Brain stimulation as a therapeutic tool for Cerebral Palsy: a multimodal study

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Acknowledgements

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Personal

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Abstract

Cerebral palsy (CP) is a heterogeneous condition, defined by shared clinical features: motor impairment due to an insult to the developing brain. As the most common cause of motor disability in childhood, clinically relevant and time-efficient interventions to improve motor function in CP are urgently needed. Transcranial direct current stimulation (tDCS) has been proposed as a therapeutic tool to augment neuroplasticity and improve function. However, tDCS study outcomes are discordant, with significant inter-individual variation. This thesis investigates the utility of this technique in CP, and explores potential contributing factors to variability.

Chapter 2 is an opportunistic study conducted during the COVID-19 lockdown. It demonstrated the negative effect of interrupting rehabilitation on motor function in CP and highlighted the need for improved rehabilitation techniques.

Chapter 3 details a pilot clinical trial of motor training and tDCS, versus training alone. While both groups showed improvement in motor function, there was no additional group-level effect of anodal primary motor cortex tDCS on outcome.

Further chapters assessed pre-intervention neuroimaging data to explain variation in response to the trial intervention, with the aim of identifying possible biomarkers of rehabilitation potential with tDCS. Chapter 4 applies a novel automated individualised probabilistic tractography technique to quantify corticospinal tract (CST) integrity. This technique is feasible and robust to structural lesions in the study population. At baseline, CST integrity correlated to hand function but not lower limb function or eventual rehabilitation outcome.

Finally, Chapter 5 interrogates the effect of electric field strength as a potential explanation for tDCS variability. Substantial variation in electric field focality and strength was demonstrated across participants. This may contribute to inter-individual variability but did not predict individual motor outcome.

This work demonstrates the positive potential of motor training in improving function in CP. There may be a role for advanced neuroimaging and computational models to introspect rehabilitation outcomes in complex heterogenous conditions such as CP in future studies to better model the effect of tDCS across the clinical and pathologic spectrum of CP.

Approximate thesis word count: 29 344

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1 Introduction

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1.1 Cerebral palsy - a clinical syndrome

The broad condition of cerebral palsy (CP) was first described and studied by Sigmund Freud, William Little and William Osler in the late 19th century[1,2,3].

Sigmund Freud was likely the first to write about cerebral palsy as a clinical entity, unifying several infantile motor deficits into one syndrome[3]. William Little, several years previously, demonstrated a relationship between birth complications and mental and physical developmental disorders. He suggested that injuries to the brain could occur during or immediately after birth, and described a clinical picture of spastic rigidity, which became one of the 4 main clinical forms of CP. Although Little never described these under the term cerebral palsy, the term "Little's disease" was used for many years as a generic term to refer to all types of cerebral palsy[3,4]. William Osler published articles in 1886 and 1888 regarding cerebral palsy, before publishing his monograph, "The Cerebral Palsies of Children", in which he described a case series of 151 children, classifying them as infantile hemiplegia, bilateral spastic hemiplegia, or spastic paraplegia[3].

1.1.1 Defining a clinical entity

Various historical descriptions and classifications of cerebral palsy have persisted to the present day, which continues to demonstrate heterogeneity in aetiology, severity, and clinical features. Since Sigmund Freud, William Little, and William Osler, several groups have come together to attempt to better define the condition.

The first unified definition was presented at the First International Study Group on Child Neurology and Cerebral Palsy, held in Oxford in 1958. The memorandum proposed that cerebral palsy be defined as: "a persisting qualitative motor disorder appearing before the age of three years, due to a non-progressive interference with development of the brain"[5]. They highlighted the practical value of a unifying diagnosis of cerebral palsy. The term could be "useful in the study of a group of conditions often of obscure origin affecting the brain and arising in early life... and a practical value largely because the affected children .. have certain special motor, intellectual and emotional difficulties and so may have certain special therapeutic and educational needs in common"[5].

Various definitions have been proposed in the decades since the First International Study Group on Child Neurology and Cerebral Palsy. In 2005, the most recent

consensus definition was proposed as follows: "Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing foetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication and behaviour, by epilepsy and by secondary musculoskeletal problems"[6].

However, the new definition, despite being welcomed by many, has been criticised for perceived ongoing ambiguity. Critics have felt that the use of "non-progressive" is not clearly defined, and that the definition fails to apply an age limit or suggest if any syndromes should be excluded[7]. Proponents, however, have argued that "non-progressive" adds sufficient meaning to the definition, as static does not imply 'unchanging', allowing for the evolution of the injury to intersect with development, which may result in changing symptoms over the lifespan[8]. There are strengths of a wide unifying diagnosis - including flexibility for the definition to continue to evolve over time and ensuring that clinical diagnosis is not hindered by burdensome criteria. The wide scope of the definition of cerebral palsy remains an important research limitation, however, resulting in differing case definitions across studies, adding complexity in comparisons across cohorts and across time, and potentially missing out on targeted therapies for subsets of patients.

1.1.2 Epidemiology of Cerebral Palsy

Globally, cerebral palsy is the most common cause of motor disability in childhood and a significant contributor to morbidity and mortality overall[9].

Population registers in Australia and Europe have historically reported a prevalence of between 1.5 – 2.5 cases per 1000 live births[10,11]. A recent systematic analysis evaluating the global birth prevalence of cerebral palsy across 27 countries reported an overall decline to 1.5 cases per 1000 children in Europe and Australia[11]. This study population included children with CP born from 1995 onwards and differentiated between pre- / perinatal cerebral palsy, in which a brain injury or malformation occurred before the 28th day of life, and postnatal CP, in which the

injury happened between day 28 and age 2 years. In developed countries, the data suggests that pre- and peri-natal CP case prevalence is declining, while post-natal CP prevalence has remained static over the preceding 2 decades. From very limited data in low to middle-income countries, the prevalence ranged from 2.3 to 3.7 per 1000 children, and unfortunately, there was not enough data was available to model the trends in prevalence over time for these regions[11].

The United Kingdom does not have a population-based registry for cerebral palsy, therefore data on prevalence is based on cohort studies across the country, as well as statistical modelling. Reported birth prevalence in the UK is estimated between 1.2 to 2.7 per 1000 for pre-/perinatal CP[12,11], and 0.8 to 3.9 per 1000 for postnatal cerebral palsy[11]. Variation in reported prevalence is likely partly explained by different inclusion criteria across studies, as well as differences in socio-economic status across regions[13].

Mortality rates in children with cerebral palsy are higher than in children without CP, with severe impairments linked to higher mortality. Modelling estimates from historical data in the UK estimate that survival to the age of 16 years ranges from 64-67% for those with the most severe manifestations of CP to between 97-100% for those with non-severe types[14,15]. Despite a declining overall prevalence, there are more children in the UK living with cerebral palsy now than there were a decade ago, attributed to improved neonatal intensive care provisions, treatments for complications, and better socio-economic provisions. The estimated number of children aged 3 to 15 years with CP was approximately 22,100 in 2020, which is a 7.5% increase since 2013[16]. Data from life expectancy models is particularly important to demonstrate the growing need for the provision of services for children with cerebral palsy, including access to rehabilitation across the lifespan. In Chapter 2, the importance of the ongoing provision of rehabilitation services and the impact of interruptions will be further explored.

1.1.3 Pathophysiology

William Little is attributed as the first to describe a causal link between birth trauma and motor and intellectual disability. In a presentation to the Obstetrical Society of London in 1861, Little presented a case series of more than 200 children who had suffered neurological complications as a result of prematurity, prolonged labour, and asphyxia[1]. The understanding of the pathways leading to cerebral palsy has since greatly increased, with evidence from population-based studies, neuroimaging data, improved perinatal care, post-mortem studies, and animal models of disease. However, a unified explanation of the large phenotypic variations in patients with cerebral palsy remains elusive. In this section, the pathological processes that are known to be related to cerebral palsy will be briefly discussed.

Prematurity and perinatal hypoxia: traditional risk factors

Perinatal hypoxia and prematurity have been proposed as two primary mechanisms of injury since William Little first demonstrated a relationship between birth trauma and developmental abnormalities. The risk of developing cerebral palsy decreases with each gestational week approaching 38 weeks, and the risk of CP for an infant born before 28 weeks is 50 times greater than the risk of an infant carried to full term[17]. In full-term infants a low Apgar score at birth, indicating birth depression (such as not breathing or moving as expected), increases their risk of developing CP[18,19]. However, this may be a symptom of an underlying issue rather than directly causative, as less than 10% of children with cerebral palsy have a history of birth asphyxia or trauma[19,20].

Further exploration of risk factors

A large meta-analysis, published in 2013, included 21 studies and identified 10 risk factors in term-born infants that were statistically significant predictors of cerebral palsy. These were: placental abnormalities, birth defects, low birthweight, meconium aspiration, instrumental/emergency Caesarean delivery, birth asphyxia, neonatal seizures, respiratory distress syndrome, hypoglycaemia, and neonatal

infection[21]. Proving causality remains an ongoing challenge with these risk factors, and many of these may be symptoms of an unknown underlying cause rather than causative in themselves.

Other major risk factors include multiple births[22,23,24], intra-uterine growth restriction[25], maternal infections such as toxoplasmosis, rubella, cytomegalovirus and herpes simplex virus[26,27,28], and developmental anomalies[29,30]. Perinatal stroke and kernicterus as a result of neonatal jaundice remain the two largest causes of cerebral palsy in the postnatal period[31,32,33].

A combination of insults: The multi-hit model

Given the large spectrum of risk factors in the perinatal period, most of which are necessary but not sufficient, two-hit and multi-hit models have been proposed that suggest two or more risk factors converge to synergistically increase the risk of cerebral palsy[34,35,36,37]. This model suggests that the combination of a hostile intrauterine environment (such as placental insuffiency, pre-eclampsia, intra-uterine infection) with neonatal complications (such as birth asphyxia), especially among premature neonates, leads to neurologic damage and the development of CP[34]. In a retrospective study of over 200 000 pregnancies, pre-eclampsia combined with neonatal infection, birth asphyxia and complications of prematurity were cumulatively associated with an increased risk of CP[35]. Wang et. al. identified that the combination of hypoxic risk factors such as birth cardiopulmonary resuscitation, patent ductus arteriosus ligation or chronic lung disease; and the addition of sepsis led to a cumulative increased risk of cerebral palsy[37].

Downstream from these primary insults, a pathophysiological mechanism that has been proposed as a “final common pathway” in cerebral palsy is maternal and neonatal inflammation. Inflammation, whether triggered by infection and/or hypoxia, is common to the majority of risk factors, and the release of pro-inflammatory cytokines has been shown to damage of neurons, disrupt vascular endothelial cells and promote microglial activation and demyelination[38,39,40].

In summary, cerebral palsy likely results from combined primary insults, underlying vulnerability, and the response/subsequent development of neural tissues. Not all insults are equal, and their impact is likely determined by the brain's underlying vulnerability, the timing of the insult, and the presence of other risk factors.

1.1.4 The spectrum of brain lesions

In parallel with the variety of CP mechanisms and risk factors, there are various possible brain imaging findings. The timing of the initial injury to the developing brain will determine the extent and location of the injury seen on imaging.

Most individuals with CP (approximately 88%) who have MR imaging will have an identifiable brain lesion[41]. However not all children with CP will have neuroimaging. The diagnosis of cerebral palsy is made on clinical findings, and imaging, such as ultrasound or MRI, is generally used to exclude other diagnoses or to assist with understanding the aetiology of CP when clinically unclear[42]. In the UK, the National Institute for Health and Care (NICE) guidelines recommend the use of MRI when the aetiology of CP is not clear from clinical history, developmental milestones, clinical examination and the results of cranial ultrasound; and highlight that subtle findings may not be visible until at least 2 years of age[43].

As a result, it can be challenging to estimate the true population spectrum and prevalence of brain lesions in children with CP. Results from observational studies may be skewed towards including cases with diagnostic uncertainty, or populations with easier access to neuroimaging.

In large European cohort studies of children with CP[41], the approximate distribution of MRI-identified brain lesions are as follows:

- white matter damage: 45%
- basal ganglia or deep grey matter damage: 13%
- congenital malformation: 10%
- focal infarcts: 7%

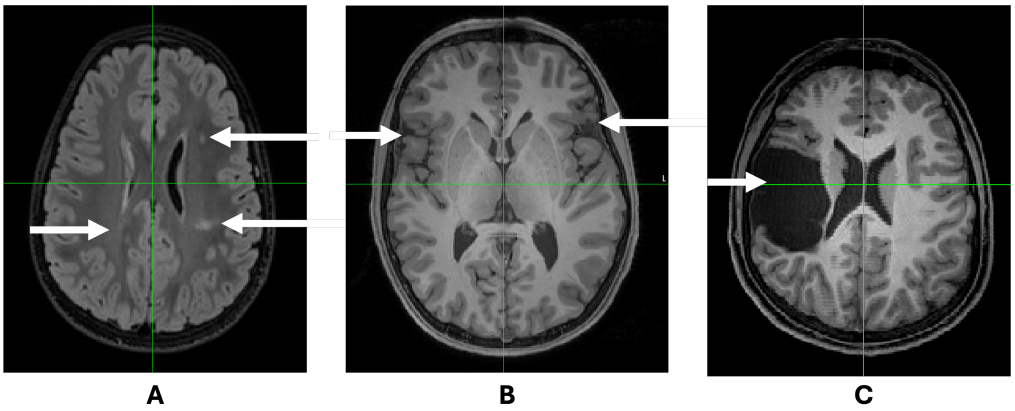


Figure 1.1: Examples of MRI patterns of injury in children with cerebral palsy. A: Predominant white matter injury; B: Maldevelopment with polymicrogyria bilaterally; C: Stroke related injury - a right-sided cystic lesion corresponding with a previous middle cerebral artery stroke, which has evolved to be a porencephalic cyst

The three most common types of brain lesions in cerebral palsy are periventricular leukomalacia, which mainly affects preterm infants; grey matter injury most commonly seen in term infants with hypoxia, and cerebrovascular injuries which may occur perinatally or postnatally.

Periventricular leukomalacia

Periventricular leukomalacia (PVL) is the most common cause of CP in premature infants[44].

The pathogenesis of PVL is closely entwined with neurodevelopment in utero, and the interplay of three important factors: 1) Incomplete development of the vascular supply to white matter; 2) Maturation-dependent impairment of cerebral autoregulation, and 3) Vulnerability of oligodendrocyte-precursor cells to ischaemia from inadequate blood supply; which results in vulnerability of the white matter to ischaemic insults if the third trimester is interrupted by preterm delivery[44,45]. In these infants, even small derangements in systemic blood pressure due to sepsis or other causes can greatly increase their risk of ischaemia to the forebrain[46].

Grey matter injury

In approximately 13% of children with cerebral palsy who undergo MRI, bilateral injury to the deep grey matter structures, cortex and subcortex, is demonstrated[47].

This pattern of injury is most associated with hypoxic-ischaemic events in term infants; but may be seen in premature infants exposed to an additional insult, such as infection, kernicterus, or hypoglycaemia[47].

Cerebrovascular insults

Focal infarcts and stroke may occur perinatally, between late gestation and 28 days after birth, or postnatally up to the age of approximately two years[48]. Perinatal stroke is the most common cause of hemiparetic cerebral palsy, but it is important to highlight that stroke only accounts for 7% of CP cases. Identifying the cause of stroke is difficult in most cases, however, causes for arterial strokes include cardiac disease and bacterial meningitis; causes for venous strokes include sepsis, meningitis, dehydration, and perinatal asphyxia[49]. Structural lesions or bleeding disorders may cause haemorrhagic strokes occurring in term-born children, while preterm children with at-risk brains experience germinal matrix and intraventricular haemorrhage.

1.1.5 Classification of cerebral palsy

The difficulty in consensus definition (outlined in section 1.1.1), poorly understood causation (section 1.1.3) and variety of brain pathology (section 1.1.4) all point to cerebral palsy being a hugely heterogenous disorder. It encompasses a range of clinical phenotypes and motor impairments ranging from mild to severe. Traditionally, CP has been classified across several domains:

Firstly, classification by motor phenotype into four main categories:

a) Spastic, the most common form, characterised by hypertonia

b) Ataxic, characterised by co-ordination difficulties

c) Dyskinetic, involving abnormal muscle contraction causing involuntary movements (athetosis and or dystonia)

d) Hypotonic, with decreased muscle tone

These motor impairments may evolve over time, and are not mutually exclusive, such that multiple types can be present simultaneously.

Secondly, classification typically includes a description of the topography of the impairment, either unilateral or bilateral, and then further subclassified as follows[50]:

a) Hemiplegia, where only one side of the body is involved, and both arm and leg of that side are affected

b) Monoplegia, where one limb is affected, usually a lower limb

c) Diplegia, where both lower limbs are involved more than upper limbs

d) Triplegia, involving both lower limbs and one upper limb

e) Quadriplegia, involving both upper and lower limbs

This classification is illustrated in Figure 1.2.

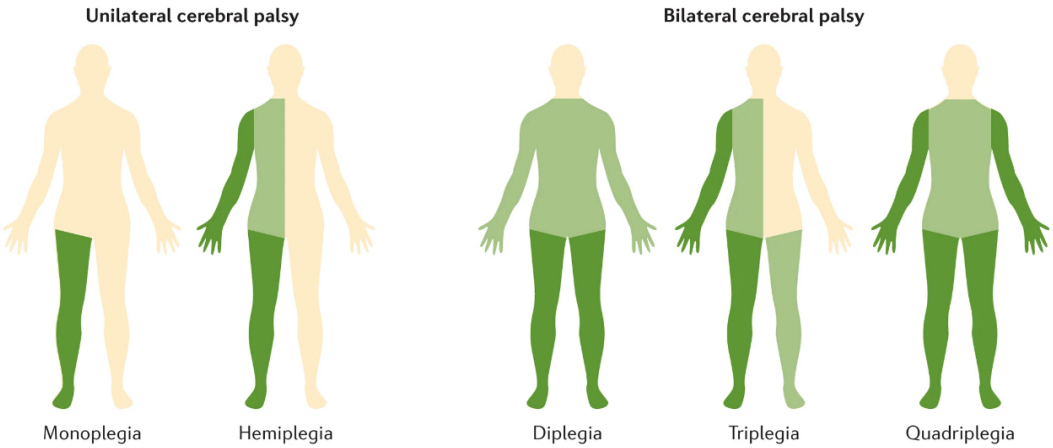


Figure 1.2: Unilateral cerebral palsy encompasses monoplegia, affecting one limb and typically a lower limb, and hemiplegia affecting one side of the body. Bilateral CP encompasses: diplegia which affects all limbs but lower limbs are more affected; triplegia with unilateral upper limb involvement and asymmetric lower limb involvement; and quadriplegia in which all four limbs are more equally involved than diplegia. *Reproduced with permission from Graham, H. K. et al. (2015) Cerebral palsy. Nat. Rev. Dis. Primers. doi:10.1038/nrdp.2015.82*

While prevalence varies between countries, the most common subtype of cerebral palsy is typically bilateral spastic CP, which includes diplegia, triplegia, and quadriplegia, accounting for approximately 50% of cases[51]. Approximately one-quarter of children with CP have unilateral hemiplegic spastic CP[51]. Approximately two-thirds of children with CP have motor deficits in more than one limb[52].

Lastly, functional status can also be employed to classify CP. The Gross Motor Function Classification System[53] classifies motor function according to ease of mobilisation, and requirement for mobility aids such as walkers and wheelchairs, illustrated in Figure 1.3.

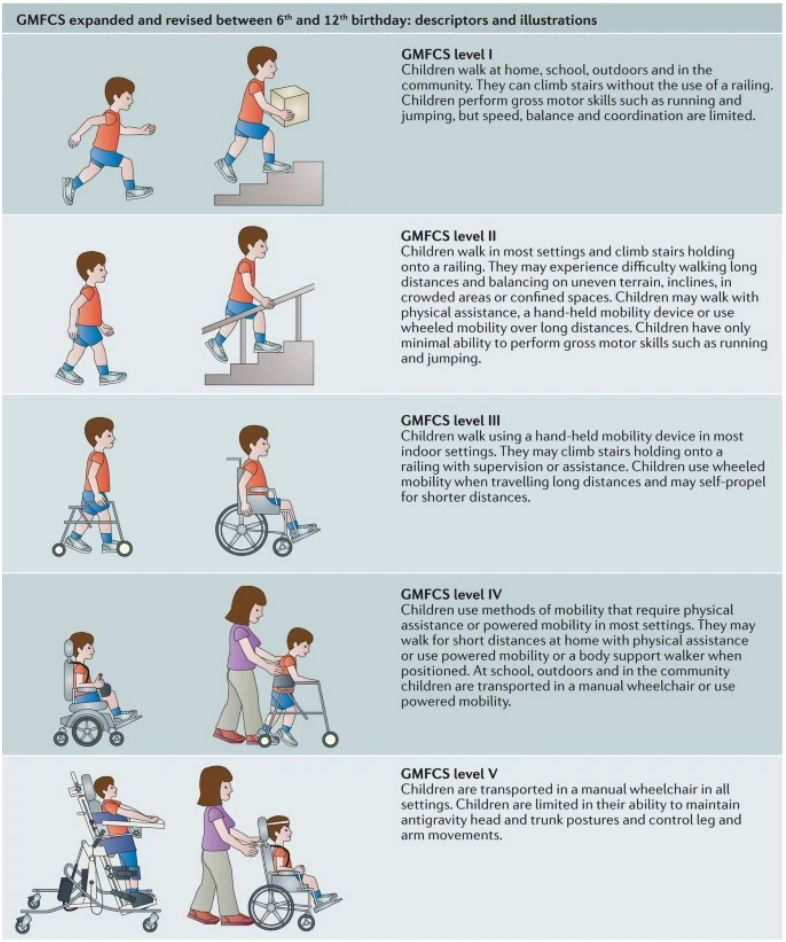


Figure 1.3: The Gross Motor Function Classification System (GMFCS) is recognised as the gold standard to classify motor function cerebral palsy. It is an ordinal classification with different descriptors appropriate to the age of the child. The descriptors illustrated are for children aged 6–12 years. The descriptors were devised by Palisano et al (1997). *Reproduced with permission from Graham, H. K. et al. (2015) Cerebral palsy. Nat. Rev. Dis. Primers. doi:10.1038/nrdp.2015.82*

The Manual Ability Classification Score describes how well children with CP are able to use their hands to handle objects of daily activity, ranging from 1 – able to handle objects easily and at most, limited in ease of performing tasks requiring speed and accuracy, to 5 – Not able to handle objects and severely limited ability to perform simple actions[54].

The MACS is similarly an ordinal scale and is rated from 1 to 5 as follows:

1. **Handles objects easily and successfully.** At most, limitations occur in activities requiring speed and accuracy, but limitations do not restrict independence

2. **Handles most objects but with reduced quality and/or speed.** Certain activities may be avoided or achieved with difficulty, but this does not usually restrict independence in activities of daily living (ADL).

3. **Handles objects with difficulty and needs help to prepare and/or modify activities.** Performance is slow and achieved with limited success. ADL's can be performed if they have been adapted.

4. **Handles a limited selection of easily managed objects in adapted situations.** Activities are performed with effort, require continuous support, and are achieved with limited success.

5. **Does not handle objects and has severely limited ability to perform even simple actions.**

1.1.6 Clinical Trajectory

Cerebral palsy is characterised by motor impairment and a delay in achieving gross motor milestones, related to abnormal muscle tone, weakness, and/or poor muscle co-ordination[55]. As a result, children with CP have limitations in performing activities of daily living (ADL), which include washing, toileting, feeding, and mobilising. These activities have been shown to be important aspects of quality of

life in this population, and achieving independence in personal care and mobility are highly rated goals for children with CP[56,57].

Children with cerebral palsy have slower trajectories of motor skill acquisition, and more difficulty in performing activities of daily living (ADLs), than typically developing children[58]. These trajectories correspond with GMFCS, such that a child with a lower GMFCS category will acquire more motor skills, and at a faster rate, than a child with a higher GMFCS score (i.e. more severe)[59]. However, despite an initial upward trajectory in motor skill function in childhood, children with CP experience an overall decline in function and mobility from adolescence into adulthood and onwards[60,61,62]. This is linked to reduced overall function in activities and lower participation in work and sport[63,61].

Participation in therapy and physical exercise is therefore particularly important for adolescents with cerebral palsy heading into adulthood, as it helps to prevent the cycle of physical impairment leading to deconditioning that, in turn, worsens the level of disability[64]. Regular stretching and loading of muscles is vital to maintaining muscle length and strength, decrease spasticity, and prevent contractures[65]. Ongoing physical training and therapy across the lifespan of children and adolescents with CP has shown to be of benefit in motor function and quality of life[66]. This evidences the critical contribution of ongoing physical training and therapy motor functioning.

1.1.7 Current therapies in Cerebral Palsy

While there is promising work ongoing regarding prevention of cerebral palsy, there remains no cure. Symptomatic management is therefore focused on maximising developmental potential and minimising complications[67]. By definition, children with cerebral palsy have motor impairments, and most treatments aim to address these.

There is substantial evidence, including good quality clinical trials, to support the use of several training-based interventions to improve motor function[68]. Two notable examples training-based interventions include constraint-induced movement therapy (CIMT) and hand–arm bimanual intensive training (HABIT). CIMT was first designed as a stroke rehabilitation therapy for adults[69], before adaptation for children

with unilateral spastic CP. It consists of intensive, unimanual practice with the more impaired upper extremity, while the less impaired upper extremity is restrained[70]. Several randomised controlled trials and reviews have found it to be effective at improving the hand function of the affected hand for children with hemiplegia[71,72,73].

Intensive bimanual training (such as HABIT) was developed as an alternative to CIMT as it was increasingly recognised that children's functional independence was related to the use of both arms in a coordinated way[74]. It has similarly been shown to improve hand function of the affected side in several randomised control trials and systematic analyses[75,76,77,78]. This training was further expanded with the introduction of Hand-Arm Bimanual Intensive Therapy Including Lower Extremities (HABIT-ILE), which includes activities which require trunk and lower extremity adaptations with upper extremity bimanual tasks[79].

Novak et al (2010) conducted a recent meta-analysis of current therapies for cerebral palsy and analysed the evidence for 63 different motor interventions[68]. They demonstrated that interventions which improve function and performance of tasks have the following shared attributes: they involve the practice of real-life tasks, using self-generated movements, at a high intensity and are goal-directed by the participant[68].

These findings are in keeping with the general principles of motor learning after brain injury, which include: repetition, specificity, increasing difficulty, intensity, time, and salience[80,81,82,83]. The proposed neuro-biological mechanism of action underlying these principles is experience-dependent plasticity[80,84].

Looking to the future of therapies

Reid et al (2015) have hypothesised that further development of clinically beneficial therapies will likely be driven by two main areas of research: Firstly, a better understanding of patterns of atypical brain development. Structural abnormalities on imaging reflect both damage to a focal brain region, as well downstream influences on processes such as neuronal migration, dendritic branching, and synaptic pruning during vital periods of brain development[85]. The effect of this early insult on

the development of interconnected brain networks and regions remains poorly understood, and is likely highly variable depending on insult timing and location[86]. These insults lead to impaired and adaptive behaviours, which differ from adult-onset insults, such as strokes, which lead to compensatory behaviours[85].

Secondly, characterisation of how and which brain networks respond to therapy may be necessary for clinical translation - including understanding the underlying conditions that facilitate response to treatment. These may include indirect factors such as genetic differences, co-existing conditions such as attention deficits, or individual network properties which prevent neuroplastic changes[86,87,88].

1.2 Defining neuroplasticity in health and disease

Neuroplasticity is broadly defined as the ability of the nervous system to reorganise its structure and/or function in response to extrinsic or intrinsic stimuli[89]. This reorganisation has been described across molecular, cellular, and behavioural levels.

Within this overarching theme of 'neuroplasticity', here we will consider two common usages relevant to this work: firstly, as part of the normal development of the nervous system, and secondly to describe activity-dependent changes in synaptic connections.

1.2.1 Developmental neuroplasticity

It is increasingly understood that the brain is continuously plastic and brain remodelling can occur, or be induced, throughout the lifespan[87,90]. However, there is also a recognition that the developing nervous system has a unique ability to undergo certain plastic changes[89]. Developmental neuroplasticity, therefore, refers to the complex interplay between neurogenesis, neuronal cell migration, synapse formation, synaptic pruning, and neuronal network formation, which all contribute to the brain's ability to acquire and refine motor and non-motor behaviours and adapt to a changing environment during development[91]. Aspects of this neuroplasticity are genetically pre-programmed and are time-sensitive during development. For example, neurogenesis is most prominent during early fetal

development, while synaptogenesis occurs mainly during the first two years of life[92]. Synaptic pruning can occur until mid-adolescence in some cortical areas[91], and processes of synapse proliferation and pruning seem to be influenced by both intrinsic control as well as environmental factors[92].

1.2.2 Hebbian Plasticity

One mechanism of neuroplasticity is Hebbian Plasticity. Hebbian Plasticity spans across the lifespan and has been described in both health and disease states. Hebb's postulate, which asserts that repeated synchronous activity in pre- and postsynaptic neurons leads to strengthened synaptic connection[93], has remained a core principle of neuroplasticity and been shown to be physiologically credible over the last century[94,95,96] The discovery of Long Term Potentiation (LTP) established a mechanism underlying the theory by which synaptic strength could be increased, facilitating plasticity[97].

Experimental LTP can be divided into an induction and expression phase. The induction phase consists of a brief event, such as a train of stimulation to a neuronal circuit. The expression phase is the subsequent time period in which specific and durable changes in synaptic transmission occur[98].

Originally, LTP was described in hippocampal neurons, and referred to a specific process thought to underlie memory formation[99,100,101]. Since this discovery, synaptic plasticity driven by LTP has been demonstrated in the neocortex, including M1, where it is referred to as LTP-like plasticity[102,103,104,105]. It is likely that the physiological effects of non-invasive brain stimulation are related to LTP-like plasticity (Section 1.3).

1.2.3 Applications to cerebral palsy

Using the development of the corticospinal tract as an example, we can illustrate how the timing of the insult affects the outcome and clinical presentation in children with cerebral palsy. Motor cortex development is driven by axon guidance molecules that are genetically programmed and then refined by activity-dependent plasticity during

pre- and perinatal life[106]. During development, the motor cortex 'wires' projections bilaterally to spinal motor neurons, before ipsilateral projections are retracted and contralateral projections are strengthened[107]. If an early, unilateral lesion occurs during development before competitive elimination of the ipsilateral corticospinal tract, ipsilateral projections from the intact motor cortex may out-compete fibres from the damaged contralateral corticospinal tract. This results in control of bilateral limb movements from the healthy hemisphere, and is demonstrated clinically by 'mirror movements': involuntary ipsilateral movements when trying to move the contralateral limb[108]. These mirror movements often impair function of the paretic limb, and are considered maladaptive. However, if the injury occurs after the elimination of ipsilateral fibres, reorganisation does not take place, and the result is a hemiparetic limb[109].

Increasingly, non-invasive methods such as magnetic resonance imaging (MRI), magnetoencephalopgraphy (MEG) and transcranial magnetic stimulation (TMS) are being used to probe structural and functional plasticity in cerebral palsy[91,110,108]. However, these studies are as yet only able to examine downstream effects on white matter microstructure and aberrant connectivity - the impact of early brain injury on LTP-like processes remains poorly understood.

1.3 Augmenting neuroplasticity with transcranial direct current stimulation

Non-invasive brain stimulation techniques have become increasingly popular in the last 3 decades for both observational and interventional studies of neurophysiology[111]. The two most commonly used techniques are transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS). While TMS uses focal magnetic fields to induce an electric field just under the coil, tDCS modulates cortical excitability more diffusely between two electrodes in a polarity-specific manner[111]. Additionally, tDCS offers several advantages over TMS, including benefits in safety/tolerability, affordability, and accessibility[112].

In tDCS, a constant low-intensity current is applied to the scalp, typically through two electrodes: an anode and a cathode. As the stimulation begins, a change in the membrane potential of neurons leads to depolarization or hyperpolarization of neurons at the target regions. Anodal tDCS depolarizes target neurons, whereas cathodal tDCS hyperpolarizes target neurons[113,114,115]. Early work demonstrated that the downstream behavioural effect of M1 stimulation, measured by motor-evoked potentials (MEPs), was polarity specific: anodal tDCS augmented MEP size in the contralateral hand muscle, while cathodal tDCS reduced MEP size[113]. Anodal stimulation is, therefore, typically seen as facilitatory and cathodal stimulation as inhibitory.

However, this dichotomy is increasingly understood to be dynamic and dependent on several factors including current intensity[116]. Several studies have shown that increasing the tDCS intensity, or 'dose' can 'flip' the effect, such that cathodal stimulation becomes excitatory at higher intensities[117,118]. Understanding the effective dose experienced by those undergoing tDCS may therefore aid in understanding individual differences in tDCS response.

1.3.1 Physiology and mechanism of action

Unlike other forms of non-invasive brain stimulation, tDCS cannot induce action potentials[119], but rather modulates spontaneous neuronal network activity by the polarity-dependent shift of resting membrane potential[120]. These changes have been shown to persist after the removal of the stimulus[121].

While the exact mechanism of action is not fully understood, changes in cortical excitability, inhibition, and plasticity underpin current explanations for the effects observed with tDCS[113,122,72]. Pharmacological and spectroscopy studies have demonstrated that tDCS modulates multiple neurotransmitter systems, including inhibitory γ-Aminobutyric acid (GABA) and facilitatory glutamate systems[122,123,124,125]. It has also been presumed that tDCS strengthens synaptic connections[126,127,128] through a mechanism similar to long-term potentiation (LTP), a cellular correlate of learning

and memory[129,130]. The ongoing effects are protein synthesis-dependent[131,122,132] and are also related to altered intracellular cAMP[133] and calcium levels[134]

1.3.2 Preclinical models of tDCS

Preclinical studies, involving both in vitro studies using slice preparations of neural tissue, and in vivo studies using rodent and primate models, have allowed for direct investigation into the cellular and molecular changes during and after stimulation and testing of safety parameters. Early electrophysiological studies in rodents demonstrated spontaneous and evoked cortical potentials were facilitated by application of anodal direct current, and inhibited by cathodal direct current stimulation[135,136,137]. In addition to being polarity-specific, these effects persisted beyond the duration of the DC (direct current) stimulation, suggesting an effect at the synaptic level[135]. Biksom et al (2004), characterised the effects of DC current on neuronal excitability using rat hippocampal slices, and showed that the direction of applied electric current parallel to neurons induced polarization, whilst perpendicular applied current did not induce polarization. Additionally, they were able to trigger epileptiform activity when large negative fields (>80 mV mm1, 1 s) were applied; helping to establish safety thresholds in clinical translation[138]. Using cortical M1 slices from mice, Fritsch et al (2010) investigated the cellular and molecular mechanisms of DC stimulation by simultaneously applying anodal DC stimulation with low frequency stimulation (0.1Hz). They demonstrated that direct current stimulation induced long-lasting synaptic potentiation which was polarity-specific, and outlasted the duration of stimulation. The effect was weaker with concurrent low frequency stimulation of a different frequency, and was not found with anodal DC alone[132]. This work demonstrated that the underlying neuronal activity, or state, is a necessary mediator of effect.

