrants charts of LI values of pSMC in the sensory motor task (abscissae) and of the AON (ordinates) of the two hands: dominant hand (DH) on panel a, nondominant hand (non-DH) on b. The first and third quadrants represent the congruence of the sign (both positive in the first, I; both negative in the third, III), while the second and the fourth represent the discordance of the sign (pSMC-negative and AON-positive in the second, II; pSMC-positive and AON-negative in the fourth, IV). TD children were represented by grey diamonds, UCP children by black stars. In the chart for the nondominant hand, labels on data of UCP children are used to identify subjects, according to Table 1.

Observation of simple and complex actions performed by the dominant hand ((C-D + S-D) > BASELINE) induced a slightly higher, but not significant, activation of contralateral hemisphere in UCP children ($LI_{UCP} = 0.35 \pm 0.22$) with respect to the TD group ($LI_{TD} = 0.27 \pm 0.17$) ($p = 0.35$, Figure 4(a)). LI mean values obtained from observation of actions performed by the nondominant hand ((C-ND + S-ND) > BASELINE) were very similar between the two groups ($LI_{UCP} = -0.04 \pm 0.40$; $LI_{TD} = -0.05 \pm 0.15$, $p = 0.98$) suggesting bilateral networks. However, in the UCP group there was a higher variability among children (LI_{UCP}range = $-0.64 \div 0.58$; LI_{TD}range = $-0.40 \div 0.12$), because single subjects presented very different lateralization indices. In particular, 4 subjects were lateralized to the hemisphere contralateral to the dominant hand, 6 subjects to the hemisphere contralateral to the nondominant hand, and only 2 subjects showed bilateral representation (Figure 4(b)). By comparing the two hands with an intragroup analysis, both groups presented significant differences in LI values between observation of the dominant and nondominant hand ($p = 0.02$ in the UCP group, $p = 4 \cdot 10^{-4}$ in TD).

For SMN, laterality indices of pSMC for stimulation of the dominant hand ((Sens-D + Mot-D) > REST) were similar between the two groups ($LI_{UCP} = 0.85 \pm 0.21$, $LI_{TD} = 0.93 \pm 0.11$, Figure 4(c)). On the contrary, there was a significant difference for values obtained with stimulation of the nondominant hand ((Sens-ND + Mot-ND) > REST; $LI_{UCP} = -0.35 \pm 0.7$; $LI_{TD} = -0.89 \pm 0.19$; $p = 0.02$; Figure 4(d)). Also for SMN, both groups presented significant differences in an intragroup analysis, when comparing LI values of both hands ($p = 0.001$ in UCP, $p < 1 \cdot 10^{-4}$ in TD).

3.4. Correlations between LI and Clinical Scores, Type of Lesion, and Reorganization in UCP Children. Due to great variability among subjects in the UCP group, a correlation analysis was performed between laterality indices and clinical scores, types of lesion, and type of reorganization.

For the AON task and all TASKS > BASELINE contrast, LIs of single UCP subjects were plotted against their respective values of clinical scales (Figure 5).

A negative correlation was found between LI and percentage MUUL and AHA scores. This finding is not significant, considering all subjects with three types of lesions in linear fit ($p = 0.06$ for MUUL; $p = 0.28$ for AHA). On the other hand, it is significant if only children with type I and type II lesions are included in the analysis ($p = 0.0007$ for MUUL; $p = 0.04$ for AHA). The same type of analysis was repeated considering only LI values obtained from observation of actions performed by the nondominant hand ((C-ND + S-ND) > BASELINE). Data showed similar trends, but statistical analysis was not significant ($p = 0.07$ for MUUL, $p = 0.08$ for AHA). No significant correlations were found in the same analysis using LI values of pSMC for sensorymotor stimulation of nondominant hand and clinical scales.

Figure 6 shows box plots of LI values of UCP children, grouped according to TMS results for different contrasts and different stimuli.

For all TASKS>BASELINE contrast of AON (Figure 6(a)), LI values of UCP children were significantly different when matched with the two types of reorganization revealed by TMS ($p = 0.023$, Mann–Whitney U test). In particular, children with IL reorganization at TMS (i.e., in the affected hemisphere) had bilateral AON activation ($LI_{IL} = -0.08 \pm 0.22$), except for one case (number 10), who presented a marked lateralization to the affected hemisphere. On the contrary, children with CL reorganization at TMS (i.e., in the unaffected hemisphere) presented either bilateral activation or higher activation in the hemisphere contralateral to the dominant hand (L $I_{CL} = 0.28 \pm 0.20$).