Animal models have not only been extensively used to test the physiological effects of tDCS, in studies ranging from measuring the effects of tDCS on cerebral blood flow[139], motor excitability[140], and visual tasks[141], but also used to study stimulation effects in models of stroke[142], epilepsy[143], depression[144], and pain[145], among others.

1.3.3 Modifying effects of tDCS

tDCS has now been extensively studied as a tool for establishing brain-behaviour relationships across motor, cognitive, social, and affective paradigms, in healthy and disease states[146].

Anodal tDCS applied to the primary motor cortex (M1) has demonstrated varying degrees of improvement in tests of motor speed, dexterity, motor learning, and adaptation[147,148,149,126,150,151]. In particular, the application of M1 anodal tDCS to modulate motor learning, the ability to acquire new skills or refine motor actions through repeated practice, may be relevant to rehabilitation interventions more widely[151,152]. These behavioural effects of anodal tDCS depend on the relative timing of the stimulation and the task. Online stimulation, where anodal tDCS and performance of an implicit learning task are done concurrently, leads to an improvement in the rate of learning of that task[126]. When the task is performed after a period of stimulation, so called offline stimulation, the rate of learning is reported to be unchanged[153].

Although most early tDCS studies were performed in the motor cortex, tDCS does not only induce long-lasting alterations of motor-evoked potentials, but also affects somatosensory[154] and visual-evoked potentials[155], dependent on the cortical area stimulated. Translational studies evaluating motor function in various disease states, including cerebral palsy, stroke, spinal cord injuries and traumatic brain injuries have predominantly stimulated the motor cortex to target motor recovery[156], but other targets for neuromodulation include the posterior parietal cortex for post-stroke neglect[157,158], the inferior frontal gyrus for aphasia[159], and the sensorimotor cortex for post-stroke dysphagia[160,161]. To date, there are no studies evaluating alternative stimulation targets for cerebral palsy. Across psychiatric disorders, tDCS has been applied to the dorsolateral prefrontal cortex (DLPFC) for depression[162,163], schizophrenia[164], addiction[165,166], autism[167] and attentional disorders[168]; and to M1 and the DLPFC for pain syndromes[169,170].

An ongoing challenge remains the range of behavioural responses to tDCS amongst healthy individuals and patient populations, even when stimulation

parameters used are the same[171,172,173,174,175]. Many of these studies report clusters of participants who show a strong behavioural response to stimulation, and those who do not[173,175]. This should not be surprising, however, given the plethora of within-subject factors that could theoretically alter both the mechanistic delivery of the modulating electrical stimulation, as well as the functional response to it. Factors that have been described thus far include brain state[176,149], pharmacological influences[126,127], biological sex and hormonal cycles[177,178], age[179], baseline neurochemistry[180,123], genetic[181,182], and anatomical factors[183,184,185].

1.3.4 Non-invasive brain stimulation in stroke

tDCS has been investigated in stroke as a promising adjunct to motor rehabilitation. Investigations of tDCS in stroke have focused on utilising anodal tDCS to increase the activity of the ipsilesional (affected) hemisphere or cathodal tDCS to reduce activity in the contralesional hemisphere, thereby seeking to restore the 'interhemispheric imbalance' of the cortices. The interhemispheric imbalance (IHI) model suggests that, in stroke, the ipsilesional hemisphere's function is reduced by the initial insult of neuronal loss and then by an increase in inhibitory signals from the contralesional hemisphere, which becomes hyperactive[88,186,187]. Anodal tDCS therefore may work by 'boosting' activity in the lesioned hemisphere, while cathodal tDCS may suppress the overactive non-lesioned hemisphere.

This model provides a rationale for applying cathodal tDCS to the contralesional (non-lesioned) M1 in an attempt to decrease excitability and thereby upregulate the ipsilesional M1 through a reduction in interhemispheric inhibition from the contralesional hemisphere.

However, this model remains controversial. The original study by Murase et al (2004) was small (n = 9), and more recent studies have been unable to replicate the findings[188]. One of the main arguments to refute the role of IHI in stroke rehabilitation is that an increase in contralesional activity could be compensatory rather than maladaptive[189,190,188]. Disruptive TMS applied to the contralesional side can lead to a decrease in performance of the affected hand[191,192],

while Xu et al (2019) demonstrated that the emergence of abnormal interhemispheric inhibition. Several reviews have therefore suggested that the interhemispheric imbalance model may be too simplistic and that a more individualised approach is needed that accounts for the extent of structural damage as well as the availability of residual motor pathways[190,192]. The largest meta-analysis of corticomotor excitability and interhemispheric inhibition post-stroke found no clear evidence for increased excitability of the unaffected hemisphere, nor evidence suggestive of imbalanced interhemispheric inhibition[193]. The authors conclude that facilitating ipsilesional M1 excitability may, therefore, be more beneficial than suppressing contralesional M1 excitability to promote recovery[193].

Despite ongoing uncertainty and efforts to elucidate the underlying mechanisms by which tDCS may improve function after stroke, a relatively large body of work on tDCS in stroke rehabilitation has now accumulated. There is evidence that both ipsilesional anodal[194,195] and contralesional cathodal tDCS[142,195] improve motor function post-stroke. However, there are also many studies that have failed to show any beneficial effect of anodal or cathodal tDCS on functional recovery post-stroke[196]. The most recent Cochrane review of tDCS in stroke highlights ongoing uncertainty in this field: they report evidence of moderate quality regarding tDCS improving activities of daily living outcomes (ADL), while ongoing disparities on the effect, if any, on upper and lower limb[197]. Variability of tDCS protocol, as well as inter-individual differences (Section 1.3.3) are cited by many as the likely cause of these discrepancies in study results[198].

1.3.5 tDCS in Cerebral Palsy

tDCS in a paediatric and adolescent population has been shown to be safe[199,200]. However, there is only a small body of literature on the use of tDCS in children and adolescents with CP, with studies of lower limb function appearing promising[201,202,203,204], but those of upper limb function still unclear as to whether there is a benefit[205,206,207,208,209].

Effect of tDCS on improving lower limb function

Two single-session studies, delivering anodal tDCS for 20 minutes and assessing lower limb function, have yielded inconsistent results between them. Grecco et al. delivered 20 minutes of anodal tDCS to the dominant hemisphere of participants with hemiparetic or diparetic CP, and assessed gait and balance. They demonstrated a significant reduction in sway and an increase in walking speed for the active group compared with sham, but no change in cadence. Follow-up only extended to 20 minutes post-intervention and stimulation was applied to participants at rest[201]. Lazzari et al. delivered anodal to the motor cortex (side unspecified) for 20 minutes in combination with virtual reality mobility training and assessed balance, demonstrating increased sway velocity (worsened balance), for both groups immediately post-intervention and no clear group effect[203]. Follow up time periods are significant limitations of both of these studies with post-stimulation assessment performed immediately[203] or at only 20 minutes[201]. It is difficult to exclude fatigue or draw any conclusions about longer term effects.

Several studies have assessed multiple sessions of anodal tDCS for promoting lower limb function and have demonstrated improvement in various measures of balance and/or gait. Duarte et al. delivered anodal tDCS of the ipsilesional side to hemiparetic and diparetic participants during 10 sessions of treadmill training[210]. They demonstrated improved scores on the paediatric balance scale (PBS), as well as reduced sway, in the experimental group. Between group comparisons are difficult to interpret as mean scores were analysed using a one way ANOVA, rather than change scores. Lazzari et al. similarly demonstrated improvements in the PBS, as well as in the TUG, following anodal tDCS (laterality unspecified) compared with mobility training using virtual reality[204]. Grecco et al. (2015) combined ipsilesional anodal tDCS with virtual reality gait training for 10 sessions and demonstrated greater improvement in walking velocity and cadence in the tDCS group compared to sham, but not in any other variables measured[202].

Effect of tDCS on improving upper limb function

Three studies have assessed the impact of a single session of anodal tDCS to improve upper limb function[208,211,212], demonstrating an improvement in a single metric of reaching with the affected hand[208], and an improvement in box and block score[211,212] . Aree-Uea et al. administered 5 sessions of ipsilesional M1 anodal tDCS and demonstrated a reduction in spasticity of the affected side of those who received anodal stimulation versus those in the sham group[205]. They did not assess any measures of upper limb function apart from spasticity. Auvichayapat et al. similarly demonstrated decreased spasticity (lower Tardieu score) of the affected arm post-intervention, and also demonstrated improved hand function (assessed by the QUEST); however, there was no control group and the sample size was small (n = 10)[206].

Two studies have evaluated the use of 10 sessions of tDCS to improve upper limb function; both utilising cathodal stimulation to the contralesional M1 and 120 minutes of CIMT per session[209,213]. Gillick et al. found a significant increase in the primary behavioural outcome of hand function (Assisting Hand Assessment) in both groups pre- and post-intervention and pre-intervention to 6 month follow up; but no significant change between groups[209]. Of note, this sample size remained small (10 in each group) and the dose of tDCS was smaller than other similar studies, using 0.7mA rather than 1mA which is more common in the literature.[213] similarly found no between-group differences, with a modest increase in hand function in both groups as assessed by the AHA. Subjective measures of upper limb performance (Canadian Occupational Performance Measure) showed significant improvement in the active group compared to sham. Multiple objective and subjective measures were included as part of the secondary outcomes with no significant effect of group found. Inclusion criteria for this study was only for a subset of children with CP - only those with perinatal stroke and hemiparetic CP.

Combined upper and lower limb interventions

To our knowledge, only one study has combined tDCS and an intervention targeted at both upper and lower limb function. Fajardo et al. assessed the effect 15 sessions of ipsilesional anodal tDCS combined with neurodevelopmental treatment (NDT)[214]. The primary outcome was change in Gross Motor Function Measurement-88 (GMFM-88) which assesses lying and rolling, sitting, and mobilising, and Modified Ashworth Scale (MAS) assessing spasticity. Both the active and control groups showed improvement over time in overall GMFM-88 scores, while the tDCS group also demonstrated an improvement in spasticity at follow-up. There are several important limitations of this study: the control group did not include sham stimulation, no between-group analysis was performed, and the rehabilitation intervention (NDT) has been consistently shown to be ineffective with low quality evidence grading on systematic review[**novak2013AEvidence**].

1.3.6 Limitations of current evidence

There are several important limitations to consider in the current literature evaluating the use of tDCS in cerebral palsy. Firstly, several studies either have no control group[215,206], or do not utilise sham stimulation for their control group, such as Radwan et al., who compared the effects of anodal tDCS with VR, with no sham condition[216], and Fajardo et al.[214]. Utilisation of a sham condition typically involves a short period of stimulation (30 seconds or less) where current is increased and then turned off, and replicates the cutaneous sensations that are associated with changing current. Sham tDCS is generally regarded as an effective blinding technique for participants, especially for those who are naive to tDCS[217,218,219], and assists with researcher blinding[146].

Two studies utilise a within-subject crossover design to probe tDCS effects, with only 24 hours between conditions[212,211]. Current consensus suggests a minimum of 7 days between stimulation sessions to enable any stimulatory effects to have been washed out between conditions[150].

One study[216] utilised a non-standard electrode placement, placing the anode over M1 on the contralesional side (rather than ipsilesional side), and the cathode over the inion, rather than supraorbitally, with no stated rationale. Type of stimulation and stimulation intensity also vary greatly between studies, ranging from 0.7 mA[209] to 1.5 mA cathodal tDCS[212], and 1 mA[206,205,208] to 1.5 mA of anodal tDCS[211]; the majority of studies using 1 mA anodal tDCS .

Several studies did not specify if participants were engaged in any activity or training during tDCS[206,212,216]. Engaging participants in a related motor task while applying a-tDCS to M1, has become a fundamental principle of tDCS, often referred to as online stimulation, or task-specific modulation[220]. This principle is based on the evidence that tDCS will preferentially enchance or consolidate a specific pattern of activity if performed during stimulation[123,221]. For example, Stagg et al. showed that tDCS applied during sequence learning task led to polarity-specific modulation of behaviour: anodal tDCS was associated with faster sequence learning, cathodal stimulation was associated with slower sequence learning. Application of tDCS prior to performing the task led to slower learning in both anodal and cathodal stimulation groups[123].

Finally, only certain subtypes of cerebral palsy are included in many of these studies - several studies include only children with perinatal stroke and hemiparetic CP[213,215,211], while Auvichayapat et al. and Aree-Uea et al. only included participants with spasticity of the right upper limb[205,206].

1.4 Aims of this thesis

Cerebral palsy is a common childhood disability that encompasses a range of underlying pathologies and clinical presentations, but is foremost a disability that affects motor function in children. Great strides have been made in prevention and treatment, but the overall number of children and adolescents with cerebral palsy continues increase, as life expectancy increases and complications decrease. There is a need for effective, time-efficient, rehabilitation strategies to improve motor function to facilitate activities of daily life.

Non-invasive brain stimulation, transcranial direct current stimulation in particular, is a potentially promising treatment avenue which could augment rehabilitation strategies by facilitating neuroplasticity. However, previous evidence in stroke, and emerging data in cerebral palsy, suggest high inter-individual variability in response to tDCS.

This thesis examines the utility of tDCS in improving motor function in a clinically relevant cerebral palsy cohort, exploring inter-individual differences in treatment response according to baseline corticospinal tract integrity and modelled electric field strength.

In Chapter 2, the impact of the COVID-19 pandemic on access to rehabilitation and motor function in children with cerebral palsy is described. This opportunistic study assessed the impact of unprecedented disruption to rehabilitation on young people with cerebral palsy.

In Chapter 3, the results of a randomised, double-blind, sham-controlled clinical trial investigating the effect of anodal tDCS and motor training in children with cerebral palsy are presented.

In Chapter 4, individualised probabilistic tractography using diffusion magnetic resonance imaging data is used to generate individual metrics of white matter tract integrity. The inter-individual variability in corticospinal tract integrity at baseline is assessed, along with correlation to the pattern of functional deficit and response to rehabilitation.

Finally, in Chapter 5, individualised electric current simulations are used to explore the inter-individual variation in electric field generated by anodal tDCS in a heterogenous cerebral palsy population, to determine if measures of electric field strength in the target primary motor cortex and field focality can predict response to tDCS.

2

Disruption in rehabilitation services: the impact of COVID-19 on children with cerebral palsy

Contents

2.1 Introduction

While the causative brain injury in cerebral palsy is static, symptoms and children's functional abilities may fluctuate over time[222]. Developmental trajectories of mobility and performance of activities of daily living (ADLs) are slower than typically developing children, and begin to plateau in early adolescence[58]. From adolescence

into adulthood, an overall decline in motor function and mobility occurs[60], which is linked to reduced overall function and lower participation in work and sport[63]. Routine monitoring and physical rehabilitation, used to maximise function and prevent secondary complications, is vital both to slow functional decline and ensure maximal improvement in domains that still show scope for improvement such as spinal alignment, joint range of motion, and strength[223].

Children with cerebral palsy have complex needs across life stages, and in the United Kingdom (UK), children may access medical and rehabilitative care through state (National Health Service) and private healthcare, and through education services at dedicated specialist schools or specialist units in mainstream schools.

When the COVID-19 pandemic led to a nationwide lockdown in the UK in March 2020, a significant reduction in healthcare support occurred[224]. For most children, this did not resume for many months, or even longer[224]. Understanding the physical consequences of the UK's lockdown on children with cerebral palsy is essential so that appropriate support can be implemented.

We gathered data following the period after the first national lockdown in the United Kingdom to record and evaluate the physical and functional effects on children and adolescents with CP.

2.1.1 Key aims of this chapter

1. Determine if children with cerebral palsy experienced a change in their ability to perform motor tasks during the nationwide lockdown

2. Describe the patterns of access to exercise and physical & occupational therapy before and during the lockdown

3. Describe how children with cerebral palsy used their time during lockdown, with a focus on time spent on exercise

2.2 Methods

This cross-sectional retrospective study was conducted in the United Kingdom between October 17 2020, and February 8 2021.

2.2.1 Study participants

A population-based convenience sample of guardians of children with CP in the UK was utilised in the CP@Home study. All guardians of children aged 16 years or younger with CP were eligible to participate. The survey included two sections; one for guardians to complete and one for children with cerebral palsy, if they were aged 10-16 years. Guardians provided electronic consent to participation and publishing of data prior to participation. Eligible children provided electronic assent.

The survey was hosted on REDCap (Research Electronic Data Capture), a secure, web-based data management software. Study information and the survey link were distributed via social media, cerebral palsy and neurodevelopmental disorder charities, and schools.

Ethical approval for the CP@Home study was obtained from the University of Oxford Research Ethics Committee (R71276/RE001).

2.2.2 Variables of interest

Demographics collected included age and gender of child, gender of guardian respondent, relationship of the respondent to child, presence of a confirmatory diagnosis of CP, and parent-reported Gross Motor Function Classification System (GMFCS) category[53]. Information related to schooling and extra provisions were obtained. A history of COVID-19 infection and illness for children and households were obtained, in order to determine if any reported changes could be associated with COVID-19 infection.

There were 4 main sections in the survey to assess the impact of the nationwide lockdown.

1. Ability to perform ADLs and motor tasks was assessed using a modified version of the Activities of Daily Living & Independence and Gait Function & Mobility section from The Gait Outcomes Assessment List (GOAL™)[225].

2. Access to exercise and physical & occupational therapy before and during the lockdown, and mode and utility of provision.

3. Time usage of children during lockdown, including estimated time spent doing low and high-intensity exercise, defined as gentle physical activity (e.g. walking or stretching); and cardiovascular workouts (e.g. running and high-intensity interval training) respectively.

4. Children's sleep patterns, understanding of the disease and lockdown and their feelings surrounding staying at home, including modified use of the Pandemic Anxiety Scale (PAS)[226]. Data not included in this thesis.

The majority of questions were presented as multiple-choice answers, with an 'other' option and free text for clarification; or Likert scales which included a 'not applicable' option. A free text box was included at the end of the survey for guardians to share any additional information that they felt was pertinent.

The optional child survey was abbreviated from the guardian survey. Similar to the guardian survey, ADL and motor task experiences based on the GOAL™[225] and a modified PAS was included.

2.2.3 Data Processing and Analysis

Data were exported from the REDCap server for statistical analysis using IBM SPSS Statistics for Mac (version 27) and plotted using GraphPad Prism (version 9.02).

Given the descriptive nature of the study, the majority of the data are described using a percentage of responses for each question. Where relevant, data were tested for normality using a Shapiro-Wilk test. Wilcoxon matched-pairs signed rank test was used as a paired difference test, and two-sided Fisher's Exact test for categorical variables. The threshold for significance was set as $p < 0.05$.

2.3 Results

2.3.1 Participants

A total of 67 guardians showed interest in completing the survey, with 54 complete or near-complete responses (defined as $as \geq 75\%$ completion). All respondents identified as parents and will be referred to as such. The mean age of the respondents' children was 8.9 ± 4.8 years and all children had a confirmed diagnosis of CP. Six adolescents completed the child survey. Participant demographics are reported in Table 2.1.

2.3.2 Access to medical care, therapy and physical activity

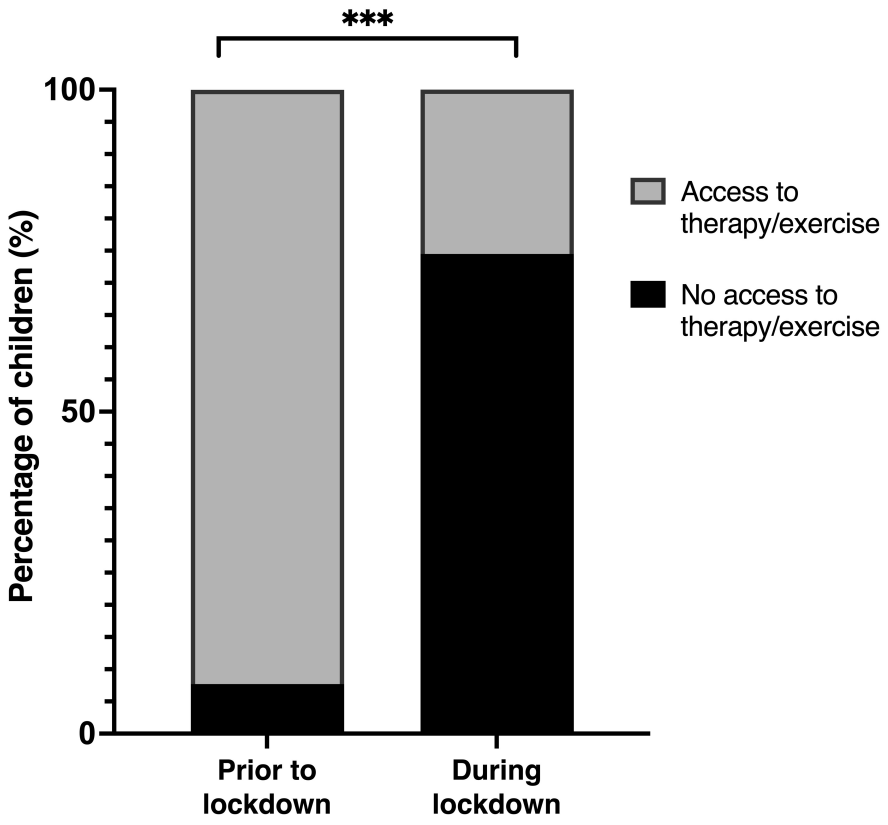


Figure 2.1: Children's access to care during the lockdown: Access to therapy and/or supervised exercise was significantly decreased during the lockdown versus prior to lockdown. (Two-sided Fisher's Exact test, $p < 0.0001$)

We asked parents about therapy attendance before lockdown for their child, and how this had changed during lockdown. Before lockdown, 82.4% reported attending

Table 2.1: Demographics of survey respondents - guardians and children, as well as characteristics of CP, therapy provisions, and illness with Covid-19 during survey period. Data are reported as n (%). *PCR: Polymerase chain reaction*

Demographic		**n (%)**
Guardian Gender		
	Female	43 (81%)
	Male	6 (11%)
	Other/prefer not to say	4 (7.5%)
Child's gender		
	Female	20 (37%)
	Male	33 (61%)
	Other/prefer not to say	1 (2%)
Gross motor function score		
	I	3 (5.9%)
	II	17 (33.3%)
	III	8 (15.69%)
	IV	13 (25.5%)
	V	10 (19.6%)
Type of school attended		
	State school	17 (44%)
	Independent school	3 (8%)
	Special provision school	13 (33%)
	Home educated	0 (0%)
	Other/not specified	6 (15%)
Primary caregiver for child		
	Respondent (parent)	31 (73.8%)
	Child's other parent	1 (2.4%)
	Joint responsibility	8 (19%)
	Respondent's partner	0 (0%)
	Family member	0 (0%)
	Child's sibling	0 (0%)
	Carer	1 (2.4%)
	N/A	1 (2.4%)
Illness with COVID-19		
PCR diagnosed and recovered	Parent	1 (2.4%)
	Child	0 (0%)
	Household	1 (2.4%)
PCR diagnosed and still ill	Parent	0 (0%)
	Child	1 (2.4%)
	Household	0 (0%)
Suspected and recovered	Parent	7 (16.6%)
	Child	2 (4.8%)
	Household	4 (9.5%)
Suspected and still ill	Parent	0 (0%)
	Child	0 (0%)
	Household	0 (0%)
Never ill	Parent	34 (81.0%)
	Child	39 (92.6%)
	Household	37 (88.1%)

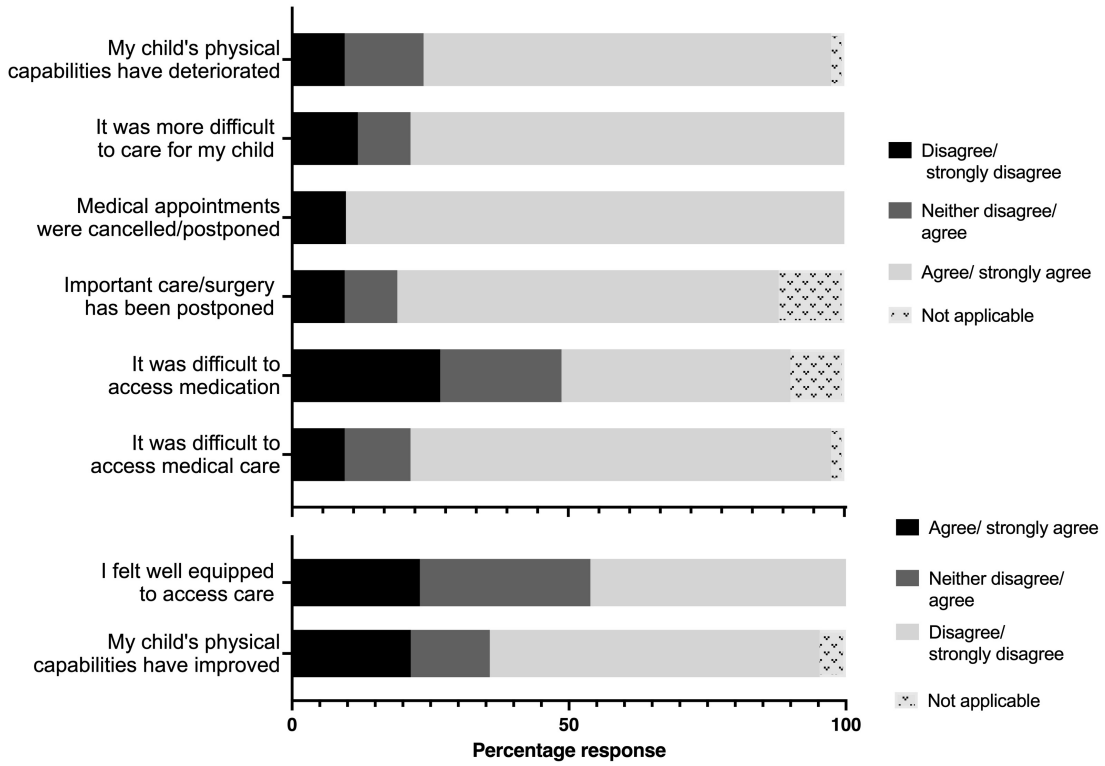


Figure 2.2: Parent reported outcomes during the lockdown: Parents reported increased difficulty in accessing care for their child during the lockdown across several domains.

physical or occupational therapy at least once a month. The majority of children attended therapy monthly (33%) or weekly (27.5%), with only 9.8% of children not receiving any therapy prior to lockdown. In terms of access to supervised exercise classes (including school and gym classes), the majority (56.9%) attended weekly, 19.6% attended daily, and 23.5% did not attend any supervised exercise prior to lockdown. These services were accessed through schools (44.7%), the NHS (28.9%), privately (19.7%), and through charities and local clubs (6.6%). In contrast to the 92.3% of children who had access to therapy and/or supervised exercise prior to lockdown, only 25.5% of children had access to these services during lockdown. The percentage of children who accessed supervised exercise or therapy differed significantly before lockdown vs. during lockdown, $p < 0.001$ (Figure 2.1).

The majority of parents reported overall negative experiences and effects of lockdown on their ability to care for, and access medical care for, their child (Figure 2.2). 76.2% of parents reported feeling that it was difficult to access medical care

for their child and 41.5% reported difficulty in accessing medication. Important care or surgery had been postponed for 69% of children and 90.2% had medical or therapy appointments cancelled or postponed.

2.3.3 Motor skills, ability to perform activities of daily living and access to exercise

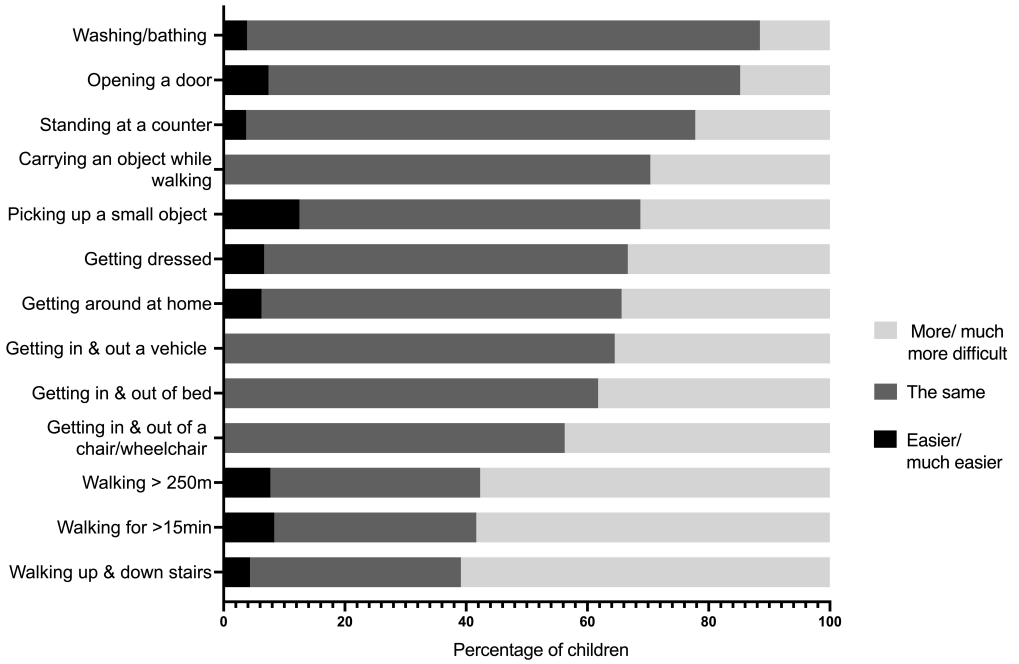


Figure 2.3: Children's activities of daily living: Parents reported substantially more difficulty experienced by their child in performing common activities of daily living when compared to their ability prior to lockdown.

A specific focus of the questionnaire was to understand the effects the lockdown had on the ease or difficulty they experienced in performing motor tasks and ADLs. Parents were asked to state if they thought their child found a series of everyday tasks, based on the GOAL™ questionnaire, easier, the same, or more difficult than before. Across the different tasks, between 12-61% reported finding activities more challenging (Figure 2.3). Mobility tasks were reported as the most challenging, with children finding it more/much more difficult to walk up and down stairs (61%), to walk for more than 250m (58%), to walk for more than 15 minutes (58%), and to get out of a chair/wheelchair (44%).

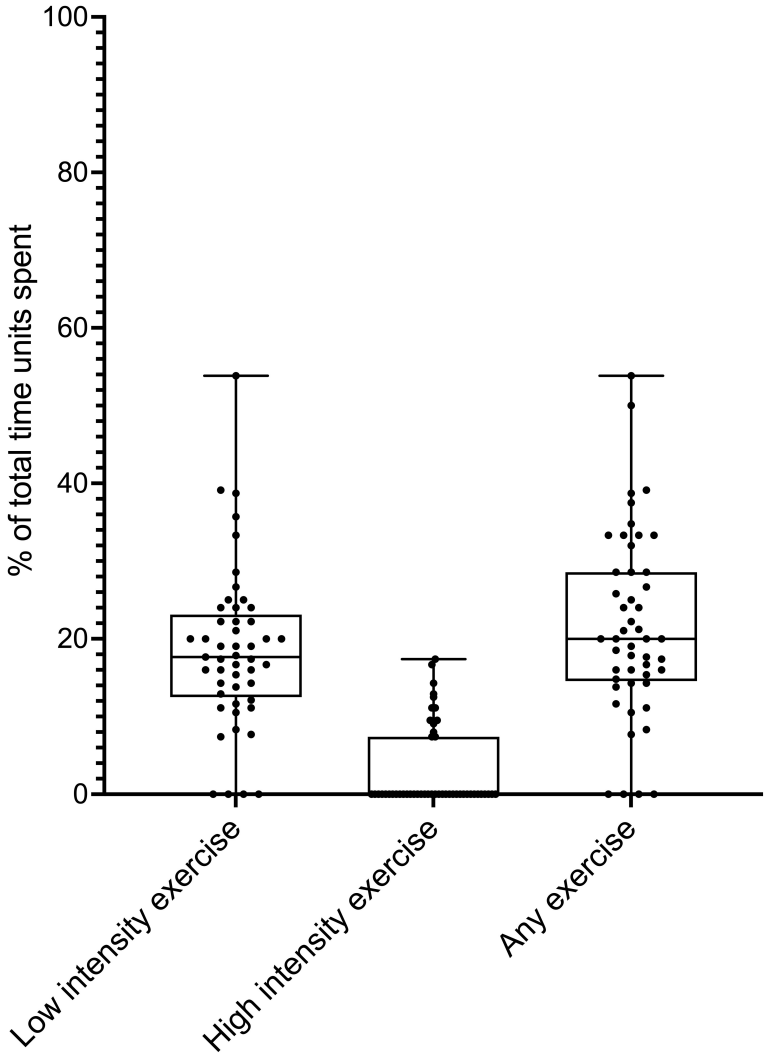


Figure 2.4: Proportion of time spent on high- and low-intensity exercise per day: Parents reported that, as a proportion of time spent on all activities in a day, very little time was spent by their child on high-intensity exercise. However, a much greater percentage recorded at least 30 minutes of low-intensity exercise on an average day.

We evaluated the time children spent on exercise during their day, as a proportion of the total time spent on all activities in a day. The majority spent little to no time on high-intensity exercise: the median proportion of time spent on high-intensity exercise was 0 (0-7.4%), and the median proportion on low-intensity exercise was 18% (13-23%). The median proportion of time spent by children on any exercise (low-intensity, high-intensity or not specified) was 20% (15-29%) (Figure 2.4).

2.3.4 Alternative methods of providing therapy: evaluation of online offerings

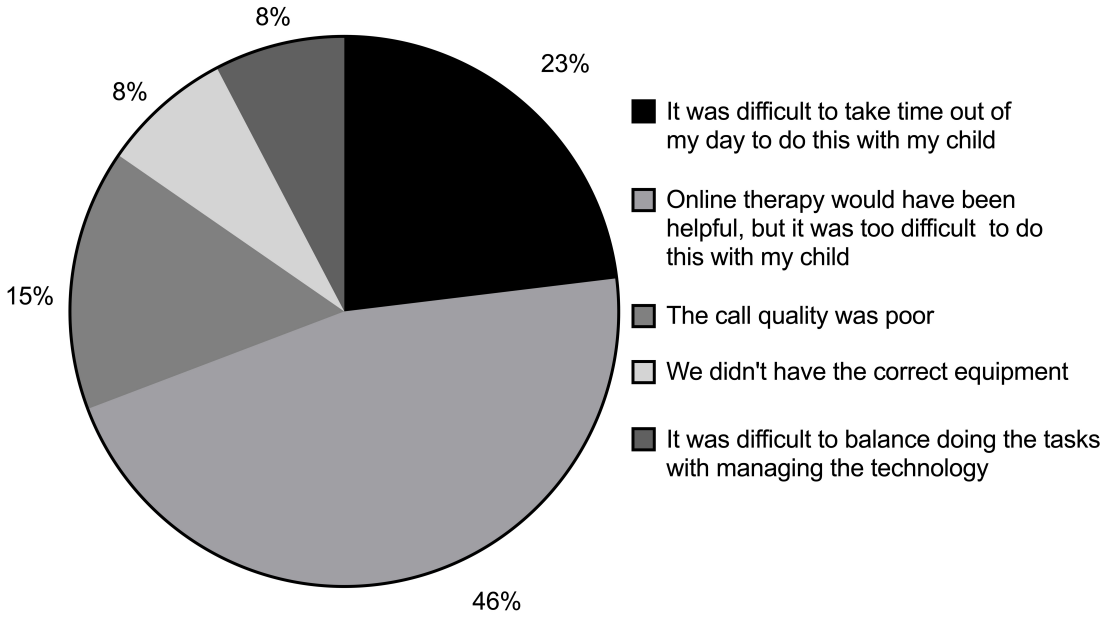


Figure 2.5: Parents experiences of the challenges associated with online therapy during lockdown. Many parents felt that online therapy was too difficult to do with their child, or it was difficult to take the time to do it.

Of the 13 children who had access to therapy or supervised exercise during lockdown, 8 were able to access it online, 3 in person, 1 both online and in person, and 1 was unspecified. Of the small sample who had access, 6 of the 9 parents reported that they felt the online offering was helpful, but not to the same extent as in person therapy (Figure 2.5). One parent reported that it was not helpful at all. When exploring the reasons for these sentiments, parents reported that it was difficult to carry out the instructions given (n=2), that technical issues made it more challenging (n = 3) and taking time to attend with their child was challenging (n = 1) (Figure 2.5). Of the adolescent respondents, only one had attended therapy since the onset of lockdown, and stated that they did not enjoy online therapy.

2.4 Discussion

The COVID-19 pandemic, and subsequent lockdowns, presented an unprecedented challenge to delivering schooling, healthcare, and physical activity to children

with Special Educational Needs and Disabilities (SEND)[224]. A rapidly changing situation made it difficult to fully understand the impact, or adequately plan for and accommodate children with neurodevelopmental disorders. However, it is crucial to ensure that we understand these impacts in order to mitigate them and support these children, as well as to inform future public health decisions.

In this dataset, parents of children with CP in the UK reported marked changes in their access to therapy and physical activity. To our knowledge, this is the first UK-wide study to evaluate the impact of the COVID-19 lockdown on motor and functional consequences in children with CP.

Our sample had low rates of COVID-19 infection and no hospital admissions were reported. This is in keeping with other studies which show that very few children with neurodevelopmental disabilities have been hospitalised with COVID-19[227,228,229], though children with CP were classed as 'clinically vulnerable', and may have been shielding.

Most respondents reported cancellation and/or postponement of medical and rehabilitative care, congruent with studies in Italy[230] and France[231] as well as charity and government reports[227,224,232]. Our study indicated that children in the UK had lower levels of access to therapy (25%), than similar reports in Italy, where 49.5% of the children with neurological illnesses had access to telerehabilitation[230], and in the USA where 72% of those surveyed had access to video-based telerehabilitation[228]. This suggests that provision was disproportionately reduced in the UK compared to other high-income countries.

Our study indicated a sub-population of approximately a third of children with a decline in ability to perform physical activities which form part of activities of daily living. In particular, gross motor activities of walking for >250m or >15 minutes, walking up and down stairs, and standing from sitting were found to be more difficult. This did not appear to correlate with GMFCS or the regularity of previous access to rehabilitative care. This is in keeping with Theis et al.[229], where 61% of respondents had observed negative physical changes in their child with a physical/intellectual disability since lockdown began. For children with CP,

it is recommended that moderate to high-intensity physical activity be undertaken at least 5 days per week, and that sedentary behaviour be limited to < 2 hours a day or be broken up regularly[233]. Our data suggest that during the lockdown 60-71% of the children spent at least 4 hours a day in sedentary activities, and only a small minority undertook any high-intensity exercise.

Participation in therapy and physical exercise is particularly important for children with CP, as it helps to prevent the cycle of physical impairment leading to deconditioning that, in turn, worsens the level of disability[64]. Regular stretching and loading of muscles is vital to maintaining muscle length and strength, decrease spasticity and prevent contractures[65]. Furthermore, the early brain injury in CP results in an overlapping window for developmental age-related motor learning, and post-injury motor recovery[234]. Both animal studies[235] and non-invasive brain stimulation studies of motor development[236] have demonstrated plastic motor organization continues through childhood and adolescence. In parallel to this, physical training and therapy across the lifespan of children and adolescents with CP has shown to be of benefit[66]. In summary, ongoing physical training and therapy are critical to support neural plasticity and motor functioning.

This study highlights several of the effects of lockdowns, with drastically reduced/disrupted service provision, on children with CP. Education and health systems are at the forefront of working with children and families to address both existing physical and psychosocial needs, as well as new issues that have arisen. This will include focusing on recovering lost motor function, especially mobility, reinstating routine monitoring for spasticity and other complications, and revising clinical and education, health and care (EHC) plans where appropriate.

It is also critical to continue to pursue better interventions to improve motor function and increase the independence of young people with CP. Non-invasive brain stimulation (NIBS) techniques, which have gained increasing favour over the last two decades, offer the potential to modulate neural activity following an acquired brain injury[237,238,239]. Previous clinical trials have demonstrated that tDCS of the primary motor cortex (M1), when applied during rehabilitation, may improve upper

limb function in stroke patients[239,142]. Chapter 3 will explore the effect of tDCS on motor outcomes in a cohort of children with CP.

3

Transcranial direct current stimulation to improve motor function in Cerebral palsy: A pilot study

Contents

3.1 Background

The clinical manifestations of Cerebral palsy are diverse, with motor deficits often accompanied by disturbances of sensation, cognition, communication and perception[41]. Regardless of the severity, or the subtype, children with CP experience a lower Quality of Life (QoL) across all domains, in particular, physical and emotional well-being[240,241]. Progress over the last 20 years has resulted in a reduction in the incidence of cerebral palsy in developed countries[11]. However, the overall prevalence of people with cerebral palsy is forecast to continue to increase as life expectancy and medical care improve[16].

The COVID-19 pandemic demonstrated that even short gaps in access to rehabilitation and physical activity can have detrimental effects on children and adolescents with cerebral palsy (Chapter 2). Therefore, it is critical to continue to pursue effective interventions to improve the quality of life, improve motor function, and increase the independence of children with cerebral palsy.

3.1.1 Current therapies for cerebral palsy

Over the last two decades, rehabilitation protocols based on motor learning principles have been developed for children with cerebral palsy. These include interventions for upper extremity function, such as constraint-induced movement therapy (CIMT)[242,243] and modified CIMT (mCIMT)[244], hand-arm bimanual intensive training (HABIT)[243,245,84] and later the development of hand and arm bimanual intensive therapy including lower extremities (HABIT-ILE) to additionally improve lower limb function[246,79,247].

Intensive bimanual training (such as HABIT and HABIT-ILE) have gained popularity as it is increasingly recognised that children's functional independence is related to the use of limbs in a coordinated way, with real-world applicability[74]. These techniques of rehabilitation have been recognised to improve function when principles

of motor learning and neuroplasticity are utilised, such as practice specificity, feedback, repetition, increasing movement complexity, motivation, and reward[84].

An important limitation of many current rehabilitation strategies, both those with evidence for benefit, and those with emerging evidence or unclear benefit, is how time- and resource-intensive they are to run[**novak2013AEvidence**]. For example, the total training time for CIMT varies between 20 to 504 hours in studies evaluating the utility of CIMT[248]; while a recent systematic review found that nearly half of all studies evaluating HABIT were based on participants having 6 hours of intervention a day for three consecutive 5-day weeks, totalling 90 hours per participant[77]. It is therefore important to investigate ways to improve the efficiency of rehabilitation.

3.1.2 Non-invasive brain stimulation as an addition to current therapy

Non-invasive brain stimulation (NIBS) techniques, which have gained increasing favour over the last decade, offer the potential to modulate neural activity and recovery following an acquired brain injury[237]. One such technique is transcranial direct current stimulation (tDCS) - a non-invasive brain stimulation technique that modulates cortical excitability[249].

Transcranial direct current stimulation offers the benefit of being low-cost and portable. As such, it has been widely studied as a therapeutic adjunct in a wide spectrum of clinical conditions. Most significantly, tDCS has been thoroughly investigated as a tool to assist with recovery of motor function post-stroke, with long-term improvement in motor function[250,251].

There is a large body of research demonstrating the potentially promising effects of both ipsilesional anodal and cathodal stimulation[194,195,252]. The underlying mechanisms remain poorly understood, and several meta-analyses have shown extensive variation in response, with a resulting estimated small-to-moderate effect size of tDCS in improving motor rehabilitation outcomes[194,197].

Whilst tDCS has been extensively investigated in adult stroke patients, it cannot be assumed that these findings will translate to children with cerebral palsy, given

that cerebral palsy is an early insult to a developing brain, while adult stroke is a late insult to a mature brain.

tDCS in cerebral palsy

tDCS in a paediatric and adolescent population has been shown to be safe[199]. However, there is only a small body of literature on the use of tDCS in children and adolescents with CP, with studies of lower limb function appearing promising[201,202,203,204], but those of upper limb function still unclear as to whether there is a benefit[205,206,207,208,209,211]. This literature is summarised in Table 3.1.

Table 3.1: Summary of tDCS studies in children and adolescents with cerebral palsy, adapted from Fleming et al (2018)[253]. M1 = motor cortex, MAS = Modified Ashworth Scale, CIMT = Constraint Induced Movement Therapy, COPM = Canadian Occupational Performance Measure, BBT = Box and Block Test, PEDI = Paediatric Evaluation of Disability Inventory, PBS = Paediatric Balance Scale, TUG = Timed Up and Go, GMFM-88 = Gross Motor Function Measure, AHA = Assisting Hand Assessment, VR = Virtual Reality

Author, Year	Stimulation Parameters	Study Participants	Motor Training?	Sessions	Outcome, tDCS effect
Upper limb only					
Moura et al., 2017	Anodal, ipsilesional M1, 1mA, 20 min	Spastic hemiplegia, 6–12 years, 10 per group	20 min reaching training with constraint	1	Decreased movement duration, no other changes
Auvichayapat et al., 2017	Left anodal M1, cathode on right shoulder, 1mA, 20 min	Right UL spasticity, 8-12 years, N=10, no control	Not mentioned	5	Decreased spasticity
Aree-Uea et al., 2014	Left anodal M1, cathode on right shoulder, 1mA, 20 min	Right UL spastic hemiplegia, 8-18 years, 23 per group	Physical therapy	5	Decreased spasticity, increased shoulder ROM
Gillick et al., 2018	Cathodal contralesional M1, 0.7mA, 20 min	Hemiparetic, 7-21 years, 10 per group	CIMT	10	Improved hand function in both groups (AHA)
Kirton et al., 2017	Cathodal contralesional M1, 1mA, 20 min	Perinatal stroke, Hemiparetic, 6-18 years, n=12 active, 11 sham	CIMT	10	Improved subjective hand function (COPM) in tDCS group
Inguaggiato et al., 2019	Anodal ipsilesional M1, 1.5mA, 20 min	Unilateral CP, 10-28 years, n=8, crossover design	Not mentioned	1 anodal, 1 sham session	Improved manual dexterity (BBT) in active group, but cross-over design
He et al., 2022	Anodal ipsilesional M1, 1.5mA, 20 min	Hemiplegic CP, 3-6 years, n=30	Not mentioned	1 anodal, 1 sham session	Improved manual dexterity (BBT) in active group

Table 3.1: Summary of tDCS studies in children and adolescents with cerebral palsy, adapted from Fleming et al (2018)[253]. M1 = motor cortex, MAS = Modified Ashworth Scale, CIMT = Constraint Induced Movement Therapy, COPM = Canadian Occupational Performance Measure, BBT = Box and Block Test, PEDI = Paediatric Evaluation of Disability Inventory, PBS = Paediatric Balance Scale, TUG = Timed Up and Go, GMFM-88 = Gross Motor Function Measure, AHA = Assisting Hand Assessment, VR = Virtual Reality

Author, Year	Stimulation Parameters	Study Participants	Motor Training?	Sessions	Outcome, tDCS effect
Lower limb only					
Grecco et al., 2014	Anodal contralesional M1, 1mA, 20 min	Hemiplegia or diplegia, 4-12 years, 10 per group	At rest	1	Increased walking speed, decreased sway
Lazzari et al., 2015	Anodal M1 (side unspecified), 1mA, 20 min	CP type unspecified, 4-12 years, 10 per group	VR mobility training	1	Increased sway velocity in both groups
Collange Grecco et al., 2015	Anodal ipsilesional M1, 1mA, 20 min	Spastic diplegia, 5-10 years, 10 per group	VR gait training	10	Increased walking velocity and cadence, increased subjective functional dependence(PEDI)
Duarte et al., 2014	Anodal ipsilesional M1, 1mA, 20 min	Spastic diplegia or hemiplegia, 5-10 years, 12 per group	Treadmill gait training	10	Improved balance (PBS), increased subjective functional dependence(PEDI) – no group difference
Lazzari et al., 2017	Anodal M1, laterality unspecified, 1mA, 20 min	CP type unspecified, 4-12 years, 10 per group	Mobility training with VR	10	Improved balance (PBS), improved TUG time
Both upper and lower limbs					
Fajardo et al., 2021	Ipsilesional anodal M1, 1mA, 20 min	Spastic unilateral & bilateral, 3-9 years, n=14 - tDCS group, n=10 - NDT only, no sham	NDT	15	Improved GMFM-88 & BBT, decreased spasticity in both groups, no between-group comparison

As outlined in Section 1.3.6, there are several important limitations of the current literature examining the impact of tDCS in motor rehabilitation in CP. Generalisability of studies is limited by studies without control groups[215,206] and those that don't utilise sham stimulation for their control group[216,214]. Without control groups utilising sham stimulation, it is not possible to blind participants and researchers to condition.

Variation in the type of stimulation, and stimulation intensity (0.7 mA to 2 mA)[209,212,206,205,208,211] make comparison between studies challenging. Many studies did not engage participants in a motor activity during training[206,212,216], which is recognised as important to consolidate the plasticity boosting effects of stimulation[123,221].

Lastly, limiting inclusion criteria to specific subtypes of cerebral palsy, for example perinatal stroke[213], or unilateral upper limb impairment[205,206] limits the generalisability of results.

While these studies have been fundamental to improving our understanding of the safety and tolerability of tDCS in children with cerebral palsy, the efficacy of tDCS in this population remains unclear. Variability in stimulation protocols, motor interventions, outcome measures, and statistical analysis makes it more difficult to interpret these findings in a broader context of clinical utility for real-world clinical populations.

Given that approximately two-thirds of children with CP have motor deficits in more than one limb[52], it is of value to investigate whether combining tDCS with therapy involving both upper and lower limb training would be feasible and have the potential to be effective. To our knowledge, no studies have evaluated the use of tDCS to improve both upper and lower limb function in the same session, coupled with well-evidenced motor training.

Additionally, there are no studies, to our knowledge, that evaluate the effect of targeting tDCS electrode placement to modulate the cortex corresponding to both upper and lower limbs, in combination with therapy interventions for both

upper limb and lower limb. This study aimed to address this gap in the literature, and the above discussed limitations of previous work.

3.1.3 Key aims of this chapter

Building on our findings in Chapter 3, which showed children with cerebral palsy experienced a decline in motor outcomes, and specifically the ability to perform activities of daily living, we aimed to:

1. Describe the effect of 10 sessions of upper and lower limb training on motor ability in children with CP.

2. Determine if anodal tDCS of the motor cortex enhances the effects, if any, of 10 sessions of training on upper and lower limb function (with a control group receiving sham stimulation).

3. Assess feasibility, safety, and tolerability for the intervention.

3.2 Methods

3.2.1 Study participants

StimCP was a randomised, double-blind, multi-centre, sham-controlled pilot study, registered at www.isrctn.com (ISRCTN74235136). The study was approved by National Research Ethics Service and the Health Research Authority (REC ref: 20/WM/0046) and carried out in accordance with the Declaration of Helsinki. Participants younger than 16 provided written assent (with parent/guardian providing written informed consent). Participants aged 16 years were considered competent youths and provided written informed consent.

Participants were recruited through participating NHS trusts, schools, and word of mouth.

Inclusion criteria were as follows:

1. Age 10-16 years

2. Clinical diagnosis of cerebral palsy

3. Gross motor function classification score (GMFCS) I to III
4. Manual ability classification score (MACS) I to III
5. Upper and/or lower limb impairment
6. Able and willing to participate, with a parent or guardian that is willing and able to provide informed consent on behalf of the participant (if under 16 years of age)

Exclusion criteria included: 1) contraindications to tDCS, including seizures within the preceding 2 years; 2) contraindication to physical therapy as determined by the clinical team; 3) cognitive impairment to the degree that the participant would be unable to interact and follow instructions; 4) scheduled elective surgery or other procedures requiring general anaesthesia during the trial; 5) life expectancy of fewer than 6 months; 6) female participant who is pregnant, or planning pregnancy during the course of the trial; 7) any other significant disease or disorder which may either put the participants at risk, or may influence the result of the trial, or the participant's ability to participate in the trial and 8) participants who have participated in another research trial involving an investigational medicinal product in the preceding 12 weeks.

3.2.2 Sample size

It was difficult to accurately estimate the expected effect size given the limited reporting from prior studies, wide variety in effect sizes reported, and differing patient profiles where these can be estimated. The median estimate from a selection of relevant previous studies is approximately d = 0.5 (Cohen's d). The proposed sample size of 30 participants would provide adequate power (80%) to detect a true effect if this estimate is correct (alpha 0.05, power 0.8, allowing for 20% attrition), or to give a better indication of the effect size if not. As a pilot study a secondary aim was to evaluate feasibility of recruitment and delivery of the intervention.

The study start date was delayed by 18 months due to the COVID-19 pandemic. Ethics approval was obtained in April 2020, and recruitment to new studies was

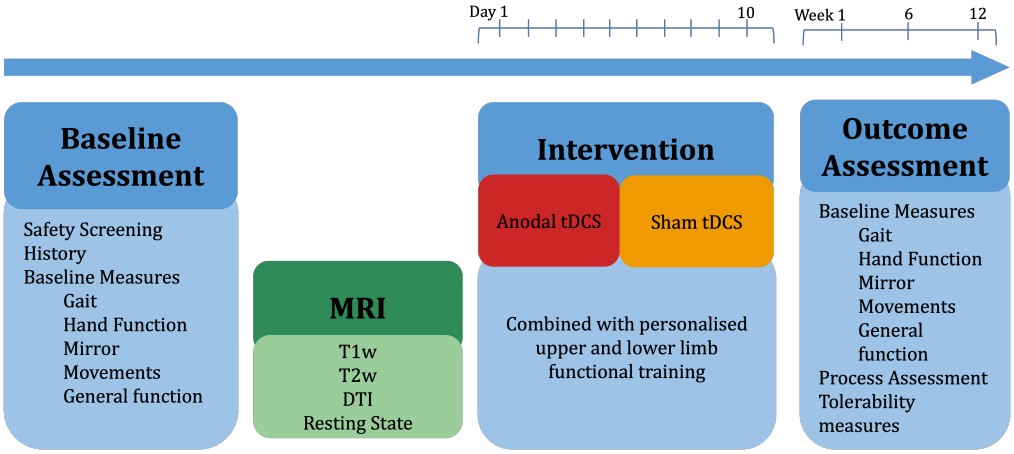


Figure 3.1: StimCP study design: Participants completed a baseline assessment which included safety screening and functional assessment. For those who opted in, a structural and functional MRI was performed before intervention. The ten-day intervention period consisted of 20 minutes of anodal or sham stimulation during a 90 min training block each day. Outcome assessments were done 1, 6, and 12 weeks after the end of the intervention

on hold until August 2021. Further information can be found in Appendix A. As a result, a smaller sample size has been obtained, however, study retention has been higher than expected, with no attrition.

3.2.3 Study Design

Enrolled participants completed 15 study visits: baseline assessment, MRI scanning session, 10 intervention sessions and 3 follow-up assessments (Figure 4.2). The MR scan was not a necessity for enrolment in the trial, and participants could opt out (for more details, see Chapter 4).

Primary outcome measures

The primary outcome for the upper limb was the change in performance time for the Jebson-Taylor hand test[254] at 1-week post-intervention. The primary outcome for the lower limb was the change in the performance time for the Timed Up and Go test[255] at 1 week. Secondary outcome assessments included:

1. 10 metre walk test[256]

2. The Children's Hand Use Experience Questionnaire[257]

3. Gait Outcomes Assessment List[225]

4. Modified Ashworth Scale[258]

The Jebson Taylor Test assessed in the standard manner. Participants were familiarised with the tasks prior to assessment at the baseline session. At each assessment session, participants completed each task three times, and the best time (in seconds) was taken. Participants were instructed to perform as quickly as they could whilst avoiding making mistakes. The maximum time allowed for each subtask was 120 seconds, and participants were given the maximum time if they were unable to complete the task. A total time was calculated by summing the best time for each of the 6 subtasks.

Participants were allowed to use any walking aids they typically required to complete the Timed Up and Go test. They were instructed to perform the task at their usual pace, and at each assessment session, participants completed the task three times if possible. The best time was used for analysis.

Secondary outcome measures

Secondary outcomes were as follows: JTT and TUG assessed across all time-points, spasticity assessed with the Modified Ashworth Scale (MAS) and 10m walk test. We also included parent/guardian and child-reported measures: The Gait Outcomes Assessment List (GOAL) and the Children's Hand Use Experience Questionnaire (CHEQ).

3.2.4 Safety and tolerability

A process evaluation questionnaire to assess the feasibility and acceptability of the study was completed by parents and participants at their final follow-up visit. Tolerability and side effects were assessed based on a pediatric non-invasive brain stimulation safety and tolerability questionnaire[259,200] which ranks the session in comparison to seven common childhood experiences ranging from playing a game to going to the dentist.

The questionnaire additionally included questions on the acceptability of session duration and number, difficulty level of motor training, and participants' perception of what effect, if any, the study had on them.

3.2.5 Study intervention

Motor activities

The intervention of ten sessions over two weeks consisted of 90 minutes of motor activities paired with 20 minutes of anodal or sham tDCS. The activities included functional upper and lower limb tasks incorporating principles of the Hand Arm Bimanual Intensive Therapy (HABIT)[260], the upper limb intensive (Magic) camp programme[261] and the HABIT Including Lower Extremities (HABIT-ILE)[246]. HABIT uses tasks that require both arms to improve the use and coordination of arms in daily function. HABIT-ILE additionally incorporates lower limb activities with the bimanual activities, aiming to improve participants' ability to carry out activities of daily living and participate in social activities. The HABIT-ILE method applies the principles of structured motor learning in functional and playful tasks, with a gradually increasing motor difficulty. Finally, the Magic camp programme incorporates learning and performing carefully selected magic tricks in an organised, easy-to-follow way, to motivate children whilst learning a bimanual magic trick.

The activity plans were designed by a multidisciplinary team including physiotherapists, occupational therapists and motor neuroscientists. Each session was delivered by a qualified physiotherapist, with additional support from physiotherapists and movement science students as needed.

Activities were individualised to accommodate for level of motor function, and incorporated a step-wise approach to increase difficulty as appropriate to their abilities. Activities were designed to be fun and engaging, while also including the participant's own functional goals wherever possible. To ensure ongoing engagement, two sets of motor activities were prepared for each participant, and were changed over after session 5. The activities remained constant between participants, however, depending on baseline function and ongoing ability, participants may progress

more or less quickly over time. Examples of lower-limb activities included carefully controlling a football through a series of obstacles, following a pattern on the floor that incorporates stepping up and down off a single or double step, and replicating a pattern with cones spread over the floor. Examples of upper-limb activities include wrapping a present, placing ping-pong balls in a pattern in a muffin tray, learning a magic trick, and building a tower with juggling balls.

(a) **(b)**

Figure 3.2: Pictured is one example of a motor activity used in the trial. This task required participants to lunge forward and pick up an object in their more affected hand (a). The object, an elastic toy, then needed to be stretched with both hands and released at a target (b). The activity targeted upper and lower limb function, as well as balance. *Images of trial physiotherapist used with permission*

Transcranial Direct Current Stimulation

Anodal tDCS was delivered during the first 20 minutes of each 90-minute intervention session. Two 5cm x 7cm conductive rubber electrodes in saline-soaked (0.9% NaCl) sponges were attached to a direct current stimulator (Nurostym, Neuro Device Group S.A., Poland). The anode was placed on the motor cortex of the contralateral hemisphere of the more affected upper/lower limb, using the EEG 10-20 system (C3/C4), and placed as close to the midline as possible. This placement is often referred to as ipsilesional. The cathode was placed on the supraorbital ridge contralateral to the anode.

Stimulation was ramped up over 30 seconds to a current of 1mA. Anodal stimulation lasted 20 minutes before being ramped down over 10 seconds. Sham stimulation was delivered in the standard manner: ramped up over 30 seconds, then ramped down and switched off for the remainder of the 20 minutes. This sham protocol is effective for establishing the initial sensations (tingling/itching) induced by tDCS which will then fade[218].

3.2.6 Randomization and blinding

An online computer-generated minimisation randomisation method (rando.la) was used by a non-participant-facing researcher to allocate participants to active or sham stimulation (1:1 treatment to control ratio). The variables considered in the minimisation of between-group differences were age, and upper and lower limb function, using Jebsen-Taylor test scores and timed up-and-go times at baseline. Participants and their parents were blinded to treatment assignment. For researcher blinding, a five-digit numerical code, linked to the sham or active condition, was generated during randomisation. Utilising the tDCS device's 'study mode', the blinding codes ensured the appropriate stimulation condition was delivered, while displaying only the generic stimulation parameters, thus maintaining blinding for the researcher delivering stimulation.

3.3 Data analysis

3.3.1 Statistical analysis

Data were analysed using GraphPad Prism (v10.2.3) and SPSS (v29.0.2, IBM inc). Data were assessed for normality of the standardised residuals using Shapiro-Wilk tests and visual inspection of frequency histograms. If data were assessed to be normally distributed, then parametric statistics were utilised, otherwise, non-parametric statistics were used. Significance was set at $p < 0.05$. For post-hoc tests, multiple comparisons were corrected for using Bonferroni correction unless otherwise specified.

To determine if there was an effect of time (1 week, 6 week, and 12 week) on outcome measures (JTT, TUG and 10m walk test), Friedman's Two Way Analysis of Variance was performed. A 'last one carried forward' approach was employed if 1 data point was absent, and the participant was excluded from analysis if 2 or more data points were missing. Where an effect was found, a pairwise comparison, with Bonferroni correction, was then performed to explore the timepoints at which differences occurred.

To determine if there was an effect of condition (anodal or sham) on outcome data, independent samples t-tests or Mann-Whitney U tests were used on the percentage change scores in outcome in the two groups, at each timepoint (1 week, 6 weeks, and 12 weeks).

3.4 Results

3.4.1 Population and demographics

Ninety-eight families were contacted to discuss enrollment. Of the 98 potential participants identified, 37 did not complete screening or lost contact, 17 were ineligible, and 15 declined participation. The most common reason to decline participation was due to the time commitment required for the study. Adherence to the study protocol was good, with 74% of participants completing all 10 intervention sessions, and 100% of participants completing the primary endpoint (1 week follow-up assessment). The CONSORT diagram is illustrated in Figure 3.3.

Participant characteristics are described in Table 3.2.

Table 3.2: Participant demographics and clinical characteristics including predominant motor type and distribution of motor impairments.

	Active	**Sham**
N	14	13
Age: mean (SD) years	13 (1.6)	12 (1.9)
Sex (F:M)	4:10	6:7
Motor cortex stimulated (R:L)	12:2	7:6
GMFCS: median (range)	2 (1-3)	2 (1-3)
MACS: median (range)	2 (1-3)	2 (1-3)
JTT, seconds: mean (SD)	99 (102)	117 (170)
TUG, seconds: mean (SD)	10 (2)	25 (31)

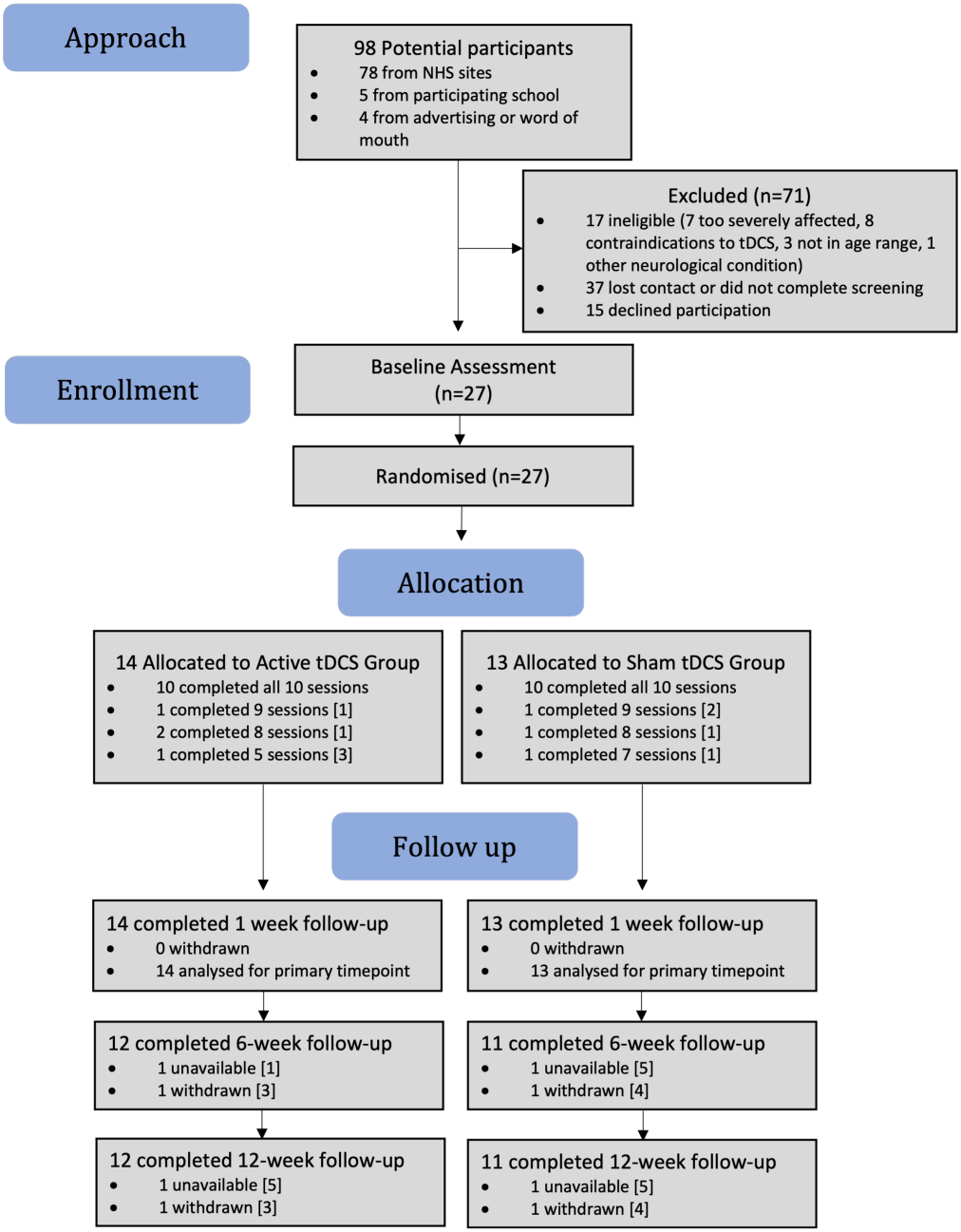


Figure 3.3: CONSORT flow diagram of study recruitment and retention. *Reasons for not attending a session were: [1] scheduling difficulties & staff availability, [2] injury (not related to study), [3] declined to continue, [4] left the UK, [5] participant on holiday*

3.4.2 Effect of 10 sessions of motor training on upper and lower limb function

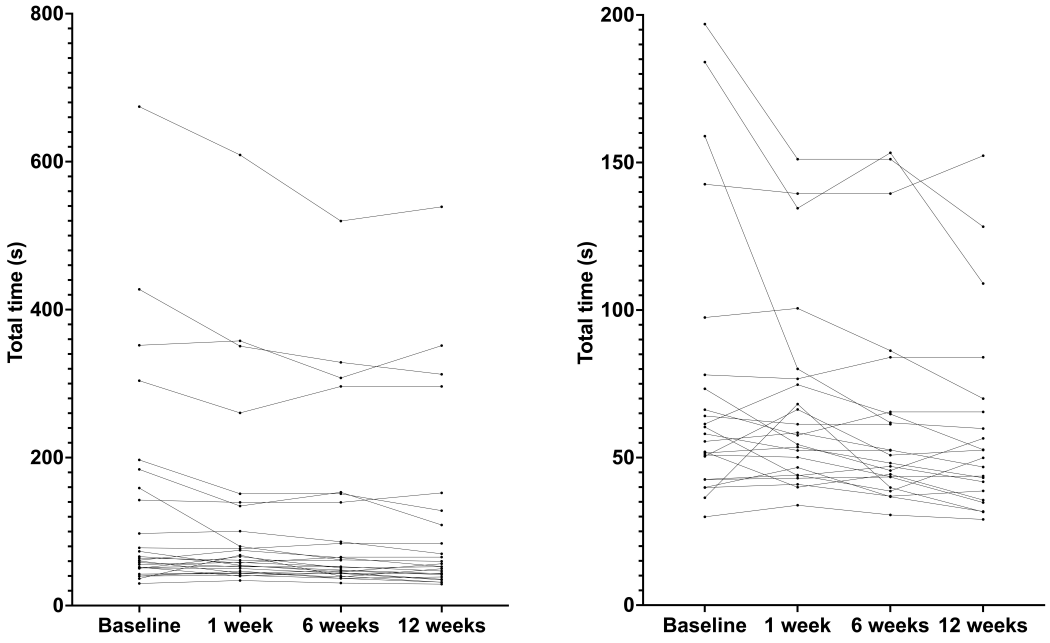


Figure 3.4: JTT times for participants across follow-up assessments in (a) all participants (b) participants with a time of less than 200s to better illustrate trends. A decrease in time denotes an improvement of function.