If the previous analysis is performed considering only observation of the nondominant hand (contrast: (C-ND + S-ND) > BASELINE, Figure 6(b)), both lateralization indices decrease accordingly (($LI_{IL} = -0.36 \pm 0.25$; $LI_{CL} = 0.18 \pm 0.33$) and their differences continue to be statistically significant ($p = 0.023$, Mann–Whitney U-test).

Regarding SMN of the nondominant hand (Figure 6(c)), UCP children with a different reorganization at TMS presented different trends of LI values of pSMC, even if globally their differences were not statistically significant ($p = 0.58$). Children with IL reorganization at TMS showed a greater activation of pSMC in the affected hemisphere ($LI_{IL} = -0.71 \pm 0.25$), in accordance to TMS. On the contrary, children with CL reorganization did not present a common behaviour, but rather a certain degree of variability ($LI_{CL} = -0.13 \pm 0.88$). Three cases (numbers 12, 2, and 8) showed lateralization of pSMC in the unaffected hemisphere (LI > 0), while the other three have it in the affected hemisphere (LI < 0): one (number 7) presented bilateral representation of pSMC, and two subjects (numbers 1 and 3) showed discordant reorganization with respect to TMS, showing an evident lateralization of pSMC to the affected hemisphere.

Similar results were found when this analysis was performed using the mirror movements data as discriminating factor (Figure in Supplementary Materials (available here)) .

3.5. Comparisons between AON and SMC in UCP and TD Children. In order to directly compare SMN and AON tasks, LI values were reported in a four-quadrants chart (SMN in *abscissae* and AON in *ordinates*), in which the first and third quadrants represent concordance of sign (both positive in the first, I, and both negative in the third, III), while the second and the fourth represent discordance of sign (one positive and one negative) (Figure 7).

In the case of stimuli performed by the dominant hand (Figure 7(a)), all subjects of both groups lie within the first or fourth quadrant, but very close to the zero axis, corresponding to a concordance between contralateral representation for pSMC and contralateral or bilateral representation for AON. Instead, scattered data were found in the case of stimuli performed by the nondominant hand (Figure 7(b)). All data of TD children are placed in the third, or second quadrant, but very close to the zero axis, meaning a contralateral representation for pSMC and contralateral or bilateral representation for AON, analogously to the dominant hand. For UCP children, the majority of data lies within the first and third quadrants, demonstrating a concordance in lateralization

of pSMC and AON, with both representations in the unaffected (first quadrant, numbers 2, 8, and 12) or in the affected hemisphere (third quadrant, numbers 3, 5, 6, 7, 9, and 10). Subject number 11 is in line with the TD group, with contralateral representation for pSMC and bilateral representation for AON, while subject number 1 is the only exception with a shifted reorganization in the contralesional hemisphere for AON (as at TMS), but with a higher activation of ipsilesional pSMC for the sensory-motor task.

4. Discussion

This study explores for the first time AON of goal-directed actions in UCP children and its relation to type and timing of lesion, sensory-motor reorganization, and clinical assessment.

The first very relevant finding is that AON in UCP children engages brain activations similar to healthy age-matched children despite the presence of brain damage. However, neural networks activated by UCP children present a higher lateralization of maps with a higher activation of the unaffected hemisphere with respect to bilateral representation in the TD group. In our previous study [5], by comparing AON of TD children with that of healthy adults, we demonstrated that lateralization of AON is age-dependent and that adults have a more lateralized activated network in the dominant hemisphere while healthy children have more bilateral and widespread AON. This early lateralization, as a consequence of reorganization due to brain damage, could be reflective of unknown mechanisms that in turn determine an exclusion of natural development through a bilateral activation phase, affecting functioning in UCP children. Our results seem to confirm this hypothesis, because higher AON lateralization was correlated with lower performances on both scales (MUUL and AHA), while UCP children with bilateral AON activation similar to that of the TD group have better performances, reaching higher MUUL and AHA values (Figure 5). This finding is in line with examples of maladaptive plasticity in the context of reorganization of the sensory-motor system where contralateral (ipsilesional) reorganization is more effective in restoring good motor function as opposed to ipsilateral (contralesional) reorganization which is associated with lower grasping and manipulation skills [6, 7].

Another possible explanation could be related to the crucial role of the mirror neuron system in early motor learning by facilitating associations between action perception and corresponding motor programs [27–29]. An altered functioning of imitation capabilities, associated with limited motor system functioning, in early infancy, could contribute to determining maladaptive plasticity.