The two primary outcome measures were the Jebson Taylor Test (JTT) for upper limb function, and the Timed Up and Go (TUG) test for lower limb function. We hypothesised that after 10 sessions of motor training, participants would demonstrate lower JTT and TUG time, indicating an improvement from baseline. We also hypothesised that this effect would persist at follow-up timepoints.

A Friedman test was conducted to determine whether there was an effect of session on JTT time across baseline, 1 week, 6 weeks and 12 week follow-up, as shown in 3.4. The effect of the 10-session motor training intervention, irrespective of tDCS condition allocation on hand function, showed a significant effect of time ($\chi^2(3) = 18.09$, p = 0.0004). To determine the pattern of differences, pairwise comparisons were performed, with Bonferroni correction for multiple comparisons, which showed a significant improvement between baseline and 12-week JTT time

(z = 3.67, p = 0.0015), as well as between 1 week and 12 week JTT (z = 2.027, p=0.01). No other comparisons were found to be statistically significant.

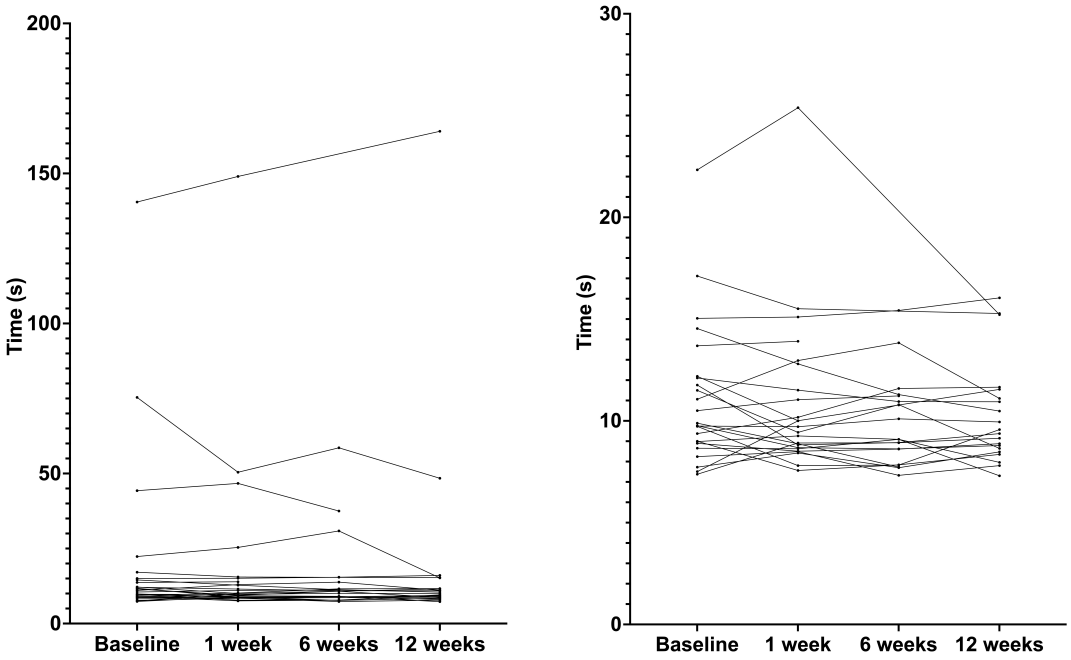


Figure 3.5: TUG times across follow-up assessments in (a) all participants (b) participants with a time of less than 35s to better illustrate trends. A decrease in time denotes an improvement of function.

To evaluate changes in lower limb function, Timed Up and Go times, as well as 10m walk test times, were evaluated. A Friedman test was conducted to test the hypothesis that there was an effect of time on TUG times, see Figure 3.5. The results show no effect of time ($x^2(3) = 2.73$, p = 0.44) and the null hypothesis was retained. However, a Friedman test conducted on the 10m data demonstrated a significant effect of time on 10m walk times ($x^2(3) = 14.59$, p = 0.0022). To determine the pattern of differences, pairwise comparisons were performed, with Bonferroni correction for multiple comparisons, which demonstrated a significant improvement between baseline and all three follow-up time points (1 week: z = 2.96, p = 0.018; 6 week: z = 2.85, p = 0.0262; 12 week: z = 3.35, p = 0.0048).

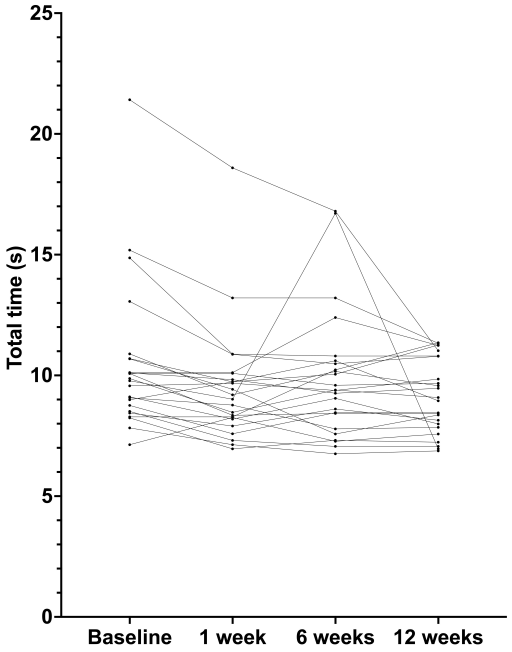


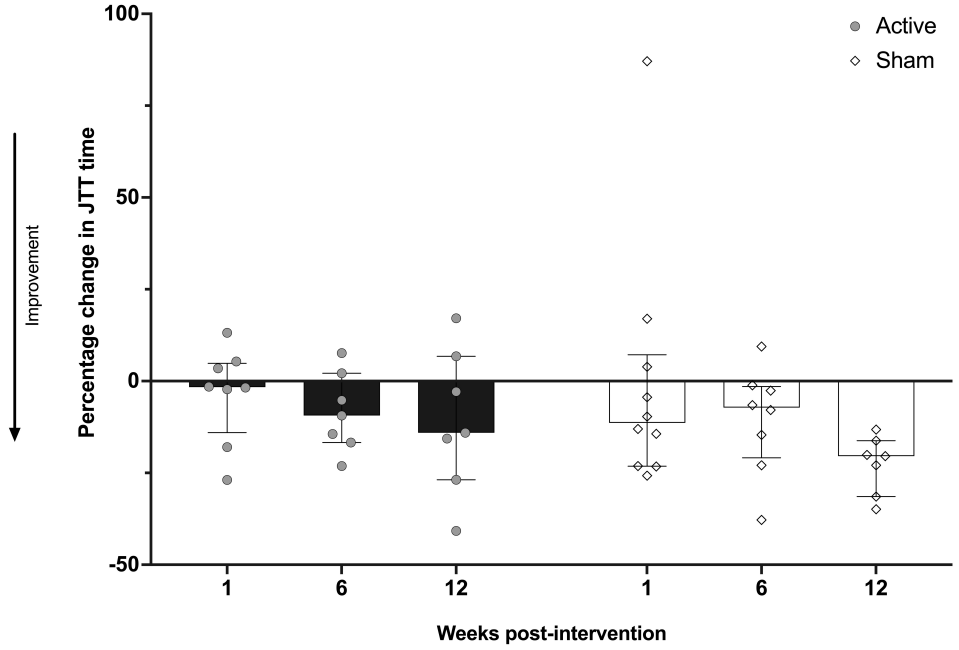
Figure 3.6: 10m walk test times across all follow-up assessments. A decrease in time denotes an improvement in function.

3.4.3 Effect of tDCS condition on upper limb outcome measures

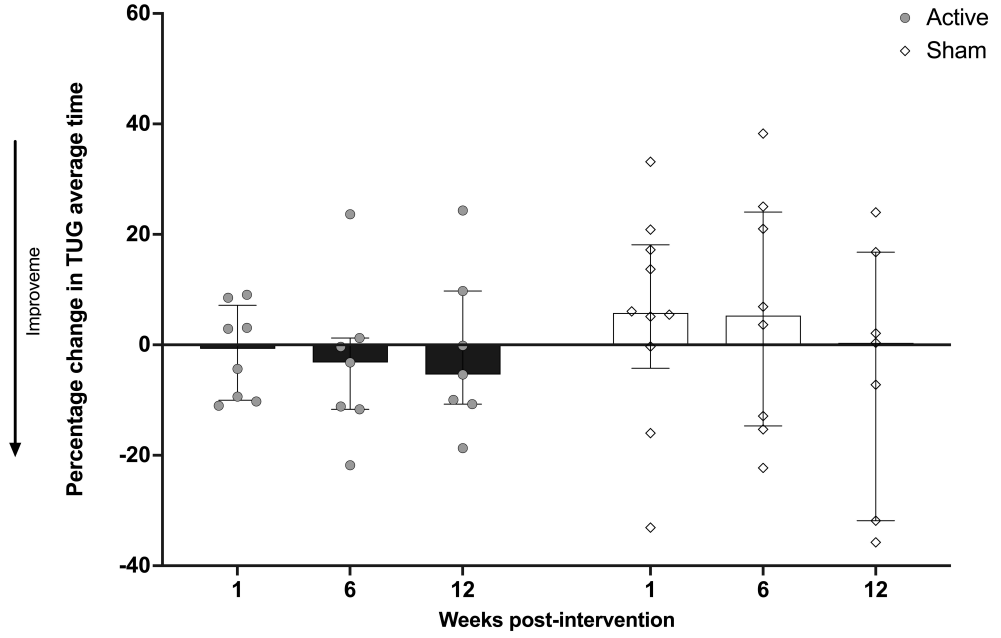
Fourteen participants were randomised to receive anodal tDCS to the motor cortex of the hemisphere contralateral to their most affected limb. We hypothesised that participants who received active tDCS would show more consistent and persistent motor improvements than sham participants (n = 13).

To probe this hypothesis for upper limb function, we performed multiple Mann-Whitney tests of percentage change in JTT time in active versus sham (condition) groups. No significant effect of condition was found for the percentage change in JTT time for any of the follow-up assessment sessions (1 week: U = 80, p = 0.62; 6 weeks: U = 64, p = 0.93; 12 weeks: U = 43, p = 0.28). See Figure 3.7a.

To test our hypothesis that participants who received anodal tDCS would show more consistent and persistent improvements in lower limb function, we assessed the percentage change in score with multiple Mann-Whitney tests in score in active versus sham (condition) groups. No significant effect of condition was found for percentage change in TUG time at any time point - Figure 3.5a (1 week: U =



(a) Percentage change in JTT from baseline, according to stimulation condition. A negative percentage change indicates an improvement in hand function



(b) Percentage change in TUG time from baseline, according to stimulation condition. A negative percentage change indicates an improvement in lower limb function

55, p = 0.08; 6 weeks U = 45, p = 0.21; 12 weeks U = 56, p = 0.82). No significant effect of condition was found for the percentage change in the 10m walk test, data shown in Figure 3.8 (1 week: U = 61, p = 0.37; 6 weeks: U = 61, p = 0.57; 12 weeks: U = 64, p = 0.69).

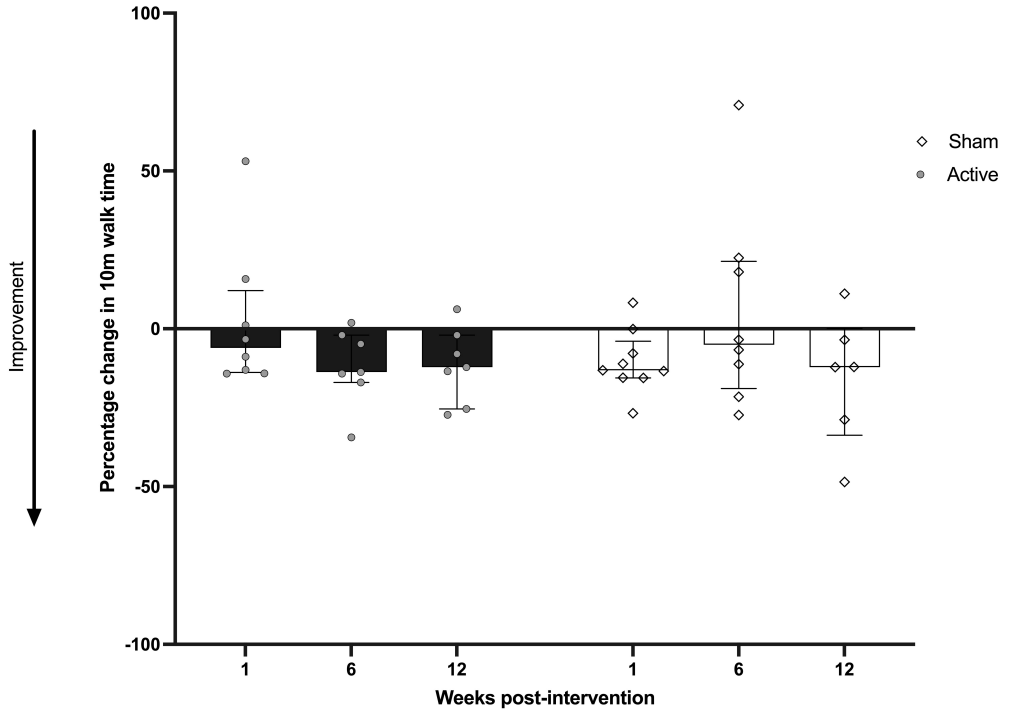


Figure 3.8: Percentage change in 10m walk time from baseline, according to stimulation condition. A negative percentage change indicates an improvement in lower limb function

3.4.4 Probing participants' perception of upper and lower limb functional changes

In addition to quantitative metrics of the JTT, TUG and 10m walk, we collected several self-reported metrics of upper and lower limb function.

Using the Children's Hand Use Experience Questionnaire (CHEQ), we evaluated the experience of participants in using the affected hand in activities where usually two hands are needed. Across both the active and sham group, participants reported increased use of both hands in bimanual activities after the intervention. There was a significant effect of time ($x^2(3) = 22.43$, $p = <0.0001$) on CHEQ score. A follow-up pairwise comparison, with Bonferroni correction, demonstrated a significant improvement between baseline and 6-week score ($z = 2.96$, $p = 0.018$), baseline and 12-week score ($z = 4.36$, $p < 0.0001$), and 1-week and 12-week score ($z = 2.74$, $p = 0.037$).

To account for large variations in baseline score across participants, percentage change scores were then used for group wise comparisons. There was no difference

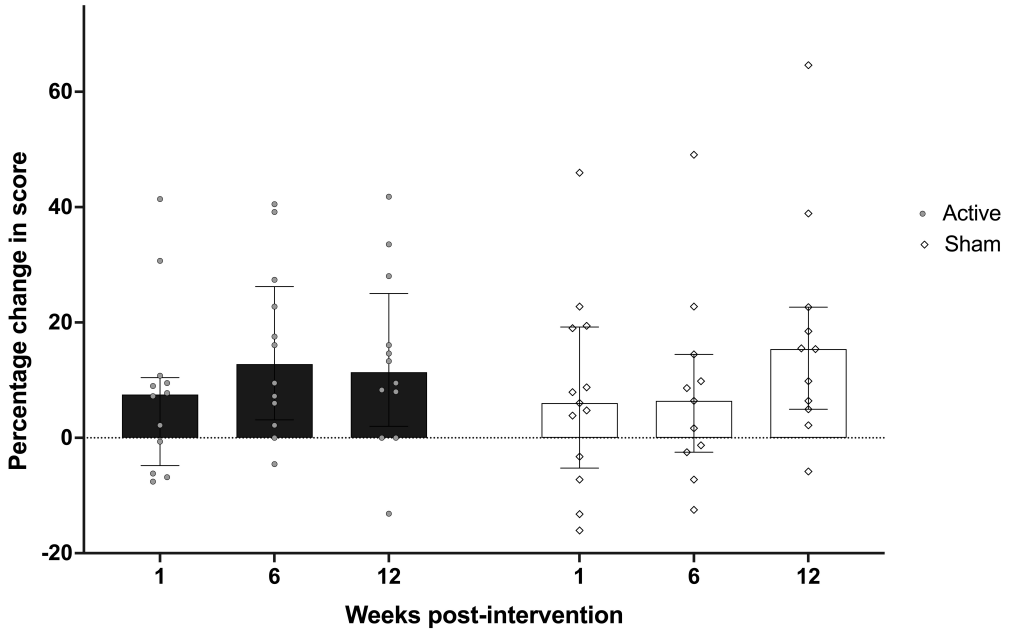
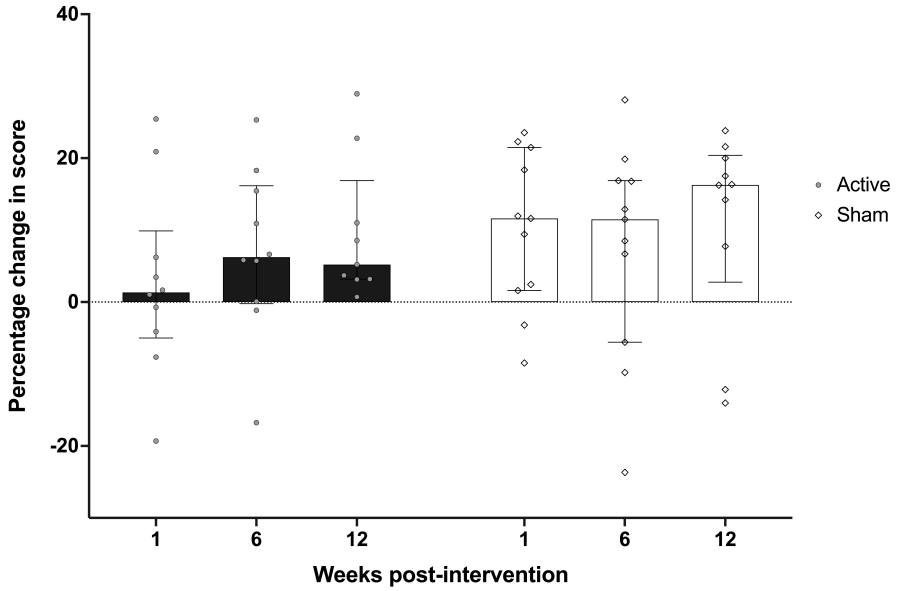


Figure 3.9: Percentage change in CHEQ score from baseline, according to stimulation condition. A positive change in score indicates increased use of both hands in bimanual activities

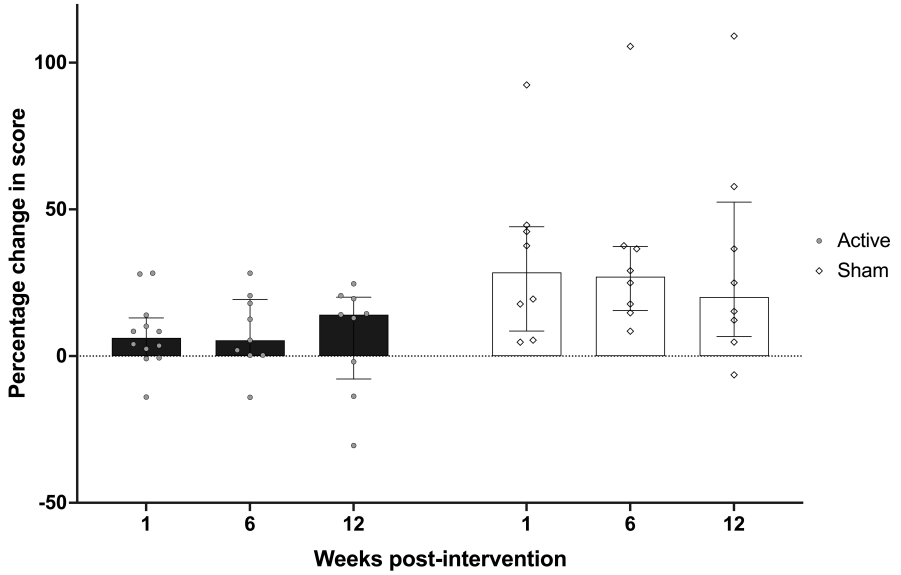
in effect across condition, reflected in Figure 3.9, which was tested using multiple Mann-Whitney tests of the percentage change in score in active versus sham (condition) groups (1 week: U = 75, p = 0.89; 6 weeks: U = 45, p = 0.21; 12 weeks: U = 59, p = 0.68).

The Gait Outcomes Assessment List (GOAL™) was used to assess self-reported functional mobility, priorities, and expectations of participants and their parents. The GOAL questionnaires (v5.0; parent and child versions) were completed on paper, with children and parents separated during completion. A researcher was present to support as needed. Scoring of the GOAL was performed automatically by a formula-protected analysis sheet provided by the GOAL developers. A higher score reflects greater ease in performing activities independently.

There was no main effect of time on the child-reported GOAL score ($x^2(2) = 1.9$, p = 0.387). The child-reported measure is illustrated in Figure 3.10a, reflecting the percentage change in score from baseline to 1, 6 and 12-week follow-up scores. The percentage change score was used for between group comparisons, and no statistically significant group differences between anodal and sham group



(a)



(b)

Figure 3.10: Percentage change in GOAL score from baseline, according to stimulation condition. (a) Child reported and (b) Parent reported. A positive percentage change indicates a self-reported improvement functional mobility, including the ability to perform ADL's involving the lower limbs.

at the follow-up assessments (1 week: U = 44, p = 0.467; 6 weeks: U = 47, p = 0.605; 12 weeks: U = 36, p=0.197).

There was no main effect of time on the parent-reported GOAL score ($x^2(2) = 0.613$, p = 0.736). For between-group comparisons, the percentage change in score

from baseline to 1, 6, and 12 week follow-up scores was used, and is illustrated in Figure 3.10b. There were statistically significant group differences between between the anodal and sham group at the 1-week and 6-week follow-up assessments (1 week: U = 16, p = 0.037; 6 weeks: U = 11, p = 0.046); but not at 12 weeks (U = 22, p = 0.599), after applying Bonferroni correction for multiple comparisons.

We included the Modified Ashworth Scale (MAS) assessment in baseline and follow-up visits, to assess if there was an effect of group on limb spasticity. Figure 3.11 demonstrates the change in MAS score from baseline for each follow-up assessment. At one week, 77% of the active group and 85% of the sham group showed no change or an improvement in spasticity, at 6 weeks this was 69% of the active group and 85% of the sham group showed and at 12 weeks 85% of the active group and 100% of the sham group showed no change or an improvement in spasticity. There was no main effect of time on spasticity ($x^2(2) = 5.56$, p = 0.062). There was no significant difference in the change in MAS score between the two groups at any of the follow-up assessments (1 week: U = 73, p = 0.56; 6 weeks: U = 70, p = 0.45; 12 weeks: U = 67, p = 0.35).

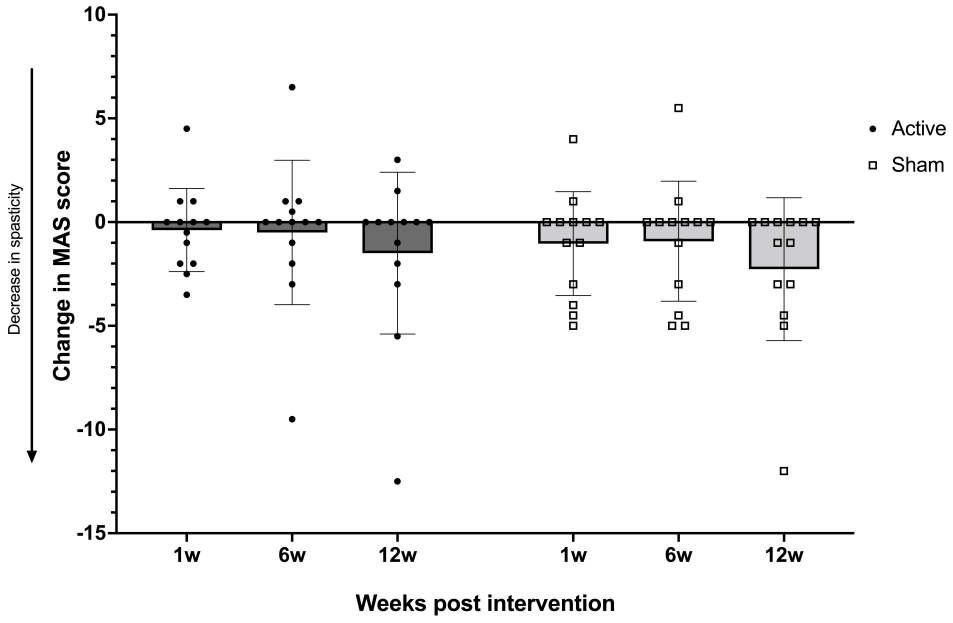


Figure 3.11: Change in Modified Ashworth Scale scores from baseline

3.4.5 Safety and Tolerability

As this was a pilot study, we additionally probed the safety and tolerability of the intervention.

At their first follow-up, participants were given a process evaluation questionnaire to gather information on their experience of the intervention. In terms of programme design, participants were asked about the length of session and difficulty level of tasks. The majority of participants in both active (71%) and sham (91%) groups reported that sessions were the appropriate length of time (90 minutes) and that the activities and exercises were targeted at the appropriate level (69% of active group and 60% of sham group). 93% of the active group reported that 10 sessions was the appropriate number of sessions, but only 55% of the sham group shared this sentiment, while 27% felt that 10 sessions were too many.

To better understand the participants' perceptions of brain stimulation, we asked them to rank how pleasant the tDCS was compared to common childhood experiences, from a birthday party (very pleasant) to getting an injection (very unpleasant). The most common experience of participants was to liken the experience to taking a long car ride (50% of the anodal group, and 42% of the sham group). 14% of the active group, and 25% of the sham group likened the experience to the most unpleasant experience on the scale - getting an injection. There was no clear relationship between receiving active stimulation and a less pleasant experience.

We also asked participants which, if any, of the commonly reported side effects they experienced after tDCS. Participants were able to report more than one side effect. Feeling tired was the most common effect reported by parents (Table 3.4) in both active and sham groups (Parents: 31% active, 27% sham, Participants: 21% active group, 64% sham), while the most common effect reported by participants was itchiness, in 50% of the active group, and 82% of the sham group.

There was no difference between groups for the proportions rating each of the common adverse effects (Table 3.4) and no other adverse effects were identified.

Table 3.3: Participant evaluation

	Active	**Sham**
The 90-minute sessions were...		
Too long	3 (21%)	1 (9%)
Too short	1 (7%)	0 (0%)
Just right	10 (71%)	10 (91%)
10 sessions of exercises were...		
Too many	0 (0%)	3 (27%)
Too few	1 (7%)	2 (18%)
The right amount	13 (93%)	6 (55%)
The activities and exercises?		
Too easy	4 (31%)	2 (20%)
Too difficult	0 (0%)	2 (20%)
It was alright	9 (69%)	6 (60%)
Do you think the study helped you?		
Yes	13 (93%)	10 (91%)
No	1 (7%)	1 (9%)
It helped me with moving my arm(s)	12 (86%)	10 (91%)
It helped me with moving my leg(s) and/or walking	13 (93%)	10 (91%)
It helped me with my balance	4 (29%)	5 (45%)
It helped me make friends	2 (14%)	1 (9%)
I think the brain stimulation was as pleasant as:		
A birthday party	0 (0%)	0 (0%)
Playing a game	2 (14%)	2 (17%)
Watching television	1 (7%)	1 (8%)
Long car ride	7 (50%)	5 (42%)
Going to the dentist	1 (7%)	1 (8%)
Throwing up	1 (7%)	0 (0%)
Getting an injection	2 (14%)	3 (25%)

Table 3.4: Frequency of side effects reported

	Participant reported		**Parent reported**	
	Active	**Sham**	**Active**	**Sham**
Headache	3 (21%)	2 (18%)	1 (8%)	0 (0%)
Itchiness	7 (50%)	9 (82%)	3 (23%)	1 (9%)
Feeling dizzy	0 (0%)	3 (27%)	0 (0%)	0 (0%)
Tingling	3 (21%)	5 (45%)	3 (23%)	0 (0%)
Neck pain	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Feeling tired	3 (21%)	7 (64%)	4 (31%)	3 (27%)

3.5 Discussion

This pilot study has demonstrated that delivering 10 sessions of tDCS and motor training for the upper and lower limbs is feasible in children with cerebral palsy.

Firstly, we hypothesised that after 10 sessions of motor training, participants would show improvements in upper and lower limb function, measured by JTT and TUG times. We also hypothesised that this effect would continue to persist at follow-up sessions. We demonstrated that hand function improved post-intervention, with a statistically significant difference between baseline and 12-week JTT times. While a familiarity/practice effect may contribute, our findings are are in keeping with prior studies and systematic reviews that have demonstrated intensive bimanual training improves upper limb function in children with CP[75,76,78,79]. These studies have assessed intensive bimanual therapy against other training modalities, such as CIMT, as well as against no-therapy controls, and shown consistent group effects of bimanual therapy. While we acknowledge that conclusions may be limited by the lack of a no-therapy control group, this was outside the scope of our study design. This is likely a true effect of the intervention based on concordance with previous studies.

We did not find an effect of time on Timed Up and Go times, our primary lower limb outcome measure, but did demonstrate an effect of time on 10m walk test across all follow-up time points (1, 6, and 12 weeks). One potential explanation for this discrepancy in lower limb outcomes may be that the TUG test evaluates a more complex task, of standing from sitting as well as walking, testing balance and mobility skills, while the 10m walk tests mobility[262].

Secondly, we wanted to determine if anodal tDCS of the motor cortex enhances the effects of the 10-day intervention. Overall, we found no effect of stimulation on motor function in any of the objective outcome variables for upper and lower limb motor function at any time-point.

Thirdly, we did not demonstrate any between-group effects on spasticity post-intervention.

There are several possible reasons for this lack of effect. While our sample size was comparatively larger than the majority of similar studies, it remains

small, with 14 participants in the active group and 13 in the sham group. Our sample size calculation (15 per group) was based on limited previous studies, which had varying methodologies and effect sizes. Our population, while more closely mirroring the clinical population of children with cerebral palsy, was more heterogenous than other studies that opted to recruit primarily participants with CP from perinatal stroke[209,207]. The diversity of underlying pathology and differences in brain anatomy as a result may have affected the current flow during stimulation and led to decreased current flow at the site of the M1 target[263]. Improved targeting of the motor cortex could potentially be achieved with neuronavigation using brain imaging, or transcranial magnetic stimulation (TMS) to localise a motor 'hotspot', which may have allowed for a more personalised and accurate electrode placement. However, the use of TMS adds additional burden to both researcher and participant in what were already logistically difficult intervention sessions, while requiring access to MRI may exclude participants and worsen recruitment challenges. Many of our participants had bilateral motor involvement, adding to the complexity of the underlying motor networks and physiology.

Anodal tDCS remains of uncertain benefit in this population. While studies on upper limb effects have demonstrated an effect on movement duration[208], spasticity[206,205], and manual dexterity[211,212], most of these effects have been secondary outcome measures and results are inconsistent between studies. Studies of cathodal stimulation on upper limb function have not demonstrated group effects of stimulation over training without stimulation[209,207]. We found no effect on primary outcome measures or spasticity. Subjective scores of upper limb function (CHEQ) demonstrated a significant effect of time but not condition and subjective parent scores of lower limb function demonstrated a significant effect on the group at 1 and 6 weeks follow-up. Studies on lower limb function have demonstrated improvements in walking speed[201], velocity and cadence,[202] and improved balance[204] in participants who received anodal M1 stimulation. However, a group effect of stimulation was not seen in all studies[203].

Our third aim for this study was to demonstrate the safety and tolerability of a 10-session tDCS intervention in children with CP. In general, both active and sham stimulation were well tolerated, with similar side effect profiles. The most common side effect reported by participants was tiredness, while headache, itching and tingling also occurred. This is in keeping with other studies, such as Gillick et al.[209]. Gillick et al. (2018) also report their side effects were headache (40% in active and 10% in sham) and itchiness (10% active and 30% sham). We had no adverse events in this study.

Overall, our study was safe and tolerable, but we found no effect of stimulation on motor function in any of the outcome variables at any study time-point. Conducting and publishing large, well designed studies of tDCS in cerebral palsy adds to the literature, and even negative results are valuable in mitigating possible publication bias in the literature.

Lack of consistent stimulation effect may also be due to participant variability. A better understanding of subject variability may help us disentangle whether tDCS can be a useful therapy in children with CP. Some potential avenues may include the development of robust and clinically relevant biomarkers to assist in predicting treatment response and electric field modelling to understand potential variability arising from abnormal underlying anatomy.

4

The potential utility of corticospinal tract integrity as a correlate of function

Contents

4.1 Introduction

Current rehabilitation interventions in cerebral palsy are predicated on motor system integrity and motor learning principles (Section 4.1.1). However, a spectrum of insults can contribute to cerebral palsy, and conventional magnetic resonance imaging (MRI) shows both gross structural lesions and 'apparently normal' structure (Section 1.1.4). Diffusion-weighted imaging and automated tractography can non-invasively measure motor network white matter tract integrity in patients with CP. In this chapter, I applied this approach to measure corticospinal tract integrity at baseline, as well as intervention-related plasticity, in a clinical rehabilitation trial for children with cerebral palsy.

4.1.1 Rehabilitation interventions in CP are predicated on motor system integrity and motor learning principles

As described in Chapter 3, many current rehabilitation protocols based on motor learning principles have been developed over the last two decades to address motor function in children with cerebral palsy. These include interventions for upper extremity function[244,243,245,84] and programmes to improve lower limb function[246,79,247].

These interventions have been developed with the underlying principle of neuroplasticity in mind, encouraging experience-dependent motor learning[79].

While there is moderate to strong evidence for functional improvement for specific programmes such as HABIT, HABIT-ILE and mCIMT[247] to assist with functional improvement, reliably demonstrating neuroplastic changes has been challenging using both non-invasive brain stimulation and neuroimaging[264].

Clinically translating the approach of optimising motor learning in cerebral palsy assumes some level of maintained integrity and function of the motor system to allow learning and plasticity. In particular, corticospinal tract integrity may be a key determinant of motor system function at baseline and in response to intervention[265].

4.1.2 White matter plasticity

In recent years, another facet of brain plasticity has been identified involving white matter and myelination. While this plastic process is distinct from synaptic plasticity, it also has similarities in common: it is activity dependent, and it is implicated in learning[266].

Activity dependence has been clearly demonstrated in extensive preclinical studies which demonstrate that myelination processes can be influenced by increases in neuronal activity. High-frequency stimulation[267,268] and pharmacological manipulation[269] which increase neuronal firing rates, result in increased myelin sheath formation and myelin compaction within 2–14 days. Hines et al (2015) demonstrated that neuronal activity guides the selection of axons for myelin wrapping and directs maintenance of sheaths of only active neurons[270]. Furthermore, preclinical evidence using transgenic mice has also demonstrated that myelin is necessary for novel motor learning[271].

Advances in neuroimaging have also allowed us to study changes to white matter structure in vivo in response to learning. Longitudinal studies have demonstrated that metrics of white matter integrity within relevant white matter tracts increase after training: examples include 6 weeks of juggling practice[272], 6 weeks of balance training[273], 4 weeks of unimanual motor training[274], 3 weeks of unicycle training[275] and 9 sessions of visuo-spatial motor training[276].

4.1.3 Conventional MRI shows both gross structural lesions and 'apparently normal' structure

Diffusion-weighted imaging, optimised for probabilistic tractography, will be utilised in this chapter to probe corticospinal tract integrity. In section 1.1.4 in Chapter 1, the current state of neuroimaging in cerebral palsy was outlined - including cranial ultrasound in neonates and conventional structural magnetic resonance imaging (MRI) in neonates and children[277]. Brain lesions in CP vary by insult and developmental period, reflecting the specific vulnerability of blood supply and developing cells[278]. Differing brain lesions contribute to clinical phenotype heterogeneity[6].

The prevalence of different patterns of injury in children with CP varies across the literature, but a recent systematic review suggests white matter lesions predominate: present in 57.8% of all children with unilateral CP (uCP), 67.0% of all children with bilateral CP (bCP), and 33% of children with mixed CP subtypes. Grey matter lesions were most frequently seen in children with dyskinetic CP (42.2%). Five percent of children with uCP were found to have brain malformations, and none were found in children with bCP. No visual abnormalities on structural MRI were reported in 5.7% of all cases[279].

These findings differ from previous studies which estimated the prevalence of a 'normal' MRI (no detectable abnormality on T1- and T2-weighted MRI) to be as high

as 14 - 17% of children with cerebral palsy with functional limitations[280,281,282,283]. The variation in estimates is likely related to between-country variations in diagnostic protocols: in many Scandinavian countries routine MRI scanning is performed in children with cerebral palsy in infancy and in childhood, and are therefore likely to better reflect the spectrum of injury. In contrast, in the UK, MRI tends to be used when there is diagnostic uncertainty.

This substantial proportion of 'apparently normal' scans highlights a limitation of MRI in CP - a focus on macroscopic lesions and an inability to probe microstructural integrity. Furthermore, even if gross pathology is identified, clinical scans do not reflect the underlying function or organisation of brain.

4.2 Diffusion MRI and its clinical translation potential in CP

4.2.1 Diffusion imaging

Diffusion weighted imaging is a form of magnetic resonance imaging which utilises differences in the random (Brownian) motion of water molecules within a voxel of tissue, to generate signal contrast[284]. Diffusion of water molecules is restricted within tissues, therefore mapping the diffusion process can reveal microscopic details about tissue architecture[285,286].

To overcome the limitations of conventional MRI, we utilised modern diffusion-weighted magnetic resonance imaging (DWI) incorporating multiple b-values and a large number of field gradient directions. Through sensitising the MR signal to water diffusion at microscopic scale - DWI indirectly probes brain tissue microstructure in-vivo to a resolution of 2–3 orders of magnitude finer than typical millimetre-scale MRI voxels[287]. In areas with few barriers to movement, such as cerebrospinal fluid (CSF), diffusion of water molecules will occur at the same rate in all directions,

known as isotropic diffusion. If diffusion is restricted, for example, due to the presence of cell membranes or myelin, water will move more freely along the principal axis of the tract rather than perpendicular, known as anisotropic diffusion. The degree of anisotropy of water molecules' diffusion directions, and the principal direction of movement, are influenced by many factors such as fibre density, fibre diameter, and myelination. By fitting computational models to the diffusion data in each voxel, these parameters and the underlying biological factors can be estimated. The validity of these models has been tested in healthy individuals[288,289], as well as post-mortem imaging in preclinical models[290] during the development of the modality. More recently, a meta-analysis by Lazari and Lipp (2021) demonstrated good concordance between various myelin histology metrics and markers from different MRI modalities, including fractional anisotropy[291].

4.2.2 The Diffusion Tensor Model

The diffusion tensor (DT) describes the diffusion of water molecules using a Gaussian model, and is the most commonly fitted model to define diffusion data[292,293,294,288] This model describes diffusion at each voxel using an ellipsoid or tensor to estimate the average diffusion in all directions, known as mean diffusivity (MD), and the degree of anisotropy of a diffusion process, known as fractional anisotropy (FA). Fractional anisotropy is a scalar measurement from 0 to 1, corresponding to completely isotropic diffusion to completely anisotropic diffusion, respectively, and is used as a measure of white matter tract integrity.

In areas with high isotropic diffusion, such as in the ventricles, FA will be the lowest and close to 0 (Figure 4.1 A), and in areas of anisotropic diffusion, such as white matter tracts, FA will be higher (Figure 4.1 B). White matter FA values in healthy adults vary from approximately 0.45 in subcortical white matter, to 0.58 in highly organised white matter tracts such as the corpus callosum[295]. FA is influenced by the size, organisation and number of myelinated axons, as well

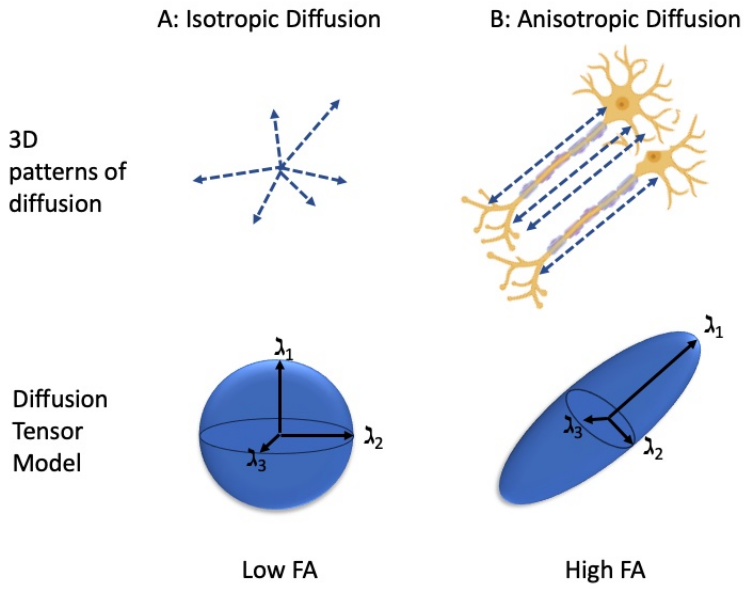


Figure 4.1: Patterns of diffusion and the corresponding diffusion tensor model. Diagram A illustrates isotropic, or unrestricted diffusion, which tends to occur in CSF. Diagram B illustrates anisotropic, or restricted diffusion, along myelinated neurons. FA Fractional anisotropy.

as by local tissue microstructure, where it is higher in areas with denser axon packing[296,297] and lower in areas of damaged myelin[286,298]. Diffusion tensor imaging (DTI) measures of white matter are therefore summary measures of integrity and do not reflect a single biological change in tissue microstructure.

The measures from DTI can be derived at the spatial resolution of individual voxels across the whole brain, or summarised in specific regions of interest. Additionally, DTI data can be used to reconstruct tracts computationally by sequentially joining together voxel-level estimates of fibre orientation to create continuous 3-dimensional tracts - a technique called tractography.

4.2.3 DTI metrics across development

The ability to probe white matter tract integrity in vivo has changed, expanded, and refined our understanding of white matter development across the lifespan. From

imaging the subtle structures of the fetal brain in utero at 18 weeks gestation[299], to understanding the temporal course of white matter maturation across the third trimester and neonatal period from inner to outer layers, and from anterior to posterior; DWI has given us far greater understanding of white matter development[300].

Beyond demonstrating that regional maturation varies in timing across development, DTI methods have also demonstrated that the maturation of white matter tracts is non-linear, with the greatest rates of change observed in the majority of tracts by 5 years of age[295,301]. Lebel et al (2008) demonstrated in a large study of 202 typically developing children, adolescents, and young adults that maximal FA measures were achieved at different time points for different tracts. While 90% of participants reached maximal (adult) FA values by the age of 11 in the corpus callosum and the inferior longitudinal fasciculus (connecting occipital and temporal lobes), maturation was only achieved between 13 to 20 years for the anterior limb of the internal capsule (connecting cortex to medulla) and by 21-24 years of age for the projection fibres in the external capsule (coursing between basal ganglia and cortex), posterior limb of the internal capsule (containing corticospinal and sensory fibres), and corticospinal tracts[295]. Further, white matter change is likely to continue throughout the lifespan[302] given it has the ability to undergo change in an activity-dependent manner[272].

If we consider the ongoing maturation of motor-related white matter tracts, such as the corticospinal tract, in the context of activity-dependent changes and boosting plasticity through anodal tDCS (as outlined in Chapter 3), this presents an ideal therapeutic target for cerebral palsy.

4.2.4 Clinical translation of DWI in Cerebral Palsy

Based on the underlying pathophysiology and timing of brain insult, many have hypothesised a correlation between lesion type and functional ability in cerebral

palsy, as well as white matter integrity and ability.

With the benefit of advanced DWI sensitive to white matter microstructure, numerous studies have linked white matter integrity of the corticospinal (CST) and somatosensory pathways (e.g. thalamocortical projections) to upper limb sensorimotor function in children with unilateral CP. In this population, white matter microstructural impairment (decreased FA and increased mean diffusivity) of the CST in an ROI (affected posterior limb of the internal capsule[303,304]; and cerebral peduncle[305]) was moderately to strongly associated with worse upper limb function.

Evidence in children with bilateral CP is more limited - there only two studies, to our knowledge, that correlate functional classification with DTI metrics. In children with spastic bilateral CP, Arrigoni et al (2016) reported reduced FA in the corticospinal tracts, posterior thalamic radiations, corona radiata, and superior longitudinal fasciculus in children with higher GMFCS and MACS levels[306]. Ballester-Plané et al (2017) utilised network-based analysis to identify reductions in FA in sensorimotor system connections, as well as prefrontal, temporal and occipital connections correlated with GMFCS[307] in children with dyskinetic CP. More research is therefore warranted in children with CP to better understand the relationship between white matter integrity and functional classification, particularly in subtypes of CP which affect more than one limb.

An important caveat of these findings is that the studies tend to be stratified by clinical features (e.g. unilateral spastic cerebral palsy) or radiological subtype (e.g. periventricular leucomalacia). Therefore, these findings are challenging to generalise to a more heterogenous real-world population of children with CP. This is a significant limitation of the field, as the majority of clinical guidelines for treatments of cerebral palsy do not sub-stratify guidance according to radiological or pathological CP type, but rather on the basis of functional classifications such as Gross Motor Function Classification (GMFCS)[308] and Manual Ability Classification Score (MACS)[54].

In the UK, this has implications for designing research trials in children with CP, as most potential participants will have a clinical but not a radiological diagnosis of CP, and therefore will not be easily classifiable into subtypes.

To our knowledge, there are currently no studies that have evaluated the relationship between white matter integrity and baseline upper and lower limb function in an **undifferentiated** CP population.

4.2.5 Reliably measuring functional change using diffusion metrics is challenging

Following on from the findings that the integrity of motor tracts in children with CP could relate to function, several studies have evaluated the sensitivity of diffusion metrics to functional improvement following rehabilitation protocols. The majority of these studies are limited by small sample sizes. Weinstein et al (2015) evaluated corticospinal and corpus callosum tract integrity before and after a 60-hour HABIT intervention in 12 children with congenital hemiparesis. They found a correlation between baseline FA in the corpus callosum and hand function, and demonstrated that the intervention resulted in group-level improvements in hand function. However, there were no group-level changes in CST or CC tract integrity following the intervention[304].

However, more recently, FA and mean diffusivity (MD) of the CST were shown to be responsive to a 2-week rehabilitation intervention in a larger study of 40 children with CP[265]. These findings are more in keeping with motor learning studies in healthy participants, which demonstrated that training in a complex visuo-motor skill could result in changes in white matter architecture[272]; and studies in stroke survivors demonstrating increased corticospinal tract FA following a rehabilitation and tDCS intervention[309].

4.2.6 Study aims

An unmet need in CP research and clinical practice is a robust, reproducible, and generalisable biomarker of underlying brain motor system integrity across disease sub-types. An effective biomarker would explain variability in function at baseline, and predict response to interventions. Diffusion tractography, as discussed, could be a promising candidate, but applying tractography in this population has been historically challenging due to protocol and analytic variability, and the challenge of reproducibly identifying tracts in brains with structural lesions.

The overarching aim of this study was therefore to utilise diffusion-weighted imaging to aid in our understanding of our study population, and determine if metrics of white matter integrity in the corticospinal tract would correlate to response to a treatment which utilises principles of motor learning and activity-dependent plasticity.

To our knowledge, there are no studies that evaluate the utility of diffusion metrics as a predictor of rehabilitation response in an **undifferentiated** CP population.

To achieve this, we utilised probabilistic tractography in a heterogenous paediatric cerebral palsy population and aimed to:

1. Demonstrate the feasibility of automated tractography in deriving corticospinal tract measures in a heterogeneous adolescent CP cohort

2. Test the hypothesis that better functional classification (GMFCS and MACS) would be associated with greater CST integrity in an undifferentiated cohort

3. Explore the relationship between baseline measures of upper and lower limb function with corticospinal tract integrity

4. Determine if baseline corticospinal tract metrics can be used to explain variability in response to a 2 week rehabilitation intervention

4.3 Methods

This chapter presents the neuroimaging component of the StimCP study, the full details of which are found in Chapter 3.

4.3.1 Study participants

Participants with a clinical diagnosis of cerebral palsy and aged between 10-16 years were recruited. Participants were required to have a Gross Motor Function Classification Score of between (GMFCS) I-III and a Manual ability classification score (MACS) between I-III; in other words, to have at least enough function in their more affected hand to handle objects with some assistance[54], and be able to walk with assistance for short distances[310].

Participants were recruited from both within the Oxfordshire area, as well as from partner sites further afield. To minimise travel time, impact on schooling and fatigue levels, participants were offered the choice of attending study visits at the nearest site to their home or our site in Oxford. As a result, several participants attended sessions outside of the Oxfordshire area. We deemed it inappropriate to exclude participants from the trial if they were unable to travel to Oxford for the baseline MRI. Additionally, we did not want a contraindication to MRI to result in the exclusion of participants from the trial. Therefore, participation in the MRI component of the study was an optional component of the full study, and standard MRI safety exclusions were applied to those who opted in.

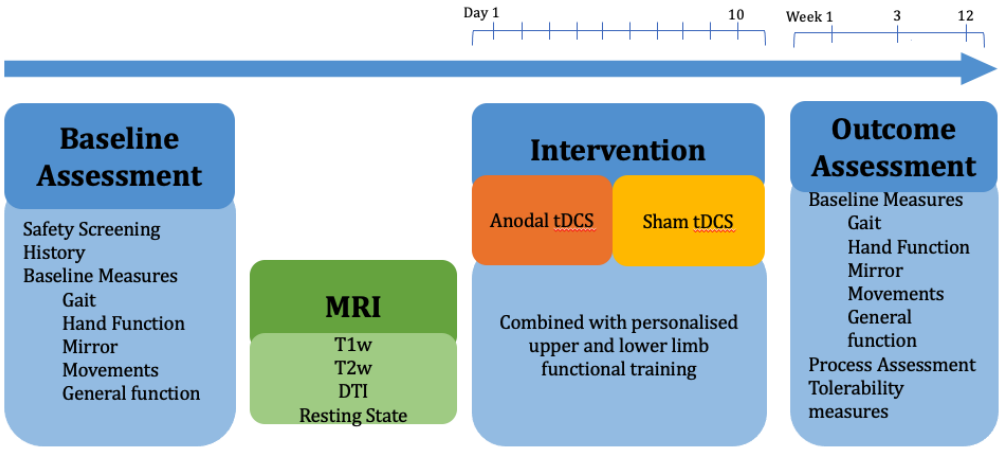


Figure 4.2: StimCP study design: Participants completed a baseline assessment which included safety screening and functional assessment. For those who opted in, a structural and functional MRI was performed before intervention. A ten-day intervention period consisted of 20 minutes of anodal or sham stimulation during a 90 min training block each day. Outcome assessments were done 1, 3 and 12 weeks after the end of the intervention

4.3.2 Study Design

Full trial design is outlined in Chapter 3 section 5.2. MR imaging was performed at baseline, before the 10-day tDCS and training intervention (Figure 4.2).

4.3.3 Magnetic Resonance Imaging (MRI)

MRI data were acquired in a single scanning session at the Wellcome Centre for Integrative Neuroimaging prior to the start of the intervention. Data were collected using a 32-channel head coil in one of two identical 3.0-T Prisma Magnetom Siemens scanners, software version VE11C (Siemens Medical Systems, Erlangen, Germany). Head movement was minimised through the use of foam padding and limb strapping as needed, on an individual basis. Structural, DWI, and resting state functional MRI data were acquired.

The T1w sequence was acquired with a Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence[311]: TR = 1900 ms, TE = 3.97 ms, voxel

size = 1.0 × 1.0 × 1.0 mm, flip angle = 8°, total slices = 192, FOV = 192mm^3.

Diffusion-weighted echo-planar imaging (EPI) data (TR = 2483ms, TE = 78.20ms, FOV = 214mm^3, voxel size = 1.8mm isotropic, multiband factor of 4) were collected for two b-values (1250 and 2500s/mm^2), over 120 directions. An additional 15 volumes were acquired at b=0, 11 in the anterior-posterior phase encoding direction and 4 in the posterior-anterior (PA) phase-encoding direction.

During image acquisition, participants watched an age-appropriate movie they selected to keep them focused and minimise movement and boredom. For participants with dyskinesia, where possible, additional padding was used around the head for stabilisation, and limbs were wrapped to the body as tightly as was comfortable. Children were given the option of having a parent present in the scanner room.

4.4 Data analysis

4.4.1 Image preprocessing

Image preprocessing and analyses were performed using the FMRIB software library (FSL version 6.0) tools[312]. Structural T1 images were processed as part of the *fsl_anat* pipeline as follows: bias-field corrected using the *FAST* tool[313], brain extracted[314], and registered (linear and non-linear) to MNI152 standard space[315,316].

4.4.2 Diffusion processing pipeline

Diffusion data were analysed using FMRIB's Diffusion Toolbox (FDT). Data were collected with reversed phase-encode directions, resulting in pairs of images with distortions in opposite directions. From these pairs, the susceptibility-induced off-resonance field was estimated using a method similar to that described in Anderson

et al. (2003) as implemented in FSL[317], and the two images were combined into a single corrected one using the *topup* tool[318,319].

Brain extraction using BET[314] was then performed on the distortion-corrected b0 output and further processed using eddy[320] to correct for eddy currents and intra-volume head motion.

4.4.3 Tractography

Then, to assess white matter integrity, we selected the corticospinal tract which is particularly affected in cerebral palsy, and performed probabilistic tractography to define the tract.

Probabilistic tractography is an automated technique for identifying white matter tracts. The advantage of this method is that it is less amenable to biases than other methods of estimating white matter tracts, such as manual segmentation using anatomical landmarks or atlas-based techniques that may be less robust in patient groups with variable anatomy. Another advantage of probabilistic tractography is that data can be analysed in native space, mitigating the potential limitation of other techniques such as TBSS which require higher quality registrations to standard space. This was an important consideration for analysis choice in this subject group, who encompass a spectrum of ages and pathologies, and who clearly have a great degree of structural variability and size on visual inspection.

Within FSL, we ran *bedpostx* (Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques for Crossing Fibers) to model the diffusion data incorporating crossing fibres within each voxel. Subject data in diffusion space were registered to MNI152 standard space by combining linear registration from native DWI to native T1w space using *epi_reg* with the nonlinear registration generated by *fsl_anat*.

Using FSL's *XTRACT* tool, probabilistic tractography with the *probtrackx2* tool was run in each subject's native space. *probtrackx2* simulates streamlines originating from each voxel in the seed region that propagate preferentially according to *bedpostx* model parameters fitted to the data in that voxel and surrounding voxels. These streamlines are terminated on reaching a voxel within the target mask or excluded if they reach a voxel within the exclusion mask. The standard *XTRACT* protocol for corticospinal tract identification was used including seed region, exclusion mask and *probtrackx2* settings (3000 streamlines, loopback checking, step length 0.5mm, 2000 steps). The seed, exclusion and target masks are transformed from standard space into each subject's native space using registrations described above. Tractography was then run in native space as specified. Summary statistics (volume, length, median fractional anisotropy, median mean diffusivity) were generated for each tract using the *xtract_stats* tool with a threshold of 0.01 applied to the tract probability map.

Data were visually inspected at each preprocessing stage, as well as post-tractography to assess seed, waypoint and endpoint position, as well as overall tract position.

Tractography has historically been limited by high analytic flexibility in the choice of seed, target and exclude regions and the configuration of streamline behaviour. The FSL tool *XTRACT* represents a large body of work in generating and testing standardised protocols in large datasets to improve the reliability and robustness of identifying important white matter tracts. The reliability of tract identification in UK Biobank data and HCP with thousands of subjects was shown, with high inter-cohort correlation of subjects scanned in both datasets. Finally, the protocols were shown to be robust to incidental and pathological structural lesions. The resultant tool incorporates standard protocols for a range of tracts by specifying seed, target, waypoint, and exclusion masks that are transformed to native space using the provided registrations in order to run tractography in native space.

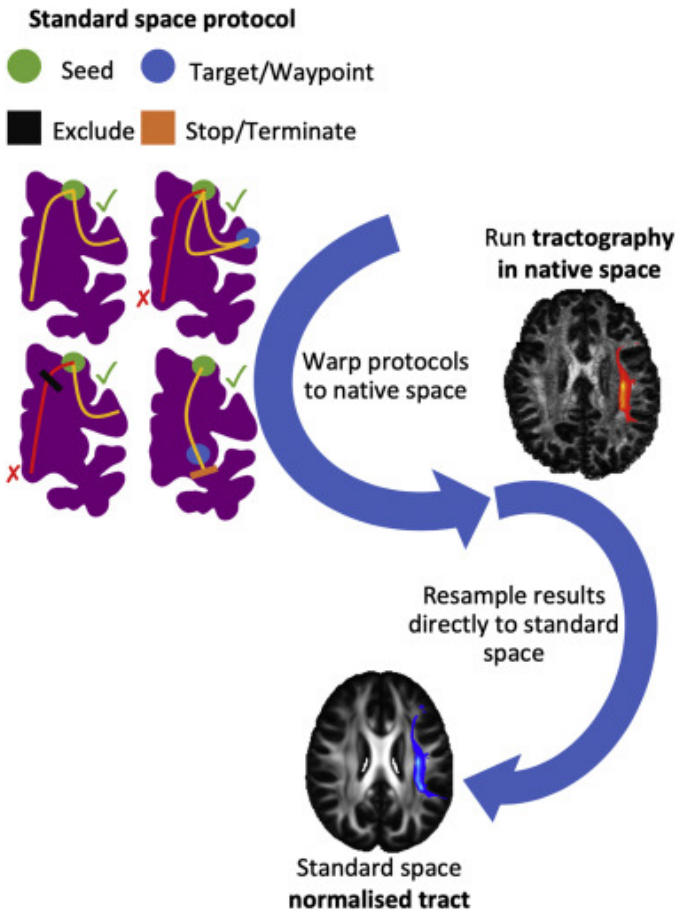


Figure 4.3: Analysis steps for automated tractography using the *XTRACT* toolbox. In this example, the left arcuate fasciculus (AF) for the human brain is shown. Reused with permission.[321]

This maintains the data resolution as acquired and allows for some degree inter-individual tract variability in native space, as opposed to a technique such as TBSS which requires tight overlap of the entire tract in standard space

4.4.4 Outcome measures

As outlined in Chapter 4, the primary outcome measures for the trial were a change in performance time for the Jebson Taylor hand test and a change in performance time for the instrumented Timed Up and Go test, at 1-week post-intervention. In this analysis, we will examine these primary outcome measures, as well as further follow-up visits at time points 6 weeks, and 12 weeks.

4.4.5 Statistical analysis

Statistical analysis was performed using GraphPad Prism (Version 9.0). Data were assessed for normality of the standardised residuals using Shapiro-Wilk tests and visual inspection of frequency histograms. If data were assessed to be normally distributed, then parametric statistics were utilised, otherwise, non-parametric statistics were used. The significance threshold was $p < 0.05$. Data are presented as individual subjects to aid interpretation or as mean ± standard deviation of the mean (SD) unless otherwise specified.

Planned comparisons using paired samples t-tests were conducted comparing the FA of right and left corticospinal tracts.

One-way ANOVA's were used to assess relationships between white matter integrity (FA) and functional classification (MACS and GMFCS). Pearson (or Spearman) correlations were used to assess for relationships between FA and baseline, and baseline and follow-up (1, 6, and 12 week) upper and lower limb outcome measures (JTT and TUG).

4.5 Results

4.5.1 Population and demographics

Full recruitment for the larger clinical trial is detailed in Chapter 4. An overview relevant to this chapter is presented in (figure:4.4). Of the 27 participants enrolled in the trial, 20 participants were able and willing to consent to undertake the MRI scan. One scan was excluded due to excessive head motion, and one scan was excluded as there was a technical problem during the acquisition of the diffusion-weighted images. Eighteen scans were therefore analysed for this section.

Seven females and eleven males are included in this subset of the data (median age = 12.9 years, range 10.2 to 15.8 years). Functional classifications, most affected side and site of stimulation are included in Table 4.1.

		Participants (n)
Sex	Male	11
	Female	7
GMFCS	1	3
	2	12
	3	3
MACS	1	9
	2	7
	3	1
Predominant motor type	Spastic	15
	Dyskinetic-Dystonic	2
	Unknown	1
Topographic distribution	Hemiplegia	5 - right (1), left (4)
	Diplegia	6
	Triplegia	5
	Tetraplegia	2
Stimulation type	Anodal	10
	Sham	8

Table 4.1: Participant demographics for this subset of data including functional classification and topographical distribution.

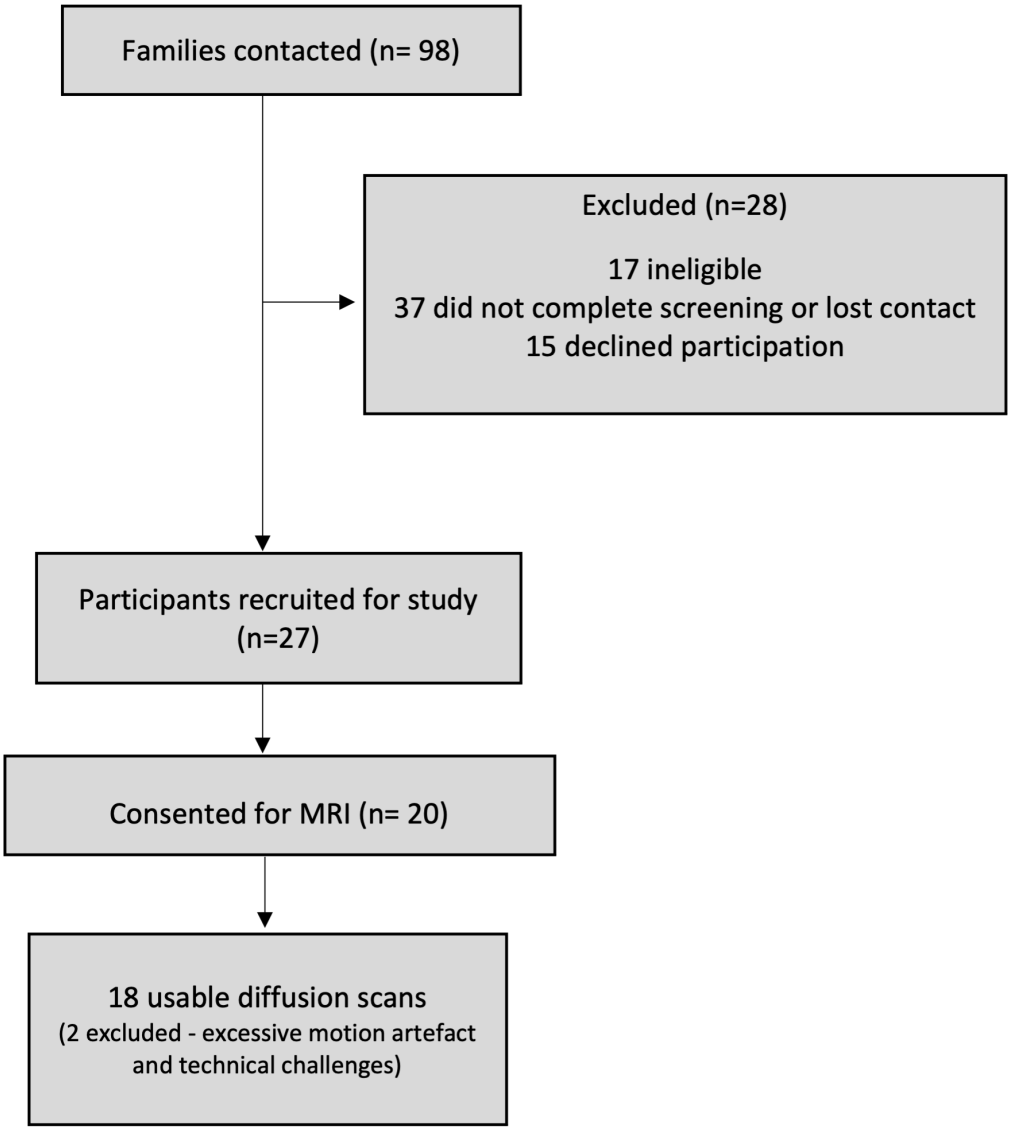


Figure 4.4: CONSORT flow diagran of recruitment into the StimCP clinical trial and optional imaging sub-study

4.5.2 Corticospinal tract integrity in an undifferentiated cerebral palsy cohort

Our automated tractography protocol was successful in generating anatomically plausible tracts in all participant scans that passed initial quality control. One scan was excluded as image acquisition failed, and one scan was excluded during the initial quality control inspection due to excessive motion artefact. Whilst being a robust automated method for the consistent reconstruction of tracts, our method also respected the underlying anatomical variation, as is demonstrated in Figure 4.7.

Fractional anisotropy was used as the primary metric of white matter integrity. FA in both corticospinal tracts was measured, with the hemisphere on the side of brain stimulation (contralateral to the weaker hand/arm) labelled as the 'more affected side', and the hemisphere contralateral to the less affected side labelled as 'less affected'. The distribution of mean FA ± standard deviation of the corticospinal tract (CST) across both hemispheres is shown in figure 4.5.

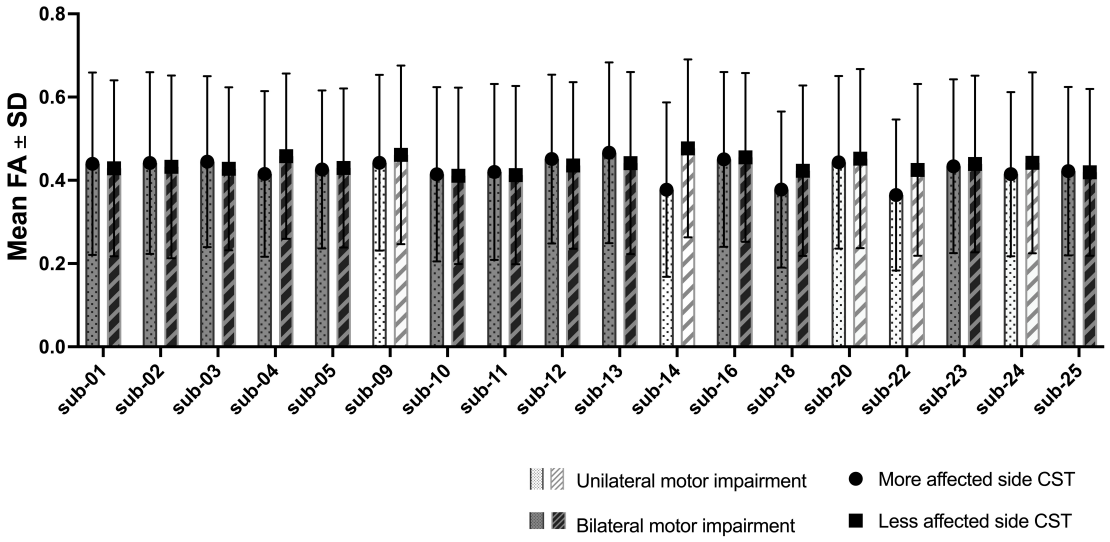


Figure 4.5: Mean fractional anisotropy ± standard deviation of the corticospinal tract in the more and less affected side. Participants with unilateral motor impairments are unshaded, and those with bilateral impairments are shaded.

A paired samples t-test was conducted to determine if there was a marked

difference in corticospinal tract integrity between the more and less affected sides. The results show no significant difference between the more affected side (mean FA = 0.43; SD = 0.024) and the less affected side (mean FA = 0.43; SD = 0.024); [t(17) = 0.18, p = 0.86], see Figure 4.6.

Representative images of participants who showed variation in brain size and shape, but no gross structural abnormality are illustrated in Figure 4.7.

Three participants had grossly abnormal imaging; representative images demonstrate structural abnormalities including loss of brain volume (porencephalic cysts in Figures 4.7e, 4.7f) and unilateral cortical atrophy (Figure 4.7g). The same three participants showed a more marked CST asymmetry than the rest of the cohort. These participants had a history of perinatal infection and resultant complications. Participant (e) suffered from a right middle cerebral artery (MCA) infarction and resultant left hemiplegia, while participant (f) developed bilateral intraventricular haemorrhages, left side more severe than the right side, and resultant triplegia (right arm and leg, and left arm).