Regarding properties of observed actions, we found a significant "hand identity" effect in AIP of the hemisphere contralateral to the nondominant hand, similar to TD children [5]. However, contrarily to age-matched controls, this area did not present a significant effect for the "complexity" factor. Lack of significance for "complexity" could be related to the fact that all observed actions may be viewed as complex for UCP children. Another possible explanation could be that AON of UCP children processes mainly for goals rather than kinematics. These explorative hypotheses need further investigation.

For the first time, this study assessed in the same group of subjects both AON and SMN by using fMRI and correlated results with TMS data. Regarding fMRI results, we found a congruence between activation of contralateral pSMC during execution of the sensory-motor task and bilateral or contralateral activation of AON.

Moreover, a good correspondence was also found between AON and TMS data. In particular, observation of the nondominant hand elicited a greater activation of the affected hemisphere in children with ipsilesional reorganization at TMS, while children with contralesional reorganization at TMS had generally either bilateral activation or higher activation in the unaffected hemisphere (Figure 6(b)). However, relevant discrepancies between fMRI results of SMN and reorganization, measured by TMS, were found in two children (numbers 1 and 3, with type I lesion, Figure 6(c)) who presented a higher activation of pSMC of the affected hemisphere despite a TMS reorganization shifted to the contralesional hemisphere. The lateralization index for SMN should be driven by prevalence of sensory contribution to the activity in pSMC of the ipsilesional hemisphere, as confirmed by an explorative post hoc analysis of fMRI data for the sensory-motor task of the nondominant hand, using different regressors for sensory and motor blocks. Considering also clinical performances of the two patients, this finding could be related to a different reorganization of the motor and sensory system and to possible sensory-motor dissociation [6].

Concerning relationships between lateralization index, type of lesion, and type of reorganization evaluated with TMS, all type I UCPs have an ipsilateral (contralesional) reorganization, while type II and III UCPs are variable with lower abilities in children with ipsilateral (contralesional) reorganization and higher abilities in those with contralateral (ipsilesional) reorganization (Table 1). This finding is in line with current literature. In addition, this study shows that type I presents lower clinical scores than the other types do and higher values of lateralization indices, and type II has higher clinical scores and LI values similar to the TD group (bilateral representation), while type III has very high variability. In particular, there are two subjects with similar intermediate MUUL values but opposite LIs, one being more lateralized to the contralateral hemisphere and the other to the ipsilateral one. These diverse findings are related to the different clinical features of these two cases. In the child (number 10) with preferred AON lateralization in the affected hemisphere ($LI < 0$), also SMN activation and reorganization at TMS were present in the affected hemisphere and her functional level at HFCS was very good. On the contrary, the other child (number 12), with a lower level at HFCS, had greater activations in the contralateral unaffected hemisphere for AON and pSMC, in accordance also to reorganization at TMS.

Another interesting result is that observation of the dominant hand induced a higher, but not significant, activation of

the contralateral hemisphere in UCP than the TD group (Figure 4(a)), suggesting a more lateralized circuit in UCP children. This is indirectly confirmed by observation of the nondominant (affected) hand which showed similar LIs between the two groups: although with bilateral activations, the UCP group showed higher variability among subjects, including single cases with high absolute LI values, indicating a specific lateralization in one of the two hemispheres (Figure 4(b)). This finding is in contrast with results of another study in which, for either side, observation of hand movements recruited the primary motor cortex contralateral to the viewed hand, while observation of the paretic side activated more strongly ipsilesional pSMC than viewing movement performed by the nonparetic side [17]. Moreover, in the same study, an engagement of AON was revealed regardless of degree of motor impairment assessed by a hand motor function Likert scale (1–4). These different findings could be related to a difference in the employed paradigm and in particular to non-goal-directed simple hand movements in the allocentric (third person) perspective of that study. Conversely, in the present study, simple and complex goal-directed movements were presented in the egocentric (first person) perspective. Concerning the issue of perspective from which action is observed, in monkeys it has been demonstrated that when comparing neural activation due to different points of view, such as first-person or third-person, first-person might be preferred [30]. An fMRI study in healthy adults showed that while the first-person perspective elicits activations in the hemisphere contralateral to the performing hand as if modelled action was mimicked with the same anatomical hand, in the third-person perspective, parietal activation ipsilateral to the modelled hand was found, indicating a specular strategy, rather than anatomical reproduction [31].