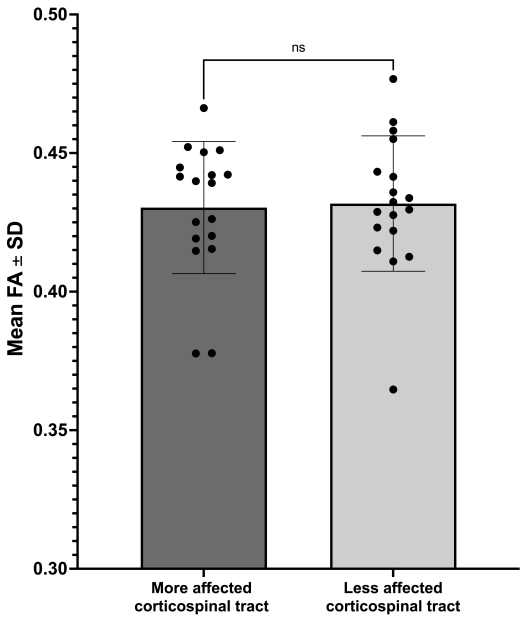
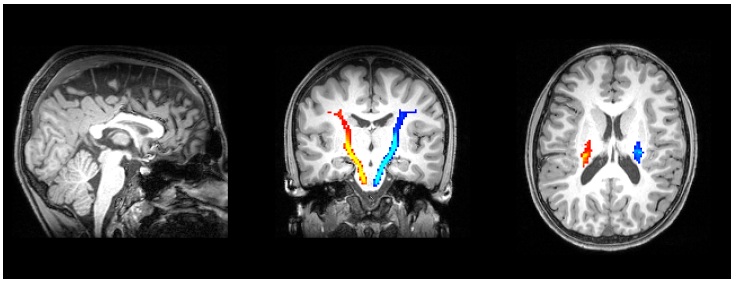
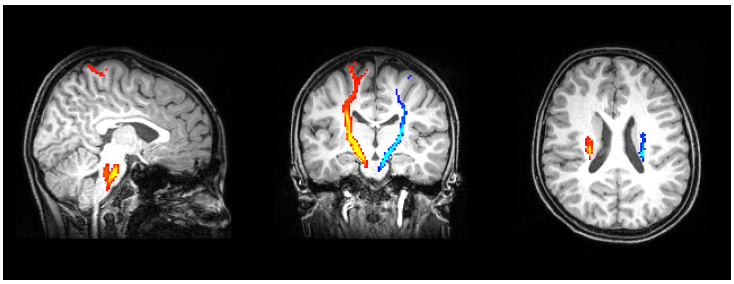


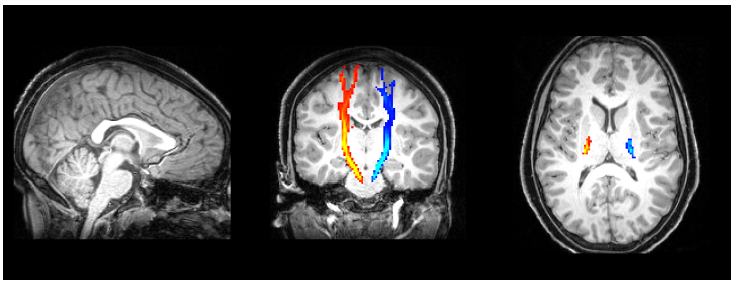
Figure 4.6: Group level comparison of corticospinal tract FA - more vs. less affected side. There was no significant difference, p>0.5



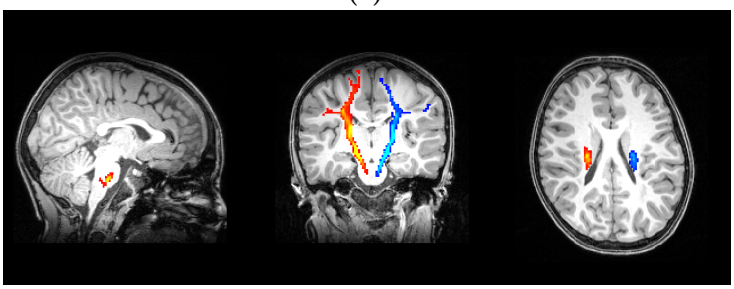
(a)



(b)

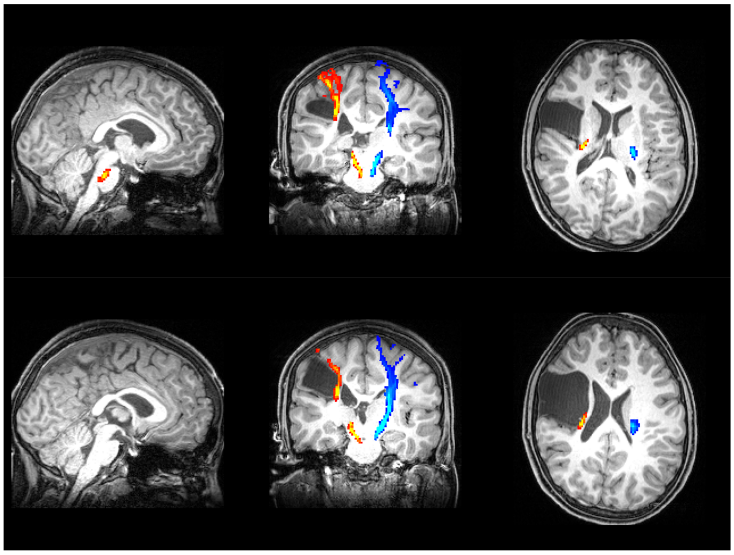


(c)

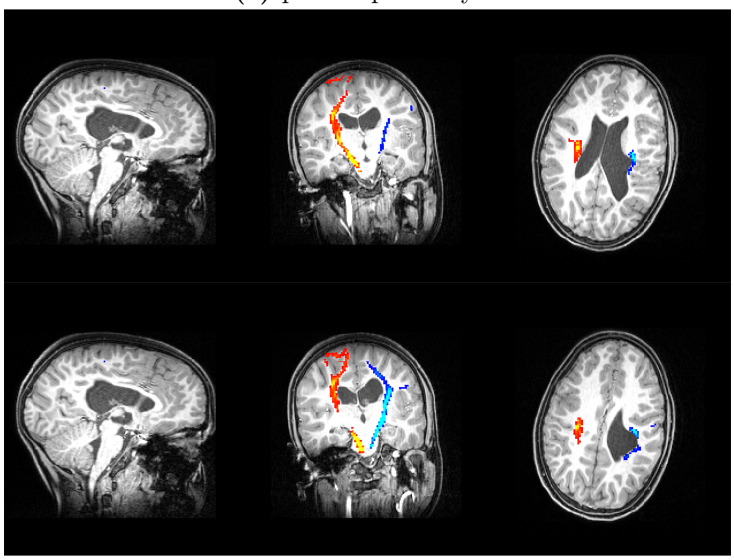


(d)

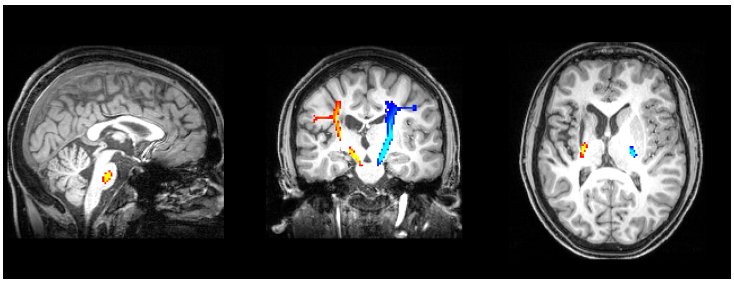
Figure 4.7



(e) porencephalic cyst



(f) porencephalic cyst

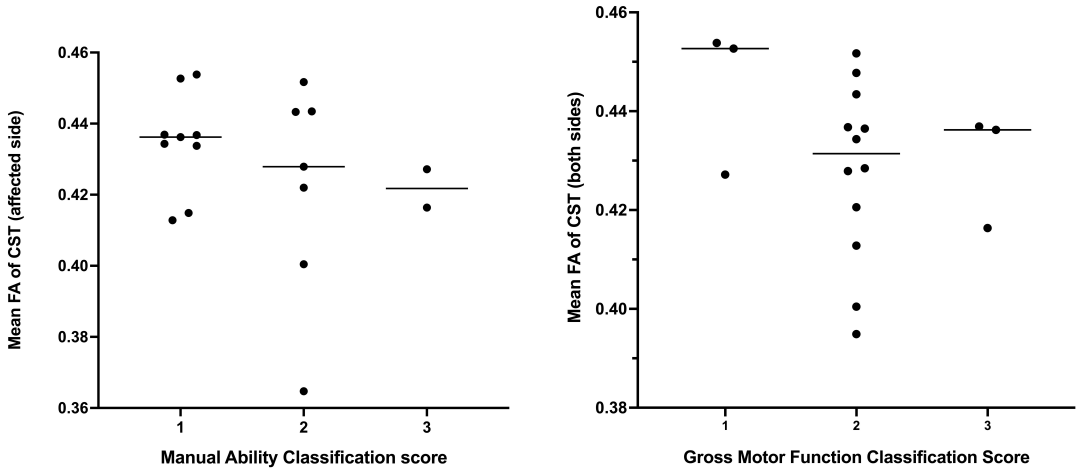


(g) unilateral cortical atrophy

Figure 4.7: Tractography of the corticospinal tracts in participants illustrating brain size, shape, and pathology variation. The tracts are overlaid against the participants' bias-corrected T1w image

4.5.3 Functional classification does not correlate with corticospinal tract integrity

One-way ANOVAs were performed to compare the effect of functional classification on corticospinal tract integrity. The two classification scales used measured upper limb (MACS) and mobility (GMFCS). Corticospinal tract FA on the more affected side was used for upper limb measures. For lower limb measures, a mean of right and left CST FA were used, as walking relies on function of both limbs. A one-way ANOVA revealed that there was no statistically significant difference in CST integrity between MACS categories ($F(2, 15) = 1.66$, $p = 0.22$), Figure 4.8a. A one-way ANOVA revealed that there was no statistically significant difference in CST integrity between GMFCS category ($F(2, 15) = 0.535$, $p = 0.6$) Figure 4.8b.



(a) (b)

Figure 4.8: Mean FA of the CST on the more affected side does not correlate to MACS. Mean FA of left and right CST does not correlate with GMFCS classification.

4.5.4 Baseline hand function correlates with corticospinal tract integrity

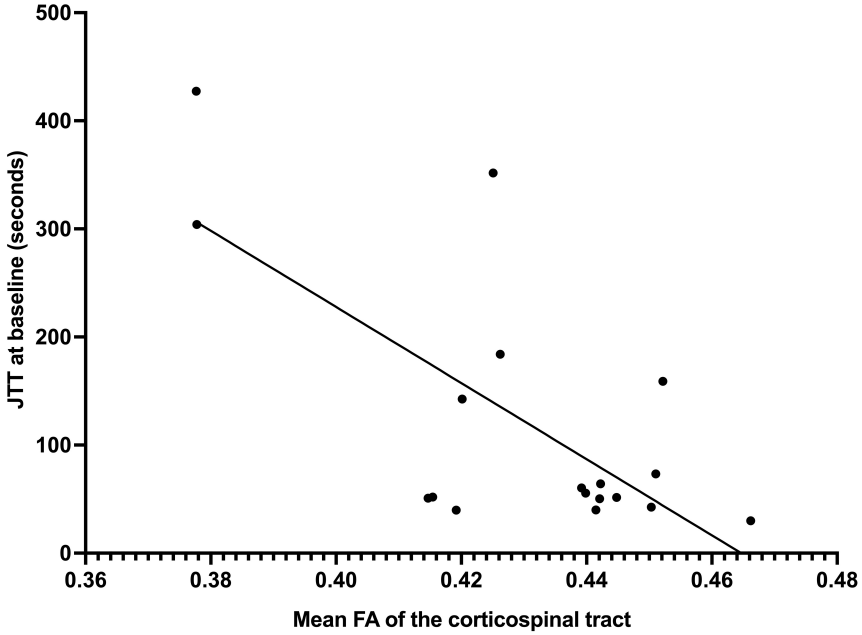


Figure 4.9: Corticospinal tract integrity of the more affected side correlates with baseline JTT.

Spearman's rank correlation was computed to assess the relationship between baseline hand function of the more affected side, measured using the Jebson-Taylor Test (JTT) and corticospinal tract integrity of the corresponding side. The relationship is demonstrated in Figure 4.9. There was a strong negative correlation between the two variables, $r(17) = -0.7$, $p = 0.0013$, which was statistically significant. In other words, higher FA is associated with faster performance time in the JTT and better hand function.

It was noted that the participants with the three slowest times might be, at least in part, driving this effect. Notably, these participants were previously noted to have a greater CST asymmetry and have lesions in their most affected hemisphere in the motor cortex and CST regions, therefore this effect is unsurprising.

To further explore this effect, we tested to see if there was a relationship between volume of the CST and baseline hand function, shown in Figure 4.10. Spearman's

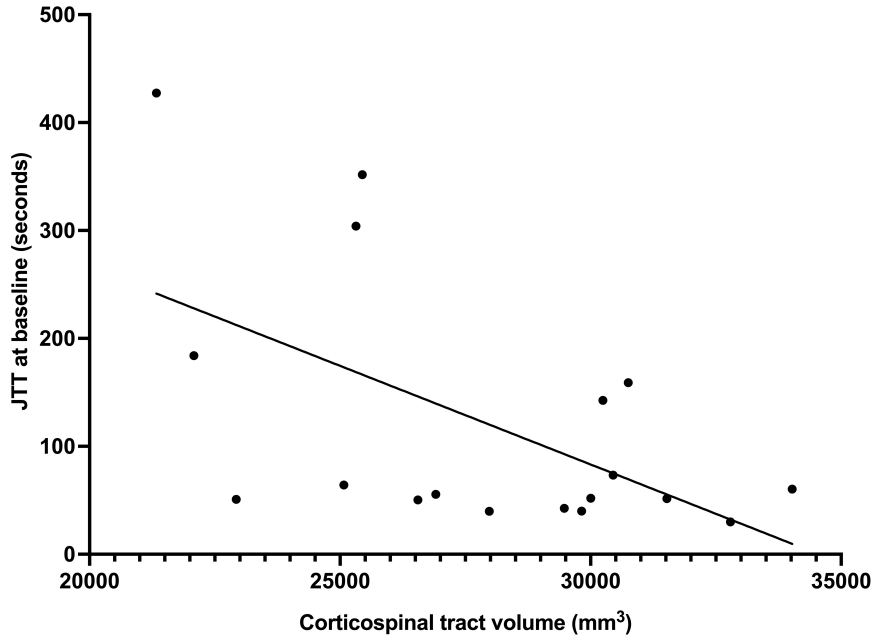


Figure 4.10: Corticospinal tract volume of the more affected side correlates with baseline JTT.

rank correlation was computed to assess the relationship between baseline JTT time of the weaker hand, and corresponding corticospinal tract volume. There was a strong negative correlation between the two variables, r(16) = -0.56, p = 0.0161, which was statistically significant. This indicates that lower CST volume is associated with slower JTT (worse upper limb function).

Spearman's rank correlation was then computed to assess the relationship between fractional anisotropy and tract volume in the corticospinal tract contralateral to the more affected side (Figure 4.11). There was a strong positive correlation between the two variables, r(11) = 0.6648, p = 0.0003, which was statistically significant.

Given that the tracts were generated using individualised tractography rather than atlas-based ROIs, where the volume is fixed, this result indicates that tracts vary in both volume and FA. Our findings demonstrate a strong linear relationship between volume and FA, indicating concordance that tracts with greater integrity

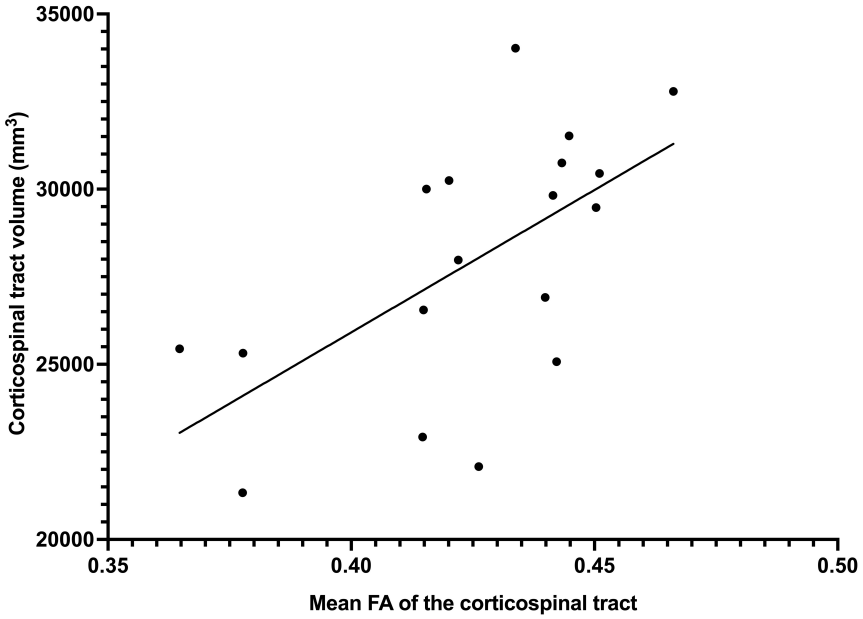


Figure 4.11: Corticospinal tract fractional anisotropy correlates with tract volume.

have both a higher volume and greater FA. Worse hand function is associated with smaller volume tracts and lower FA in this cohort.

4.5.5 Baseline lower limb function does not correlate with corticospinal tract integrity

For lower limb function, the Timed Up and Go Test (TUG) measure is not specific to one side, but rather measures the overall function and coordination of the right and left leg as a unit. Therefore, the mean of the right and left corticospinal tracts was used for analysis.

The relationship between baseline lower limb function, measured using the TUG and corticospinal tract integrity of both sides was assessed using a Spearman's rank correlation, and plotted in Figure 4.12. There was a weak negative correlation between the two variables, r(16) = -0.24, p = 0.33 which was not statistically significant.

Given that 10m walk time, but not TUG time, improved with the rehabilitation

outlined in Chapter 3, we additionally assessed the correlation between baseline 10m time and FA. However, there was no statistically significant correlation: r(16) = 0.05, p = 0.87.

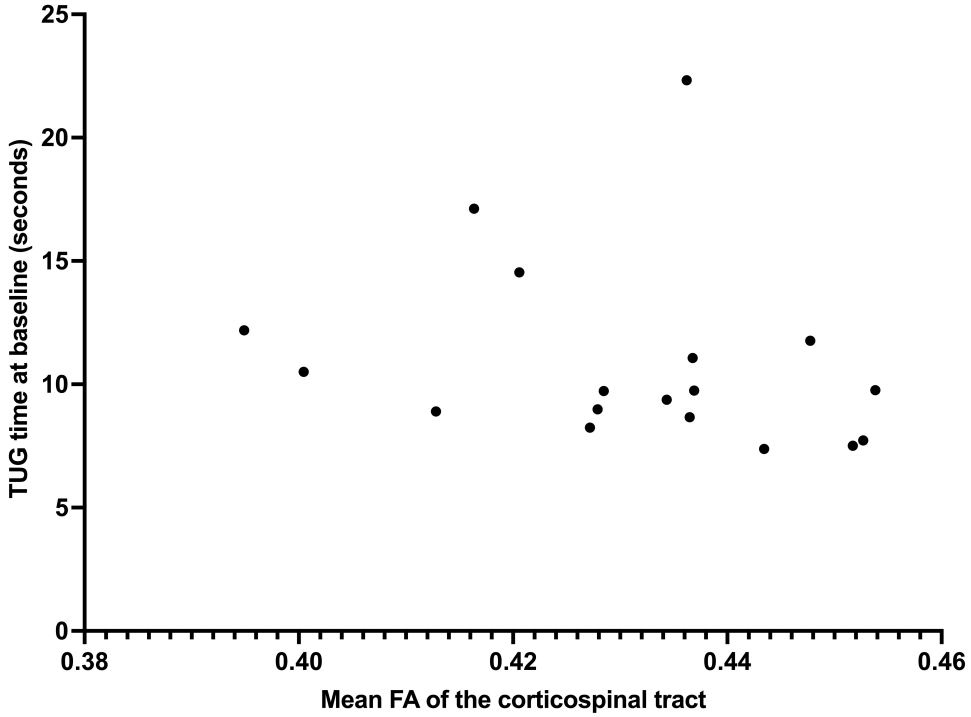


Figure 4.12: Mean FA across left and right corticospinal tracts does not correlate with baseline TUG time.

The relationship between TUG time and corticospinal tract volume (right and left side) was assessed using Spearman's rank correlation (Figure 4.13). There was a weak positive correlation between the two variables, r(16) = 0.25, p = 0.32 which was not statistically significant.

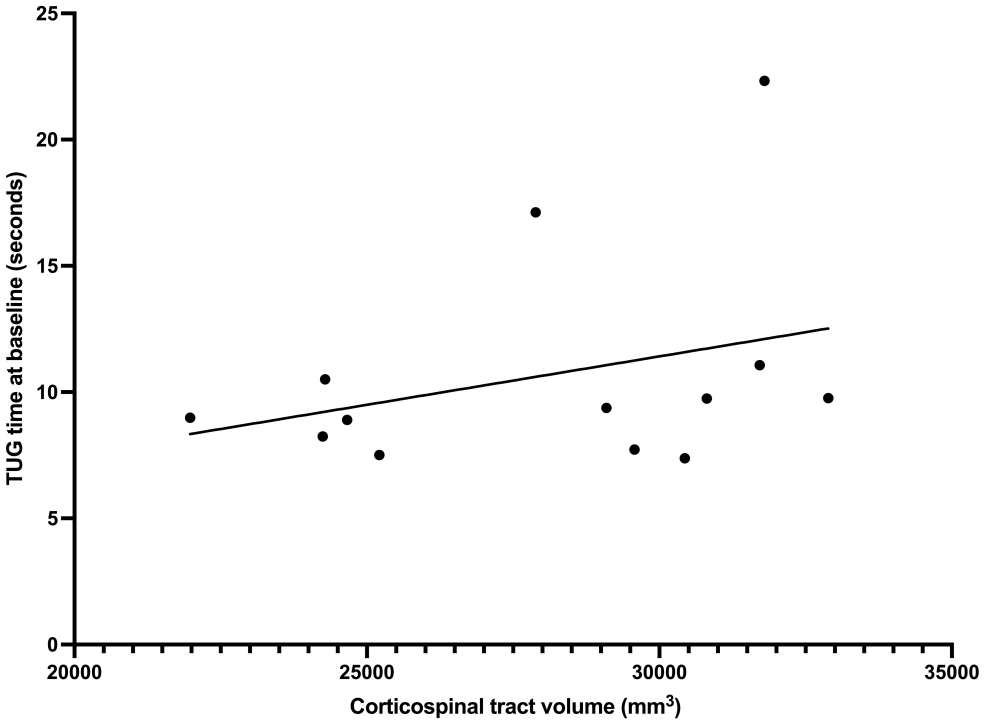


Figure 4.13: Tract volume of the left and right corticospinal tracts does not correlate with baseline TUG time.

4.5.6 Corticospinal tract integrity does not correlate with response to rehabilitation

Given the range of baseline scores for both the JTT and TUG times across participants, the percentage change in test time was used in preference to raw time. Percentage change was calculated as [follow-up time] - [baseline time] / [baseline time] x 100. A negative percentage change indicates an improvement in time to complete the assessment compared to baseline.

The relationship between baseline corticospinal tract FA, of the more affected side, and change in JTT time was assessed using Spearman's rank correlations for each time point (1 week, 6 weeks and 12 weeks). There were no statistically significant correlations between the two variables at 1 week (r(16) = [0.18], p = [0.46]), 6 weeks (r(14) = -0.11, p = 0.68) and 12 weeks (r(14) = 0.072, p = 0.79), demonstrated in Figure 4.14.

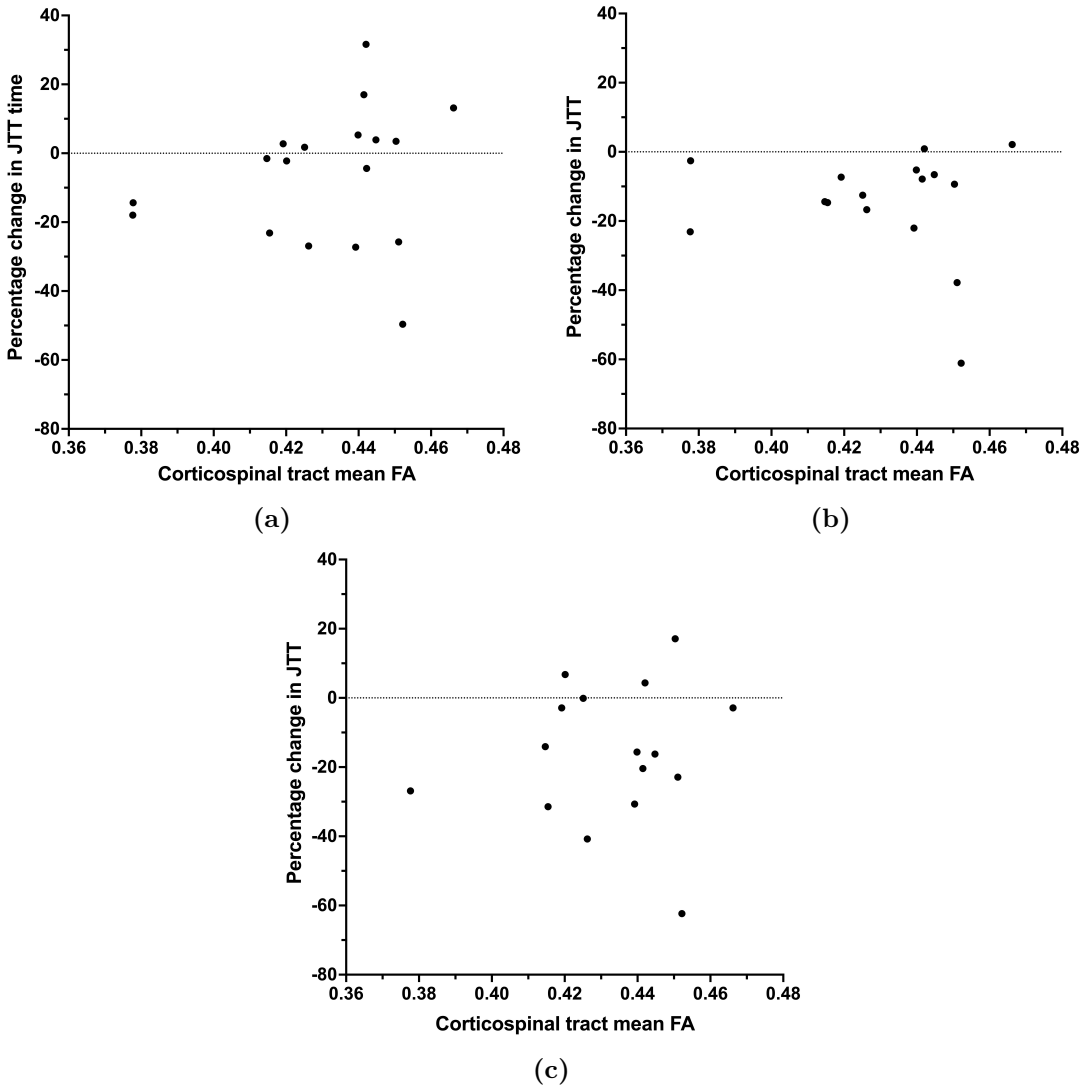


Figure 4.14: More affected CST integrity in relation to the percentage change in JTT time across timepoints: a) 1 week follow up, b) 6 weeks and c) 12 weeks

The relationship between corticospinal tract FA (mean of left and right side), and percentage change in TUG time was assessed using Spearman's rank correlations for each time point (1 week, 6 weeks and 12 weeks)(Figure 4.15). There were no statistically significant correlations between the two variables at 1 week (r(16) = [0.348], p = [0.16]), at 6 weeks (r(14) = 0.14, p = 0.60) and at 12 weeks (r(14) = 0.165, p = 0.54).

Similarly, there was no statistically significant correlation between corticospinal tract volume and change in JTT score (1 week (r(16) = -0.023, p = 0.92), 6 weeks

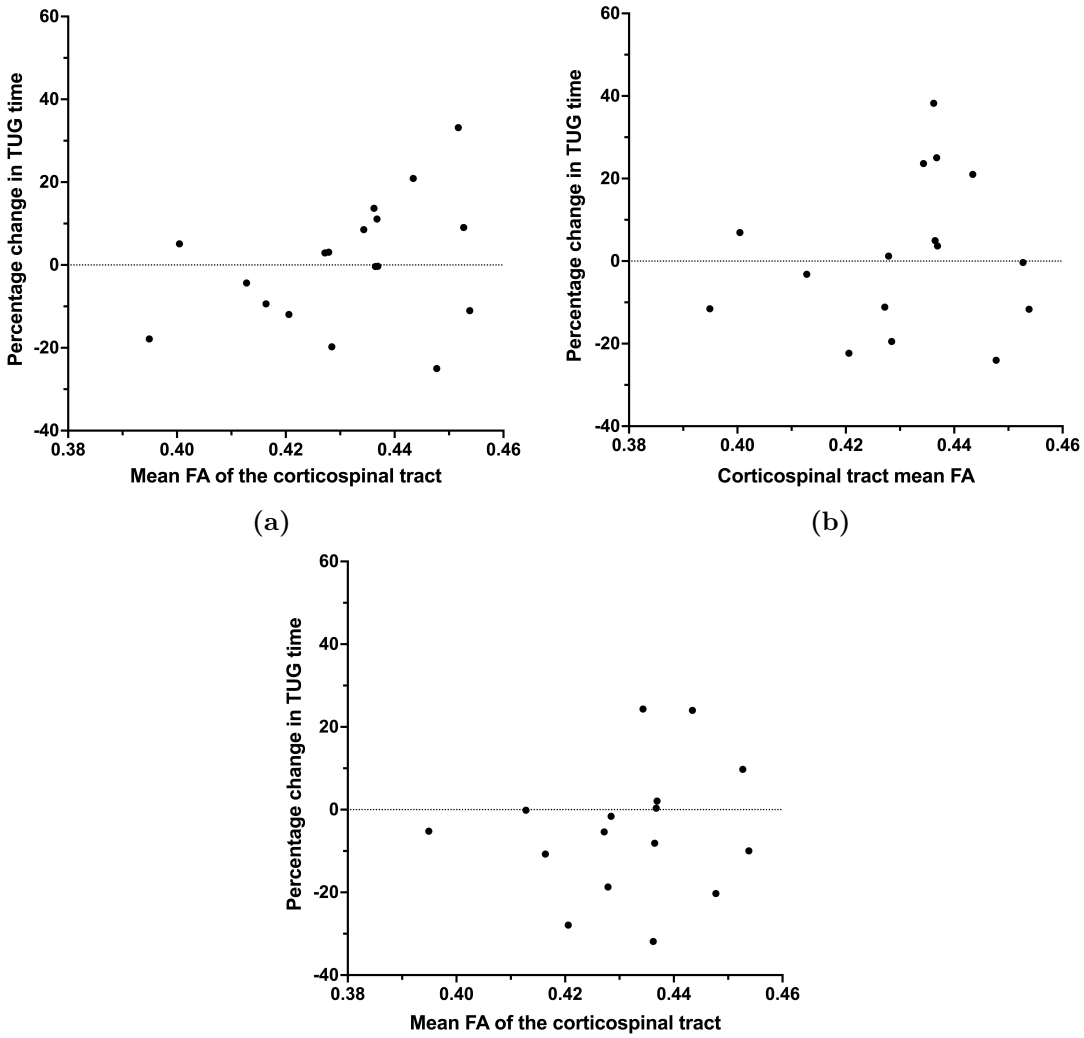


Figure 4.15: Mean FA of the right and left CSTs in relationship to the percentage change in TUG time across timepoints: a) 1 week follow up, b) 6 weeks, and c) 12 weeks

(r(14) = -0.11, p = 0.67), 12 weeks (r(14) = 0.026, p = 0.92). There was no correlation between CST volume and change in TUG scores (1 week (r(16) = -0.15, p = 0.54), 6 weeks (r(14) = 0.19, p = 0.48), 12 weeks (r(14) = 0.037, p = 0.89).

4.6 Discussion

In this chapter, probabilistic tractography was used to characterise CST integrity in a heterogenous clinical cohort of cerebral palsy patients. Additionally, the relationship between baseline function, as well as treatment response, to metrics of CST integrity was explored.

Implementation of an automated tractography tool in a paediatric patient population

Cutting-edge neuroimaging analysis tools are often designed for, and validated in, cohorts of healthy adults. Through careful implementation and meticulous inspection, I have demonstrated that an automated probabilistic tractography tool, XTRACT, can be successfully utilised in a paediatric population with structural pathology. No participants were excluded, despite several having large lesions in the affected hemisphere.

This is a significant advance, as much of the previous work on tractography in cerebral palsy has relied on manual delineation of tracts or ROIs[322,323,324,325]; or has excluded participants with large lesions[326,327,328,325].

In our sample of 18 individuals, we have demonstrated the feasibility of automated tractography in deriving corticospinal tract measures in a heterogeneous, clinically relevant, adolescent CP cohort.

Corticospinal tract variation

Our patient cohort demonstrated less variability of CST morphology than expected. Of the 18 participants, three showed marked CST asymmetry, all having evident lesions.

The mean CST FA of the more and less affected sides did not differ significantly, with values of 0.430 and 0.432, respectively. These do not appear to be markedly lower than FA values of CST in typically developing adolescents in the same age group in a longitudinal study conducted by Lebel et al (2011); though exact values were not reported[329]. There does not seem to be a consensus on what is considered the normal range in typically developing adolescents, with reports varying from 0.38[276], to 0.57[330] and 0.67[331]; and at least partially dependent on scanner magnetic field strength and processing pipeline.

These findings, while limited by the small sample size, allude to the more global nature of brain involvement in patients with cerebral palsy and demonstrate the importance of rehabilitation techniques, such as HABIT and HABIT-ILE, that focus on bimanual, co-ordinated tasks involving the left and right limbs. In future work, additional assessment of corpus callosum integrity may add value in characterising response to these rehabilitation techniques.

Corticospinal tract integrity and baseline functional classification

Previous studies have demonstrated a possible correlation between CST integrity and motor function classifications such as the MACS for hand function[303,304]. However, these studies included only children with unilateral upper limb involvement. In our cohort, we found no correlation between MACS and CST FA, and no correlation between GMFCS and CST FA. We feel that our sample benefits from being more reflective of the typical clinical caseload of children with cerebral palsy, but acknowledge that the small sample size might be underpowered to detect a correlation between FA and functional classification. Furthermore, we included participants with mild to moderate CP, with MACS and GMFCS classifications of 1-3. More severe CP, with classifications of 4 and 5, was not included in our study, and may have markedly different tracts.

Corticospinal tract integrity, tract volume, and baseline hand function

Next, we evaluated the relationship between baseline upper and lower limb function, with corticospinal tract integrity. We found a strong, statistically significant, negative correlation between baseline CST FA and baseline Jebson Taylor Test time, which measures hand function, on the more affected side. Worse hand function was associated with lower FA values. We also identified that the three participants with the lowest FA and worst hand function had large lesions.

Next, we demonstrated that this correlation was also present between JTT time and CST tract volume. Our results suggest that greater impairment is associated with both lower FA and smaller tract volumes.

We then demonstrated that CST tract volume and FA were strongly positively correlated. One possible explanation is that the fibre structure of the tract itself is abnormal - with higher FA driven by having larger or less densely packed fibres. In the context of this population who have had an early insult, with subsequent development and maturation, we propose an alternative hypothesis: in participants with injuries to areas through which the CST normally passes, it is likely that the CST did not develop or mature fully and, therefore, has a lower volume and lower FA. This explanation would be in keeping with the previously demonstrated longitudinal findings of Lebel et al (2013,) which demonstrated that during normal developmental changes of the corticospinal tract, in typically developing adolescents, increased tract volume over time correlated with increased or static FA, rather than decreased FA. Given CP occurs during brain development, the tracts with a lower FA, which represents the integrity of the tract (such as fibre density, axonal diameter, and myelination) are likely to be less well developed/more degenerated as well.