Moreover, the lack of correlation with degree of motor impairment in the study of [17] could be related to the narrower range of the Likert scale with respect to MUUL and AHA. The relationship between MUUL scale and type of sensory-motor reorganization has been previously reported [6], and both MUUL and AHA are highly correlated to lesion extension [32]. We have shown that not only the type of reorganization assessed with TMS but also the laterality index of AON (ALL TASKS > BASELINE) is related to MUUL and AHA scores if we consider type I and type II brain lesions (Figure 5). A similar result was found considering observation of actions performed by the nondominant hand ((C-ND + S-ND) > BASELINE); however, the lack of significance ($p = 0.07$) could be due to high variability of type III lesion (e.g., similar MUUL values with different types of reorganization and opposite laterality indices values).

As far as the relationship between SMN of each hand and laterality index is concerned, we have shown that in the TD group, beyond dominance, there is activation of pSMC of the contralateral hemisphere, reaching values of complete lateralization. In the UCP group, the dominant/unaffected hand induces similar activations while the nondominant/affected hand induces very variable activation with values varying from activation of the contralateral (lesioned) hemisphere to prevalent activation of the ipsilateral, unaffected hemisphere (Figure 4(d)). Another important finding is the lack of relationship between LI values of the nondominant hand and clinical scales, in contrast to the previous interesting relationship of clinical scales with LI of AON. This finding is in accordance with previous studies [33], and it could be related to different reorganization patterns among subjects that determine a huge variability of data with widespread values. All enrolled subjects did not undergo any intensive treatment for the upper limb, and from literature it seems that intensive treatments induce higher LI values with more lateralization in the affected hemisphere [34]. Another possible explanation could be related to the possibility of associated movements (e.g., mirror movements) that can alter SMN data. The solution of excluding UCP children with mirror movements from fMRI studies of SMN is not practicable since it would limit applicability to a small number of subjects. In our sample, the mirror movements, even if assessed with a nonstandardized method [35, 36], were present in 8 out of 12 UCP children. Further studies in UCP children with quantitative and standardized assessment of MM could shed light on the role of MM in SMN reorganization and clinical outcome.

Moreover, the sensorimotor tasks, especially for the affected hand, are often challenging for UCP children, and their execution can generate and be accompanied by an excessive head motion during fMRI sessions. In this study, we paid careful attention to the analysis and compensation of motion and we succeeded in obtaining fMRI data from all subjects for AON and from 11 out of 12 subjects for SMN. Taking into account these issues and considering the good concordance of lateralization indexes for pSMC and AON (Figure 7), the paradigm for exploring AON seems more reliable for studying the motor system in all UCP children, due to its greater feasibility. In fact, for AON tasks, children must only observe actions without doing any physical movement. Plasticity of the AON system with respect to the sensorimotor system still requires greater investigation.

5. Conclusion

This fMRI study explores, for the first time, AON of goal-directed actions and SMN in UCP children and their relation to type and timing of lesion, sensory-motor reorganization (TMS), and clinical assessment.

A good congruence was found between bilateral or contralateral activation of AON and SMN activation, TMS data, and clinical scores, suggesting that our paradigm might be useful in exploring AON and adaptive mechanisms or maladaptive plasticity. All these results, based on a small and variable group of UCP children, are necessarily exploratory and need to be extended to and confirmed by other studies. However, collectively, they indicate that, despite congenital and large brain lesions, AON is very active in these children, although with some characteristic differences when compared to TD children. These findings support clinical trials that have been carried out and are in line with numerous ones in progress using action observation therapy (AOT) as a tool to improve manual function, also in chronic phases of

these children. Our explorative attempts to correlate manual proficiencies as shown by clinical scales and fMRI, TMS, and other findings may indicate ways to explain and predict efficacy of rehabilitation in UCP children. The fMRI paradigm could also be particularly suitable to investigate effects of plasticity induced by this specific rehabilitation program [37–39].

Data Availability

All the data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have not conflict of interests.

Authors' Contributions

Giuseppina Sgandurra and Laura Biagi contributed equally to this work.

Acknowledgments

The authors would like to thank the children and parents who participated in this study and Vincent Corsentino for reviewing the English language of the manuscript. The authors would also like to thank Elena Beani for scoring the mirror movements. This trial has been partially funded by the Italian Ministry of Health to Giuseppina Sgandurra (GR-2011-02350053).

Supplementary Materials

Figure S1: box plots of LI values obtained by different contrasts in UCP children, grouped according to the absence or presence of mirror movements. *(Supplementary Materials)*

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