Interpretation of DTI metrics

While DTI is highly sensitive to changes in white matter microstructure, it has significant limitations. The tensor model is most useful in regions of highly coherent fibres, and is more difficult to accurately interpret where fibres cross or have complex paths[332]. Diffusion tensor metrics from a given voxel, though derived from microstructural properties at $\sim$ micrometre scale, are a voxel mean at $\sim$ millimetre scale. FA is a summary measure of white matter integrity, and can be influenced by various underlying microstructural properties or pathologies, including axon packing, axon diameter, oedema, and fibre numbers[296,297,333]. DTI measures of white matter therefore cannot be attributed to one specific microstructural change. As cerebral palsy is an umbrella condition, DTI metric alterations may have varying contributions from different underlying pathologies in different patients. In this work, fractional anisotropy is treated as a broad overall measure of white matter integrity, for which it is robust[332]

Corticospinal tract integrity, tract volume, and baseline lower limb function

We did not demonstrate any statistically significant relationships between baseline TUG time and CST metrics. The TUG test has been shown to be a reliable test to assess balance, anticipatory postural control, and functional mobility[334,335,336,337]. Our results may imply that there is no relationship between overall CST integrity and lower limb function, or it may be that the TUG test is not sensitive enough to test leg function, as balance and coordination contribute to results. The corticospinal tract is somatotopically organised[338], and quantifying tract integrity in the more superior parts of the tract corresponding to the lower limb as it fans out across the motor cortex might be more sensitive to lower limb function.

4.6.1 CST as a predictive tool

Finally, we aimed to determine if baseline corticospinal tract metrics would be a sensitive metric to be used as a predictor of response to rehabilitation.

Our findings do not show a correlation between a change in upper or lower limb function with metrics of corticospinal tract integrity. Previous studies have found reduced FA in the lesioned CST in children with hemiparesis correlated with simple motor assessments such as the Gross Motor Function Classification System[324]. Others have demonstrated that reduced FA and increased MD correlate with bimanual motor function as measured by the Assisting Hand Assessment and Melbourne Assessment of Unilateral Upper Limb Function[339,340]. However, these studies are similarly limited by small sample sizes, are restricted to the hemiparetic cerebral palsy subtype rather than undifferentiated CP, and do not assess for correlations with treatment response or outcome.

It is possible that the lack of relationship between CST diffusion metrics and treatment response may be due to the heterogenous underlying pathology in our sample. Kuxzynski et al (2018) have described differences between two specific stroke types (AIS and PVI), demonstrating disease-specific differences in CST structural connectivity relative to controls. It is possible that there is not enough common pathology between participants to be united by one measure. Another explanation may be that the relationship between corticospinal tract integrity and rehabilitation potential may not increase linearly. Significant motor improvements may be possible across a wide range of corticospinal tract integrity, as long as it is above a critical threshold.

Despite this, children with CP have a shared syndrome, with significant shared clinical features and shared responses to rehabilitation, regardless of the subtype. Work should continue to be done to probe possible interactions between more metrics of white matter integrity, as well as different tracts, and treatment responses.

5

Individualised electric field modelling to explain variability in tDCS effect

Contents

5.1 Introduction

As outlined in Chapter 3, our clinical trial investigating the effects of 10 sessions of ipsilesional anodal tDCS did not demonstrate an effect of condition over time. Studies of transcranial direct current stimulation in the last two decades have often demonstrated inconsistent effects, with many avenues of inter-subject variability investigated[198].

The universally held adage of paediatricians is that "children are not just small adults" - and this is particularly important to bear in mind with regard to the developing brain. Differences in skull size and thickness, brain volume, grey/white matter ratios, and cerebrospinal fluid (CSF) volume could all impact the electric current flow of tDCS[341,184,342]. Given that there is growing interest in using tDCS in children with neurological disorders, including CP, it is important to better understand the impact of structural variation on current flow and treatment effects.

This chapter will use computation modelling to generate personalised electric field models for a subset of participants from the StimCP trial and explore whether these models can provide insight into treatment response.

5.1.1 Understanding variance in tDCS response

Protocol variations

In the adult population, tDCS has been shown to modulate neuronal membrane potentials, and induce long-lasting, polarity-dependent changes in cortical excitabil-

ity in humans[147]. Polarity of the effect is determined primarily through electrode placement, with membrane depolarisation induced through anodal stimulation, and membrane hyperpolarisation induced through cathodal stimulation[113,147,126]. This same early work by Nitsche and Paulus interrogated the optimal tDCS stimulation duration, current intensity, and electrode placement on motor-evoked potentials (MEPs)[113]. They demonstrated the critical role for electrode position in achieving the behavioural effects of anodal stimulation, with only one configuration (motor cortex and contralateral forehead) resulting in significant excitability changes[113]. Secondly, they demonstrated that higher current intensity and greater stimulation time resulted in higher amplitude MEPs which persisted for a longer duration after stimulation[113,147]. In other words, the dosage of current materially affects the behavioural outcome, and selecting the correct stimulation site, current dosage, and stimulation duration are vital to maximising possible effects.

Individual factors

It is increasingly recognised that beyond protocol variations, there are also many possible inter-individual factors which lead to variation in response, including neurochemical, genetic, developmental, and anatomical factors[198], which are discussed in greater detail in Chapter 1.

5.1.2 Anatomical differences between adults and children

Anatomical factors are particularly relevant to our study population, as the adolescent brain is still undergoing developmental changes, while also having underlying pathology as a result of the early insult which led to cerebral palsy. These may influence brain volume, grey/white matter ratios, and cerebrospinal fluid (CSF) volume, which all impact electric current flow during tDCS[342].

Skull and scalp changes through the lifespan

Skull thickness and size undergo rapid changes during the newborn to toddler phase, where skull thickness increases 6-fold in the first three years of life[343]. Skull and overall head size show a logarithmic increase in early childhood[343] before growth slows, but continues to increase until approximately 16 years of age[344].

Early current modelling studies showed that both scalp and skull thickness differed between a 12-year-old adolescent and a 35-year-old adult and were important factors in explaining differences in current flow as well as peak current intensities[345].

Brain volume increases and ratio changes of CSF, grey, and white matter

At birth, the brain is ∼25% of the volume of an adult brain, and undergoes rapid growth in the first two years of life[346] (Figure 5.1). Thereafter, it continues to increase in size until adolescence (age 12–15 years, sex-dependent) before gradually declining across the rest of the lifespan[346]. Both grey and white matter volume changes reflect overall brain volume changes across the lifespan; however, grey matter volume declines with age more than white matter volume, resulting in a high grey:white matter ratio at birth, declining over childhood and reaching a plateau in ageing. Unlike brain volume, cerebrospinal fluid (CSF) volume increases steadily over the lifespan from birth to advanced age[344,346].

5.1.3 Computational modelling of current flow in tDCS

Computational finite-element method (FEM) modelling has enabled us to begin to untangle the effect of anatomical differences on current flow in both clinical populations[184] and healthy adults[185]. These models have advanced our mechanistic

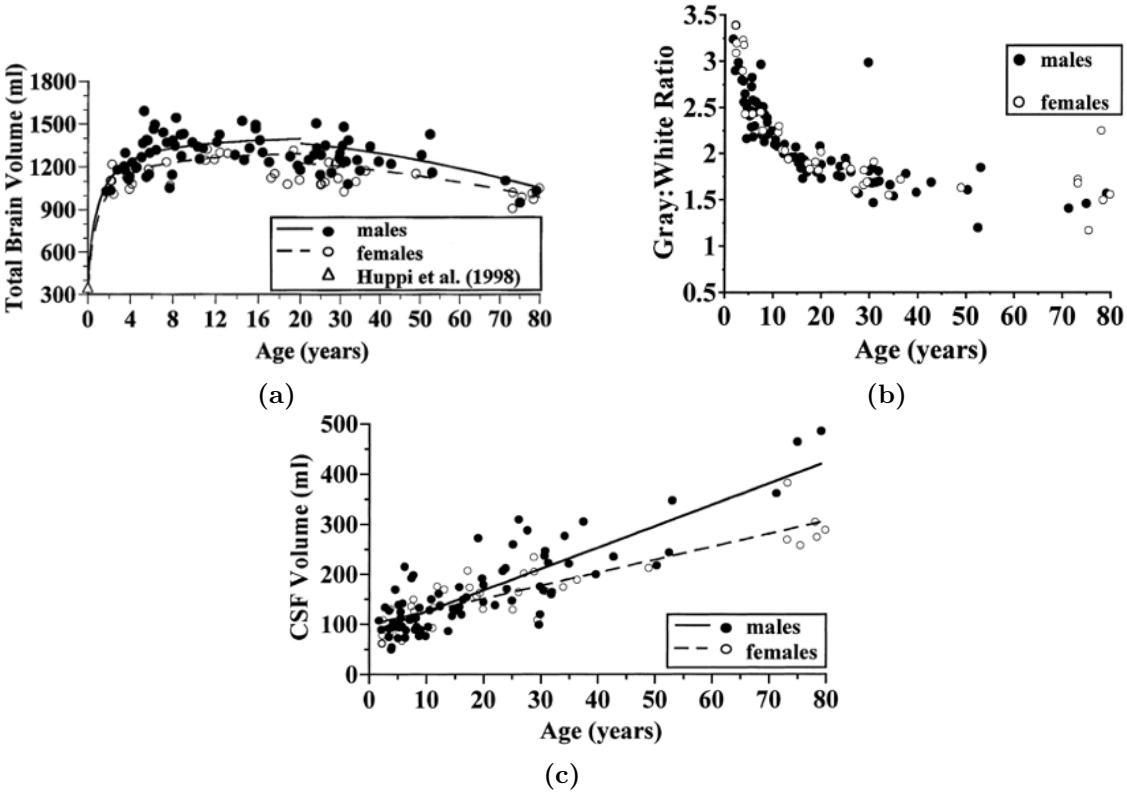


Figure 5.1: Total brain volume (a) across the lifespan increases rapidly in childhood and declines slowly after adolescence. The immature brain has a greater grey:white matter ratio, which declines rapidly during childhood and plateaus in adulthood (b). CSF volume increases linearly across the lifespan (c). Reused with permission from Chourchesne et al, Neuroradiology (2014)

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understanding of the current flow during the application of transcranial direct current stimulation (tDCS), particularly regarding how lesions affect current flow[184].

The effect of these anatomical differences, including brain and CSF volume, and skull thickness, on electric field strength and distribution is an area of ongoing investigation, and is likely non-linear. Increased electric field peak strength was found to be strongly correlated with skull thickness in children in a study of 58 healthy children, adolescents and adults,[342], but not correlated to skull thickness or grey/white matter volume in a more recent study of 32 children and adolescents[263]. Despite no clear correlation between discrete anatomical differences and field strength, several modelling studies have found higher peak electric field in children than in adults, when applying the same tDCS current and montage[347,345,348,342,349], as

well as more expansive electric field spread across the brain[342]. This is likely resultant of a complex interplay of anatomical factors that are not clearly understood.

Nearly all modelling studies have been performed in adults, with very few studies evaluating how anatomically abnormal paediatric brains alter electric field strength and distribution of current. Cerebrospinal fluid is a particularly good electrical conductor, and modelling studies in adults have shown that differences in CSF distribution dramatically change tDCS current flow estimates[184,347]. Large CSF-filled areas post-infarction, or changes in ventricle shape and size, both common findings in cerebral palsy, are likely important factors determining current flow[184]. It is, therefore, important to strive to ensure that current modelling studies are inclusive of the full spectrum of pathologies for which tDCS could be applicable.

5.1.4 Electric field modelling to explain response variation

While it is clear that the emerging technique of modelling current flow, particularly in children and patients, can provide useful insights into the biomechanics of current flow; it is not yet clear if there is a direct relationship between expected current flow and response to tDCS. To date, very few studies have examined electric field modelling parameters in relation to study outcomes. Of the handful of published studies, it appears promising that electric field strength in the proposed tDCS target area may correlate to outcome measures in depression[350] and working memory[351,352]. One study has evaluated the relationship between electric field and functional connectivity changes, in a cohort of stroke patients[353]. Yuan et al. (2022) demonstrated a positive correlation between functional connectivity of the ipsilesional sensorimotor region after anodal tDCS, and electric field strength of the ipsilesional M1, hypothesising that anodal tDCS facilitates improved functional connectivity in chronic stroke subjects, and individual electric field predicts the functional outcomes[353]. Unfortunately, the study did not include any behavioural outcome scores to further evaluate this claim.

5.1.5 Key aims of this chapter

Electric field modelling is a tool that facilitates better understanding of how individual anatomical factors can affect current flow. This may be a promising avenue to further our understanding of the causal reasons for variation in response to tDCS.

In this chapter, I have tested a model of electric current strength simulation in a clinical paediatric population to address the following questions:

1. Is electric field modelling feasible in a real-world paediatric cerebral palsy population?

2. Does electric field modelling suggest consistent target engagement of M1?

3. Does electric field modelling help to predict motor outcome after an anodal tDCS intervention?

5.2 Methods

5.2.1 Study design

Data presented in this chapter were collected as part of a larger randomised control trial, outlined in Chapter 3. In brief, children aged 10-16 with cerebral palsy underwent a 10-day motor training. Each session consisted of upper and lower limb training for 90 minutes, and participants received anodal or sham tDCS during the first 20 minutes of the training. An optional baseline MRI was performed before the intervention, and outcome measures were collected at baseline, 1, 6, and 12 week follow-ups (Figure 4.2).

5.2.2 Magnetic Resonance Imaging (MRI)

MRI data were acquired in a single scanning session at the Wellcome Centre for Integrative Neuroimaging prior to the start of the intervention. Data were collected using a 32-channel head coil in one of two identical 3T Prisma Magnetom Siemens scanners, software version VE11C (Siemens Medical Systems, Erlangen, Germany). Head movement was minimised through the use of foam padding and limb strapping as needed on an individual basis. Data from the structural scans were utilised in this chapter.

The T1-weighted structural image was acquired with a Magnetization Prepared Rapid Acquisition Gradient Echo (MPRAGE) sequence[311]: TR = 1900 ms, TE = 3.97 ms, voxel size = 1.0 × 1.0 × 1.0 mm, flip angle = 8°, total slices = 192. A field view of 192mm^3 was set as standard; if this FoV did not include the full scalp perimeter, an additional T1w scan was acquired for use in the computational modelling of the electric fields.

T2-weighted images were acquired in the sagittal plane: TR = 5000ms; TE = 397ms; voxel size = 1.0 × 1.0 × 1.0 mm; slice thickness = 1.05mm; 192 slices; FOV = 256mm^3.

Transcranial Direct Current Stimulation

Anodal transcranial direct current stimulation was delivered during the first 20 minutes of each 90-minute intervention session. Two 5 x 7cm conductive rubber electrodes in saline-soaked (0.9% NaCl) sponges were attached to a direct current stimulator (Nurostym, Neuro Device Group S.A., Poland). The anode was placed on the contralateral hemisphere of the more affected upper/lower limb, using the EEG 10-20 system (C3/C4), and placed as close to the midline as possible. For

simplicity, this is referred to as the ipsilesional side. The cathode was placed on the contralesional supraorbital region.

5.3 Data analysis

5.3.1 Current Modelling

Electric field current modelling was generated using the standard SimNIBS pipeline [version 4][354]. In brief, T1w and T2w FLAIR scans were bias-corrected and affinely co-registered. Next, using the SimNIBS tool *CHARM* (Complete Head Anatomy Reconstruction Method)[355] the anatomical volumes are segmented into 15 tissue types, employing a combination of intensity, atlas, and machine learning based segmentation. The tissue types are listed in Figure 5.2.

Segmented volumes were visually inspected for accuracy, and manual clean-up of inaccurately segmented masks was performed. In particular, segmentation errors were seen at CSF / brain interfaces. In these cases, Freesurfer Freeview v7.1.1 was used to manually reclassify tissue within the brain, and FSLeyes v1.0.13[356] to delete erroneously classified voxels at the skin/air interface. To generate the volume conductor from voxel segmentations, surface meshes are generated using triangular elements, and the volumes between the surfaces are filled in with tetrahedral elements, using Gmsh[357]. The surface meshes represent the boundaries between tissue types. Together, these volumes and surfaces form the basis of the finite element model (FEM) of the electric field generated by tDCS. Previously established conductivity values for each of the 15 tissue types were assigned to the appropriate meshes.

Tissue	Details
Air Internal (AI)	Air cavities, such as sinuses and the throat.
Artery (AR)	Large arteries in the head and neck.
Cerebrum gray matter (GM)	Cerebral and cerebellum cortex.
Cerebrum white matter (WM)	Cerebral and cerebellum WM including subcortical structures.
Cerebrospinal fluid (CSF)	Cortical CSF, ventricles, CSF around spinal cord, meninges.
Eyes (EY)	Vitreous body, cornea, lens.
Other tissues (OT)	Soft tissues not belonging to any of the other classes.
Rectus Muscles (RM)	Muscles around the eyes.
Mucosa (MU)	Mucous tissue in the nasal cavity.
Visual Nerve (VN)	Visual nerve from the eyes to the optic chiasm.
Skin (SK)	Outermost tissue layer of fixed thickness on the head.
Spinal cord (SC)	From the inferior part of the scan to the beginning of the brain stem.
Veins (VE)	Major veins in the head and neck.
Bone cortical (BC)	Compact bone in the skull and vertebrae.
Bone cancellous (BS)	Spongy bone in the skull and vertebrae.

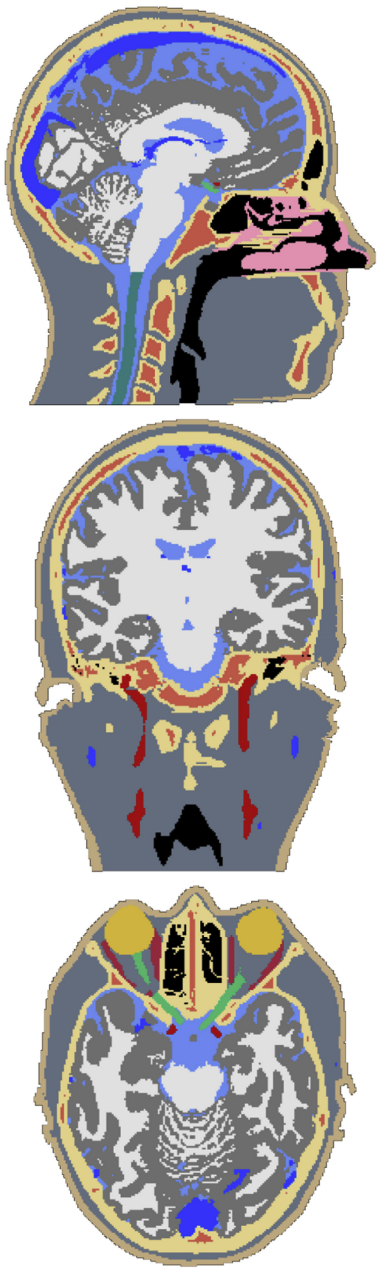
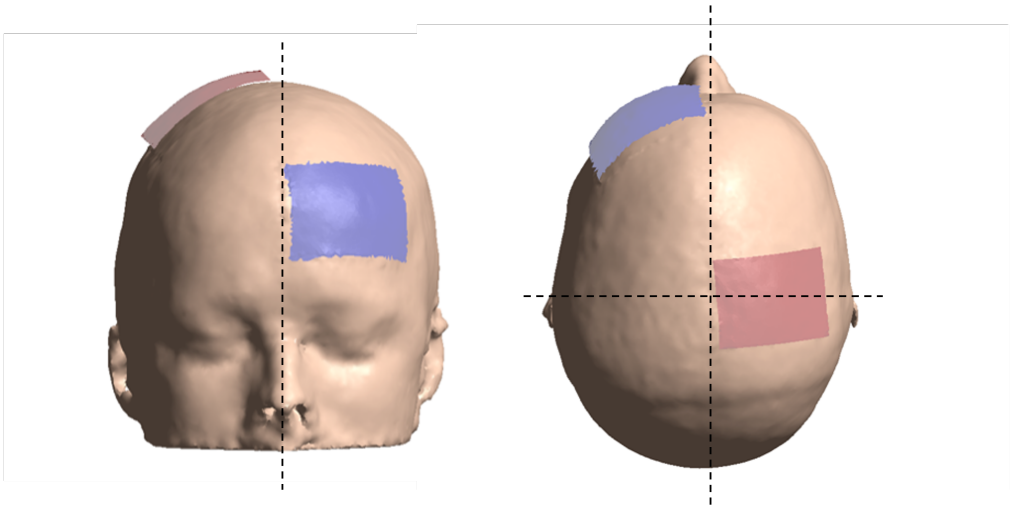


Figure 5.2: Tissue types segmented with SimNIBS CHARM: A reference segmentation with corresponding colour-coded tissue labels for the 15 tissue types

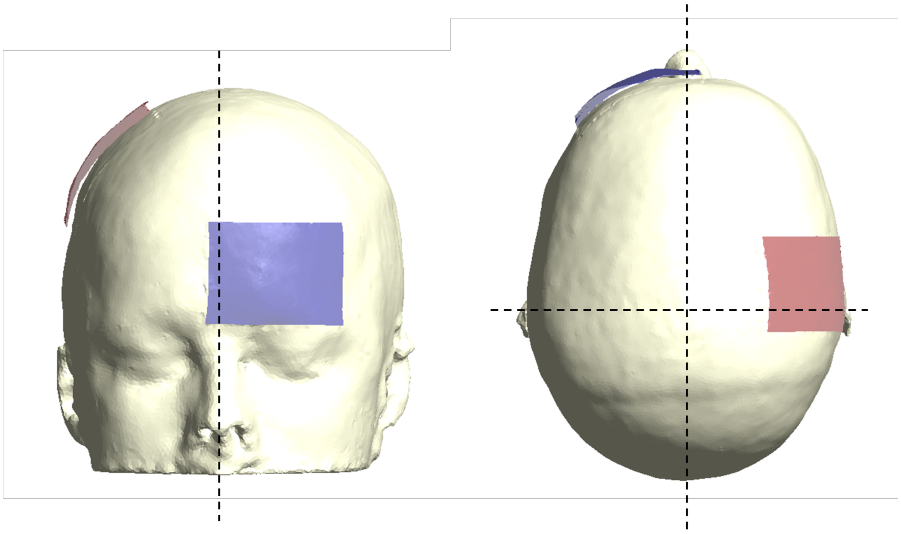
5.3.2 tDCS configuration

Simulated electrodes were placed on the reconstructed head meshes of individual participants to model anodal tDCS of M1. Electrode placement was guided by the standard 10/20 EEG system but manually corrected where needed. M1 electrodes were placed on C2 (right-sided, for participants with a weaker left side) and C1 (left-sided, for participants with a weaker right side). The cathode was centred on the contralateral forehead at AF3 (right-sided stimulation) or AF4 (left-sided stimulation). These landmarks differ from the previously reported studies which utilise C3 and C4 for M1, and Fp1 and Fp2 as co-ordinates for the anode and cathode respectively. The anode in our study was deliberately placed in a coronal orientation with the aim of capturing both upper and lower limb regions of the motor cortex. Furthermore, given the smaller head sizes in our cohort, traditional placement of the frontal electrode on Fp1 or Fp2 resulted in obscuring parts of the eyebrow and eye, thus we opted for a higher-up placement. This is illustrated in Figure 5.3. All simulations were visually inspected and manually corrected to ensure electrode placement mirrored real-life placement.

Electrodes were modelled within SimNIBS to represent those produced by NeuroStym. Rectangular sponges (7 x 5cm) of 6mm thickness and a rubber electrode of 1 mm were specified. Standard conductivity values as recommended by SIMNIBS were used: Rubber electrode conductivity was set to 0.100S/m, and saline-soaked sponges at 1.00S/m. Current strength was set to 1mA.



(a)



(b)

Figure 5.3: Electrode placement for right M1 anodal stimulation as used (a) for this study versus (b) previously reported electrode placement illustrating the frontal electrode obscuring part of the left eyebrow, as well as the differences in placement of the anode. The red electrode represents the anode, and blue electrode represents the cathode

Region	Atlas labels
Motor	4
Supplementary motor	6mp, 6ma, SCEF
Premotor	FEF, PEF, 55b, 6d, 6v, 6r
Occipital	V1

Table 5.1: Summary of atlas regions selected for subsequent ROI-based analyses

5.3.3 Regions of interest analysis

The HCP MMP1 atlas is a multimodal atlas derived from structural T1w and T2w data, as well as task and resting state fMRI data[358]. The atlas was chosen for this reason as its parcellations may have functional, in addition to anatomical, relevance, and StimCP was a trial of neuromodulation of motor function. This atlas is also notable for fine-grained parcellations of motor regions of interest (ROIs), including primary motor areas, premotor, and supplementary motor areas. Table 5.1 summarises the atlas regions that made up the ROIs used for analysis. The occipital ROI was included as a control.

As part of segmentation and creation of mesh surfaces, SimNIBS calculates 6 degrees-of-freedom, 12 degrees-of-freedom and non-linear MNI transformations. For specified ROI labels, SimNIBS transforms atlas regions from MNI space to subject space. Mean electrical field strength of all the triangular nodes in each ROI in subject space was calculated, weighted by the area of each node.

5.3.4 Variables of interest

Heat maps of electric field strength were generated for each participant. Variables of interest included:

1. Peak electric field strength (in V/m), which corresponds to the 99.9th percentile value of the electric field strength values associated with each

mesh element

2. Field focality was extracted as the volume (cm^3) of grey and/or white matter tissue that had electric field strength values greater than or equal to the peak electric field strength.

3. Regions of interest, namely motor, premotor, supplementary, and occipital regions, were used to extract the mean electric field strength in each region.

5.3.5 Outcome scores

Motor outcomes were assessed at 1, 6, and 12 weeks post-intervention. The percentage change in upper limb outcome, measured by the Jebson Taylor Test (JTT) and lower limb outcome, measured by timed up and go time (TUG) were calculated at each follow-up period. Further details are outlined in 3.2.3.

5.3.6 Statistical analysis

Data were analysed using GraphPad Prism (Version 10.2.3) and SPSS (v29.0.2, IBM inc). Data were assessed for normality of the standardised residuals using Shapiro-Wilk tests and visual inspection of frequency histograms. If data were assessed to be normally distributed, then parametric statistics were utilised, otherwise, non-parametric statistics were used. Significance was set at $p < 0.05$. Multiple comparisons were corrected for using Bonferroni correction unless otherwise specified.

All participants (sham and active stimulation) were included in analyses evaluating electric field distribution and strength. Independent samples t-tests were used to determine if there was an effect of stimulation laterality on the mean electric field in specified regions of interest.

Only participants who received active stimulation were analysed in the outcomes section. Primary outcomes, the JTT and TUG, were assessed by using the percentage change in score from baseline to each time point (1 week, 6 weeks, and 12 weeks) Pearson's correlation analyses were run to determine whether mean electric field of each ROI was related to outcome (upper and lower limb outcome).

5.4 Results

5.4.1 Demographics

Data in this chapter were analysed prior to completion of the full StimCP study, therefore this represents a subset of the first 18 participants in the study. Four participants did not consent to the baseline MRI and 3 participants were excluded after initial MRI quality checking due to movement artefact and inadequate field of view. Five females and six males are included in this analysis (median age = 12.7 years, range 10.2 to 15.8 years). Functional classifications, most affected side, and site of stimulation are included in Table 5.2.

5.4.2 Electric field modelling in children with cerebral palsy can be achieved with standard tools

Our results show that current modelling was feasible in this population with robust quality control of segmentation and tissue classification employed. Two main technical challenges arose in preparation of the head models for the electric field simulation. Firstly, segmentation errors of CSF occurred in two participants, similar to those previously described by Carlson et al (2022). The first participant, illustrated in Figure 5.4, had a large CSF-filled defect at the site of a previous right middle cerebral artery stroke. Initial segmentation (Figure 5.4a) incorrectly classified this region as white matter, which could be manually re-classified correctly, as seen

Table 5.2: Participant demographics for this subset of data, including those who received sham stimulation. The site of active stimulation and the number of participants receiving active stimulation are also included.

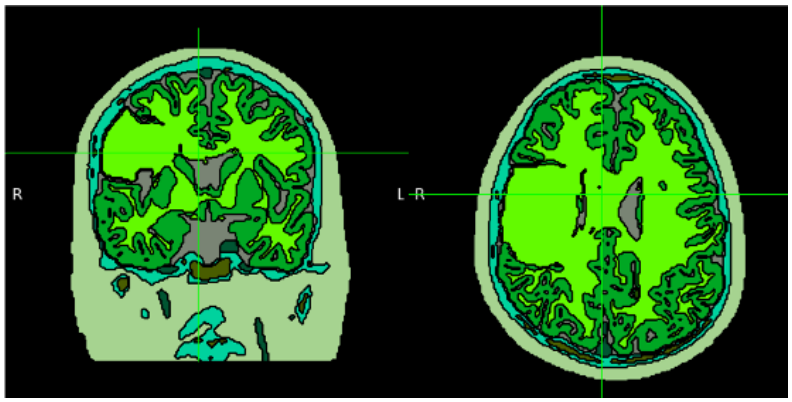
Sex		
	Male	6
	Female	5
GMFCS		
	Level I	3
	Level II	6
	Level III	0
MACS		
	Level I	5
	Level II	6
	Level III	0
Most Affected Side		
	Left - Leg	3
	Left - Arm	3
	Right - Leg	2
	Right - Arm	3
Site of Stimulation		
	Right	6
	Left	5
Active Stimulation		
	Right	5
	Left	2

in Figure 5.4b. The electric field simulation for this participant is demonstrated in Figure 5.6. The second participant similarly had a misclassification of CSF at the boundary of an irregularly shaped ventricle, which could be manually re-classified. The remaining 9 participants had no segmentation errors on visual inspection.

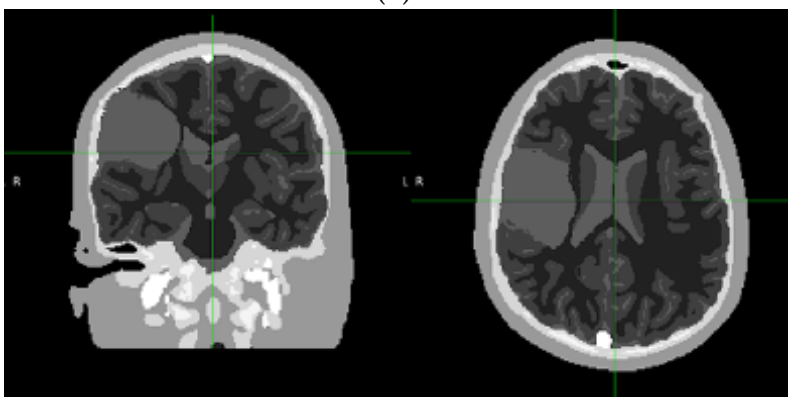
Secondly, owing to the smaller and irregular head shapes in this cohort, the specification of the electrode position in each participant had to be visually checked prior to running the simulation. Two participants required manual rotation of electrodes (less than 30 degrees) to accurately match the real-world placement of electrodes.

Biologically plausible simulations were generated in all included participants (Figures 5.5 and 5.6). These are encouraging results, which demonstrate that, while

more labour-intensive, it is feasible to include participants with larger lesions.



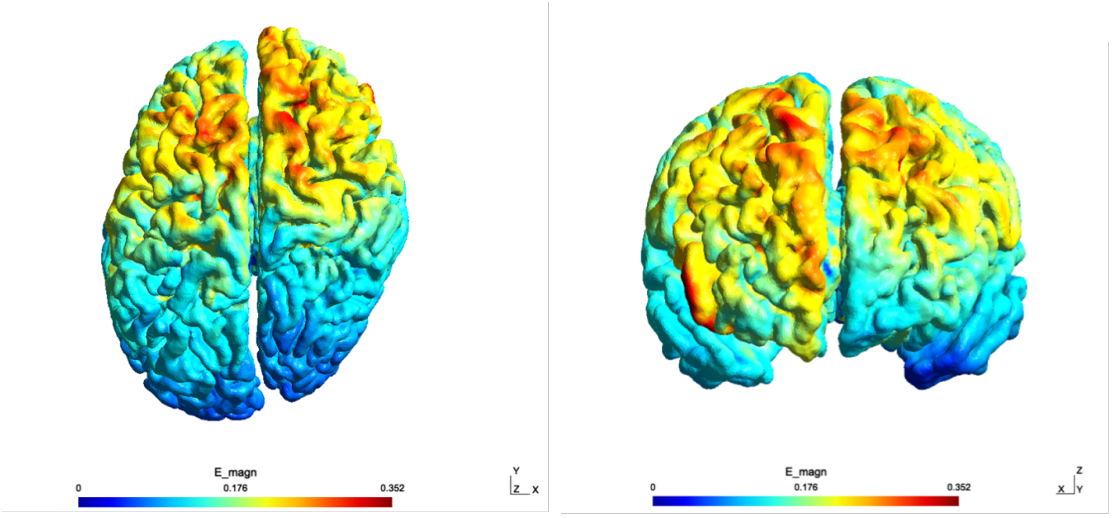
(a)



(b)

Figure 5.4: Representative example of segmentation errors. In this example, figure (a) demonstrates that CSF has been misclassified as white matter on the right, while (b) illustrates the correction post manual segmentation of the CSF/white matter boundary

Sub-01



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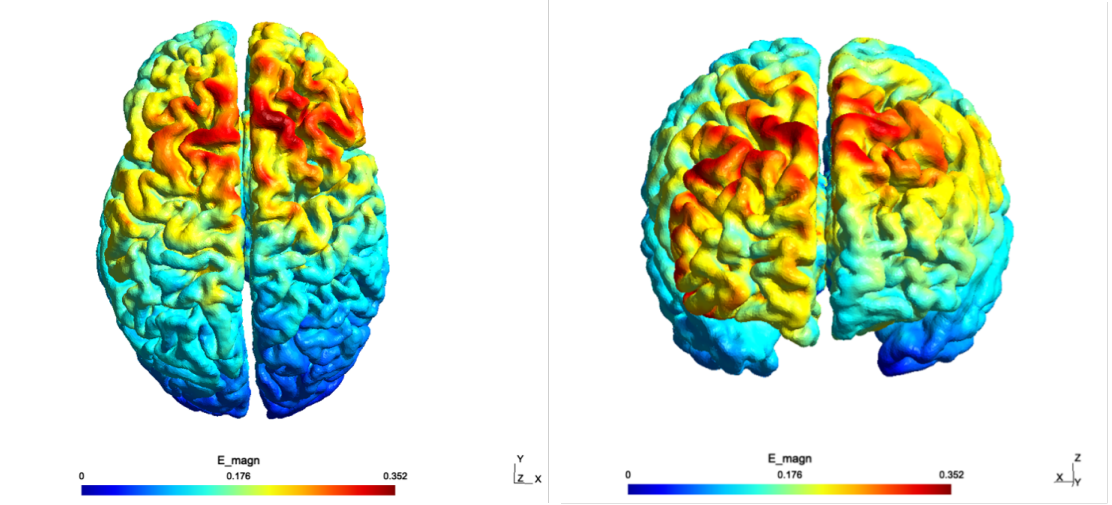

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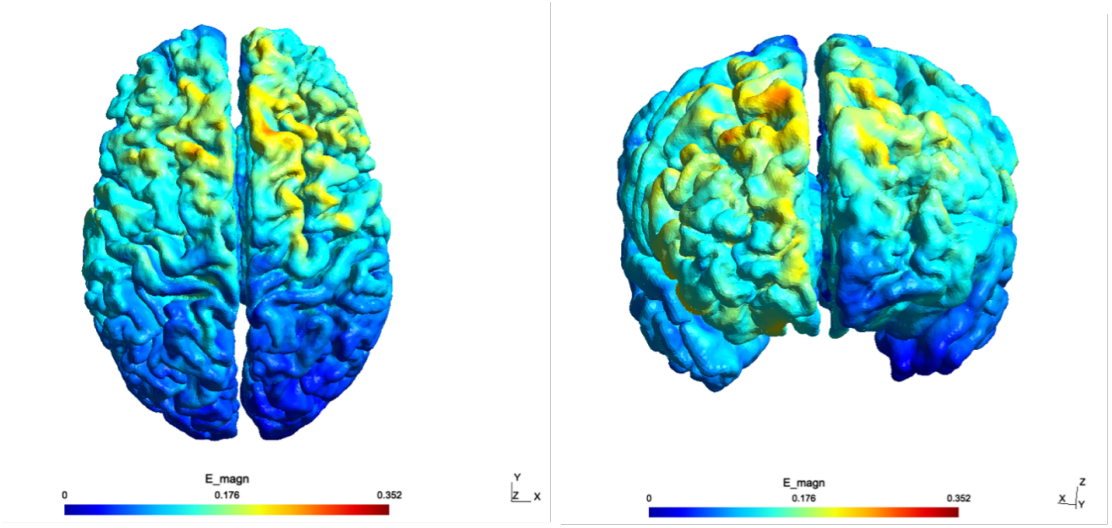


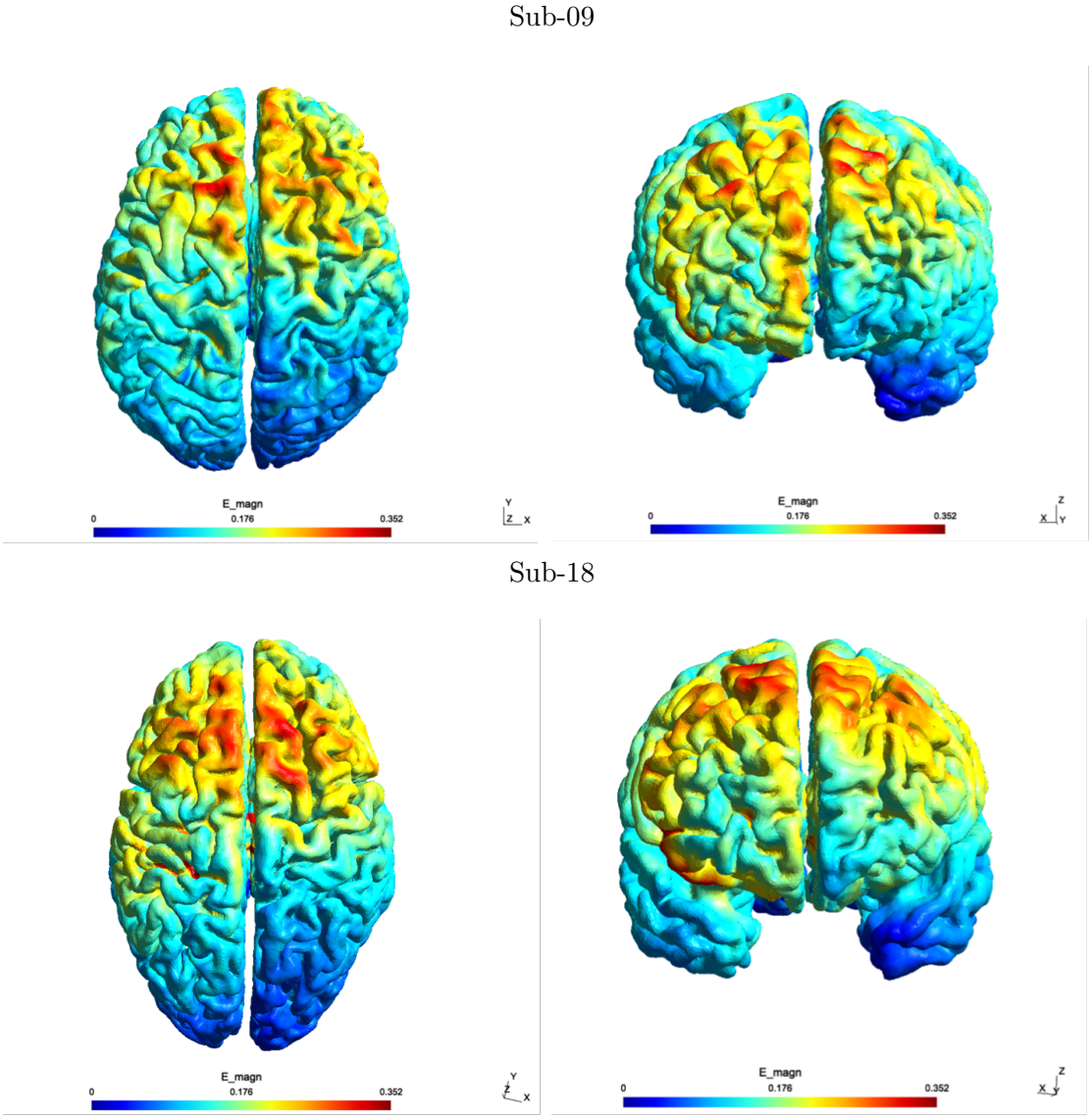
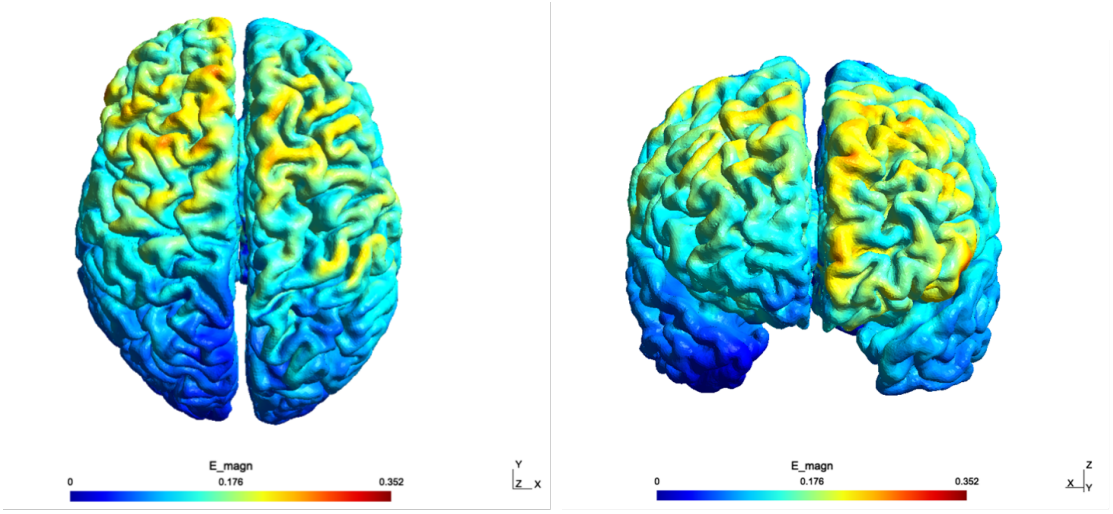


Figure 5.5: Electric field models for left-sided anodal stimulation in CP (same colourmap scale for all participants). This illustration includes participants who received sham stimulation to demonstrate inter-individual variability.

Sub-02



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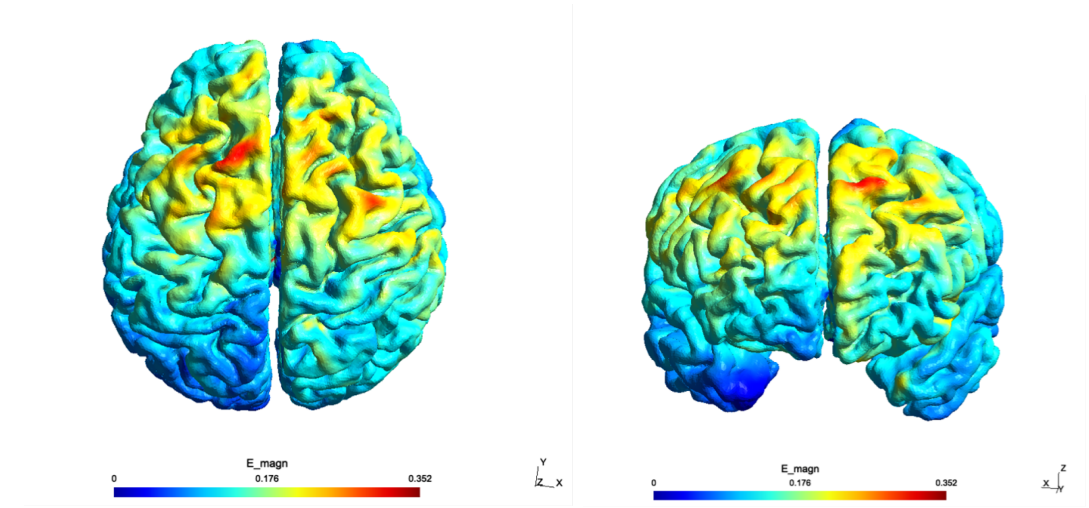

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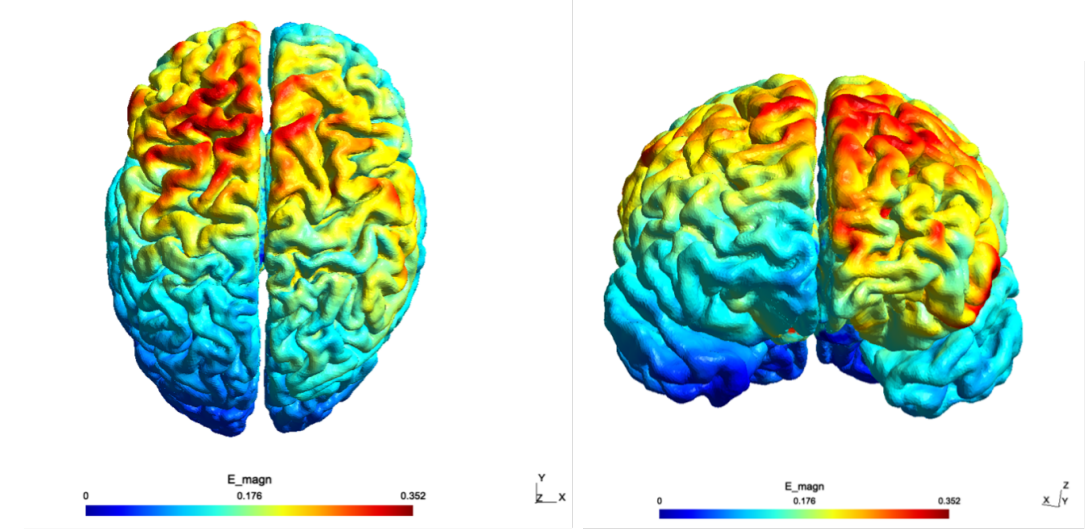


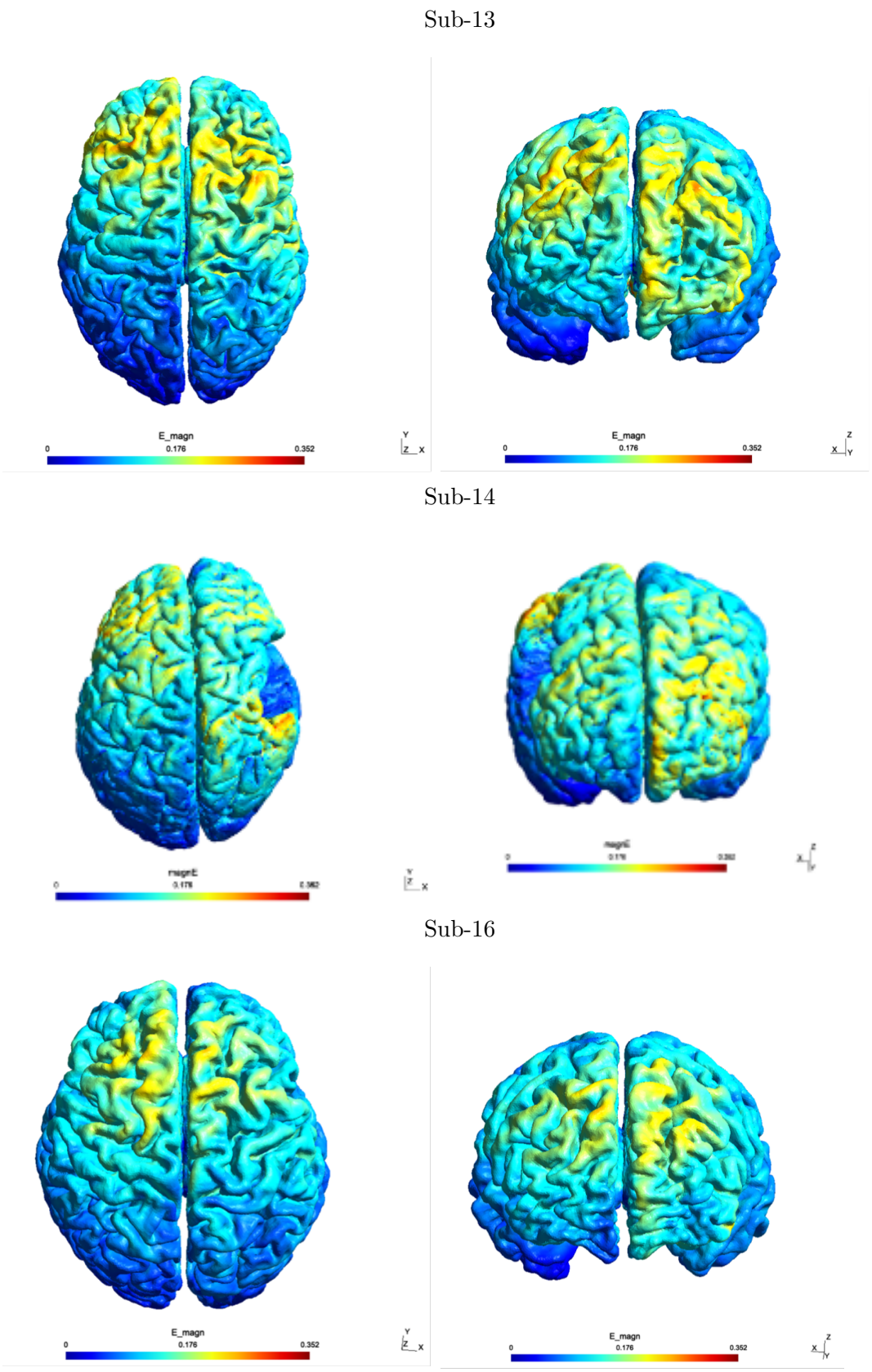


Figure 5.6: Electric field models for right-sided anodal stimulation in CP (same colourmap scale for all participants). This illustration includes participants who received sham stimulation to demonstrate inter-individual variability.

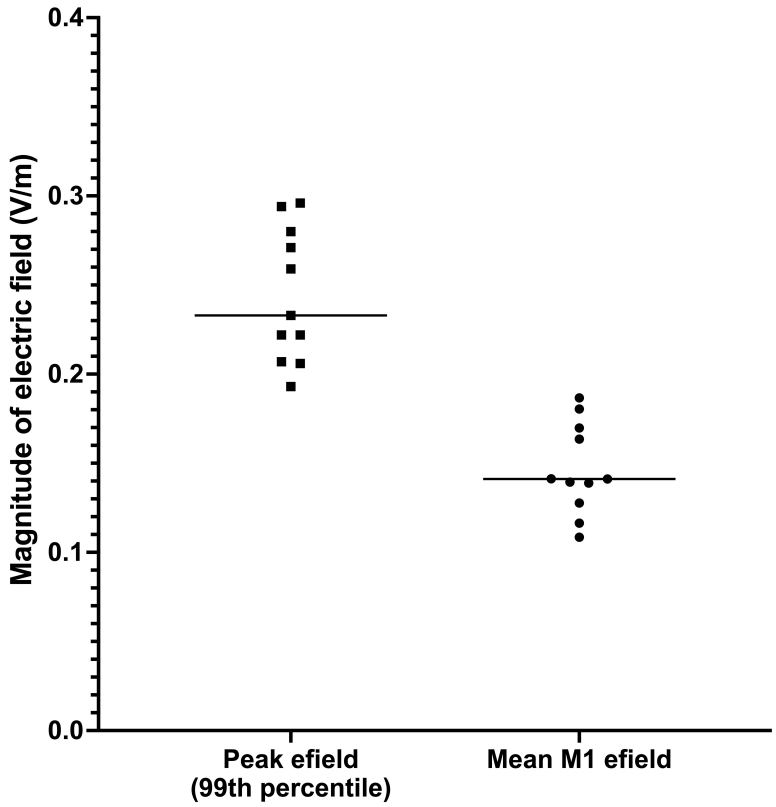


Figure 5.7: Peak electric field strength and mean electric field strength in the stimulated motor cortex (M1) across all participants

Electric field strength and distribution is highly variable between individuals

The electric field strength and distribution seen in Figure 5.5 and Figure 5.6 demonstrate highly variable maps across participants who received left- and right-sided anodal stimulation, respectively. The minimum mean electric field strength of the stimulated motor cortex was 0.1085 V/m, and the maximum was 0.187 V/m (Figure 5.7).

Stimulation site does not correlate with site of maximal electric field intensity

Determination of the mean electric field strength in the motor, premotor, and supplementary motor areas for the stimulated and non-stimulated hemisphere is demonstrated in Figure 5.8. While visually there appears to be a trend for left sided anodal stimulation to produce higher mean electric field strengths, this was not found to be a statistically significant difference across M1 ($t(9) = 0.69$, $p = 0.51$), the premotor area ($t(9) = 1.09$, $p = 0.30$) or the supplementary motor area ($t(9) = 1.11$, $p = 0.30$).

The supplementary motor area demonstrated the highest mean electric field strength. There was a statistically significant difference between the mean efield strength in the stimulated M1 versus the SMA ($t(10) = 5.4$, $p = 0.0003$) across all participants (Figure 5.9). There was no statistically significant difference between mean electric field strength of M1 and the premotor region ($t(10) = 2.181$, $p = 0.054$).

Electric field strength was predictably low in both occipital cortices for both groups.

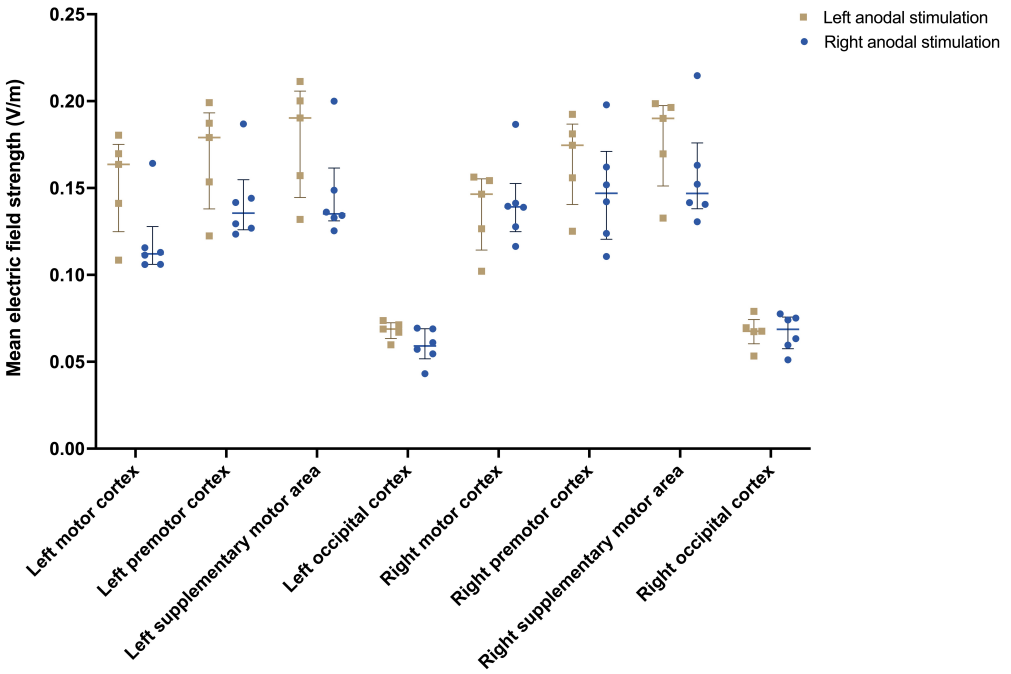


Figure 5.8: Mean electric field strength across the motor cortex (M1), premotor cortex, and supplementary motor area (SMA) illustrates the range of simulated currents across participants. Occipital cortex included as a control. Simulations were based on anodal stimulation to the ipsilesional hemisphere, and are categorised into ROIs in the stimulated and non-stimulated hemisphere, as well as to stimulation in the left or right hemisphere.

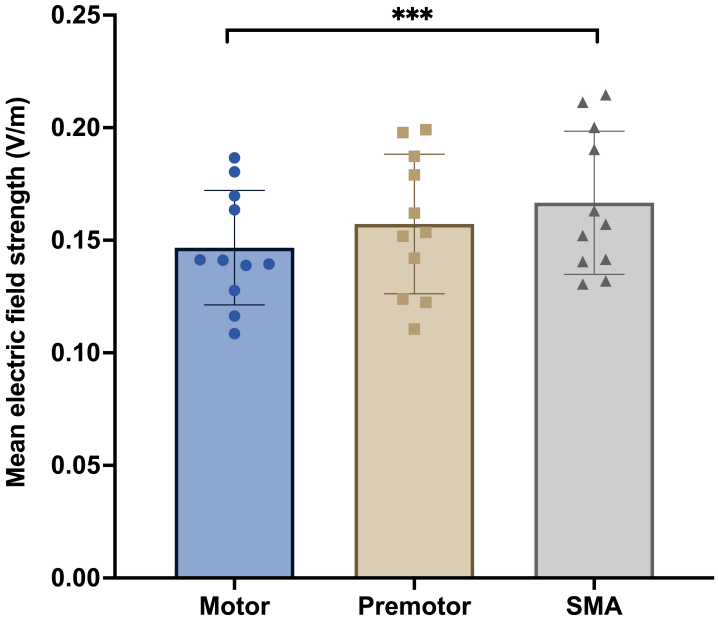


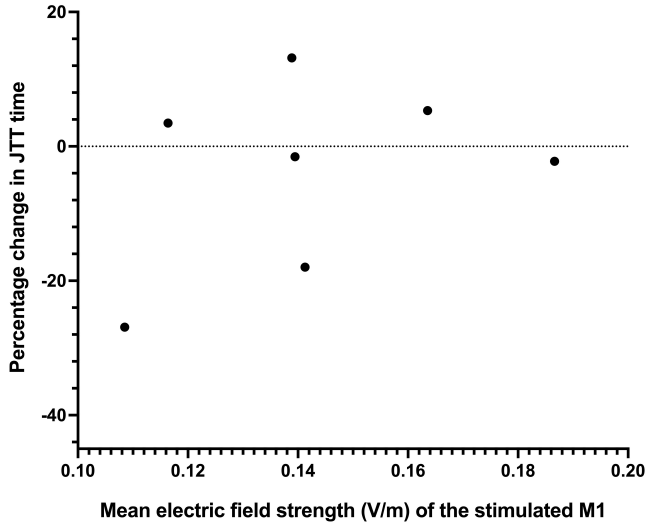
Figure 5.9: Mean electric field strength in the stimulated motor cortex (M1), premotor, and supplementary motor area across all participants. There is a statistically significant difference between mean EF of M1 and the SMA, p = 0.0003

5.4.3 Electric field correlations with motor outcome

For this analysis, only participants who received active stimulation were included, thus 5 participants who underwent right-sided stimulation and 2 who underwent left-sided stimulation were included. The region of interest used was specified as the area on the side where anodal stimulation was applied.

Spearman's correlations were calculated to determine the relationship between electric field strength and upper and lower limb motor outcomes. There was no significant correlation between change in JTT time and M1 mean electric field strength at 1- ($r(6) = 0.11$, $p = 0.84$), 6- ($r(5) = 0.08$, $p = 0.92$), or 12- ($r(6) = 0.14$, $p = 0.78$) weeks. Similarly, there was no significant correlation between change in TUG time and M1 mean electric field strength at 1- ($r(6) = -0.29$, $p = 0.56$), 6- ($r(5) = 0.02$, $p = > 0.99$), or 12- ($r(6) = 0.21$, $p = 0.66$) weeks (Figure 5.10).

Further exploratory analysis was performed to determine if there was a correlation between peak electric field strength and motor outcome (Figure 5.11), and field focality and motor outcomes (Figure 5.12). No statistically significant relationships were found.



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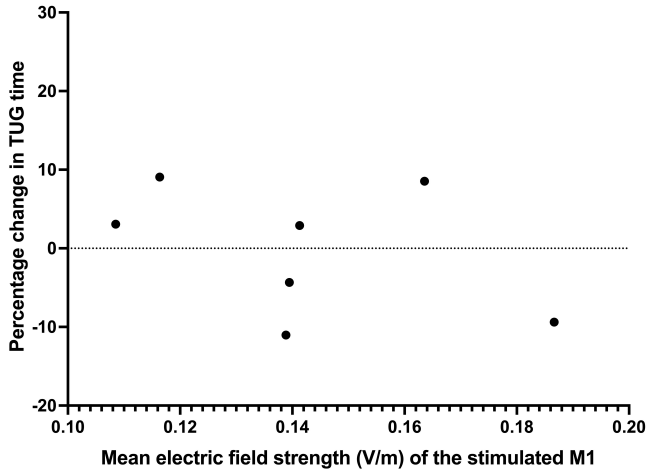
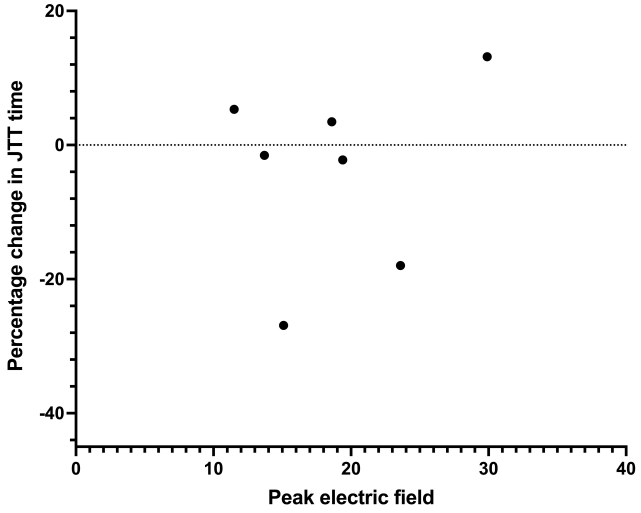
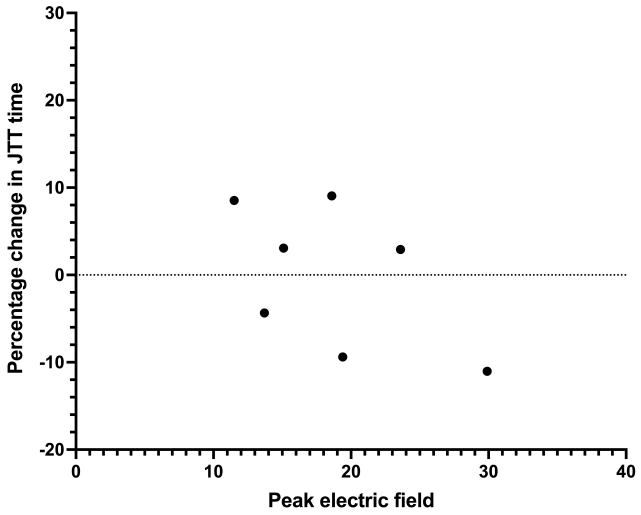


(b)

Figure 5.10: Percentage change in (a) upper limb score and (b) lower limb score at one week follow up demonstrated no significant correlation with mean electric field strength in M1. A negative percentage change indicates an improved score at 1 week.

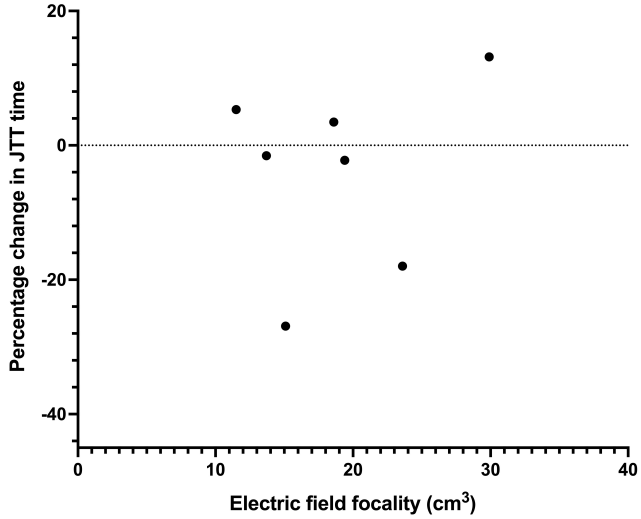


(a)

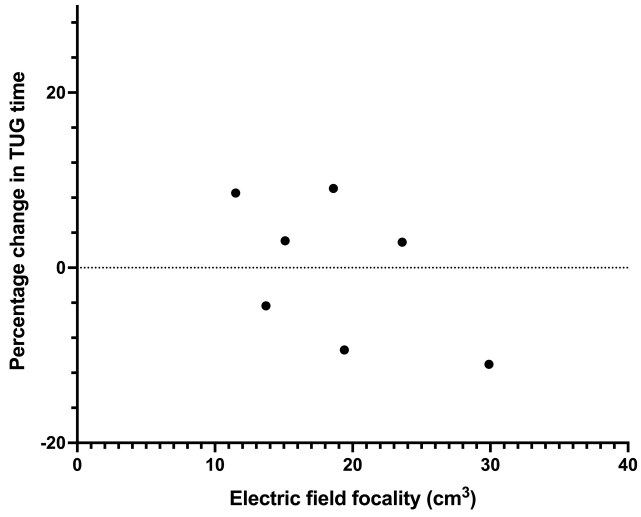


(b)

Figure 5.11: Percentage change in (a) upper limb score and (b) lower limb score at one week follow up demonstrated no significant correlation with peak electric field strength. A negative percentage change indicates an improved score at 1 week.



(a)



(b)

Figure 5.12: Percentage change in (a) upper limb score and (b) lower limb score at one week follow up demonstrated no significant correlation with field focality. Field focality is the tissue volume (in cm^3) that had electric field values greater than or equal to 75% of the 99.9th percentile. A negative percentage change in score indicates an improved score at 1 week.

5.5 Discussion

5.5.1 Demonstration of feasibility

In this chapter, individualised electric field current models were successfully generated for 11 participants with cerebral palsy, as part of exploratory analysis to better understand variation in response to transcranial direct current stimulation.

Through the adaptation of existing tools, it is feasible to generate biologically plausible and anatomically accurate head models and electric field simulations. It is possible to include participants with large lesions in the analysis of electric fields, even when automated tools fail to accurately segment tissues, through meticulous inspection and manual mask correction. This analysis addresses key limitations of previous work by assessing variability between individuals. Additionally, this analysis fills in part of the gap between the application of tDCS at a standard "dose" on the scalp and models what "effective dose" is delivered to the target motor regions.

5.5.2 Stimulation of adjacent motor areas

In this cohort of participants, we have demonstrated large variability in peak and mean electric field (EF) strength in regions of interest. This mirrors previous literature which similarly found high inter-individual variability in typically developing children[342] and adults[359,185]

Our findings are also in keeping with Carlson et al (2023), who have have recently reported a large series of electric field modelling using MRI data from 83 children with arterial ischemic stroke (AIS), periventricular infarction (PVI), and typically developing controls (TDC). They demonstrated variability in peak electric field strength across all participants, in keeping with our findings. Of interest, they also reported statistically significant differences in peak electric field

strength between the AIS and TDC groups, demonstrating group-based variability based on pathology. Furthermore, they demonstrated significant variations in mean electric field strength in M1, dependent on the electrode montage[263]. Importantly, despite this being a large sample, 19% of the initial 108 participants were excluded as their lesions were incorrectly classified during the modelling pipeline[263]. The results, therefore, likely underestimate the extent to which larger lesions affect current strength and distribution.

Of particular interest is our finding that the mean EF strength in M1 is lower than in both the premotor cortex and supplementary motor area. This reflects the overall lack of focality of transcranial direct current stimulation. However, more widespread stimulation does not necessarily imply lower efficacy, as it may be through stimulating a wider motor network involving primary motor, premotor, and supplementary motor areas that motor learning could be modulated[360]. Lefebvre et al (2019), in a study evaluating changes in cortical motor excitability through stimulation of either SMA or the motor hand "hotspot", demonstrated that stimulating the SMA led to a higher number of responders and greater changes in motor excitability[360].

5.5.3 EF modelling to predict motor outcome

This is the first study, to our knowledge, that explores the relationship between simulated electric field parameters and response to a tDCS intervention. In this cohort, there were no correlations between motor outcome and electric field stimulation parameters including mean EF in M1, peak EF, and field focality. This is a small sample size, and therefore, the conclusions that can be drawn from this data are limited.

While ours is the first study to evaluate the relationship with outcome metrics, a study by Nandi et al (2022) utilised electric field modelling and MR spectroscopy

to assess if EF variability correlates with variability in tDCS-induced changes in GABA. They demonstrated that mean EF magnitude was associated with greater decreases GABA, and that this effect was moderated by grey matter volume in the MRS voxel[361]. This is compelling work, given that decreased GABA is one of the proposed mechanisms of action for anodal tDCS (Section 1.3.1).

Future work would benefit from utilising multimodal techniques, such as MRS, while also evaluating relationships between EF strength, neurotransmitter changes, and behavioural responses to tDCS.

Another potential application of electric field modelling is dose and electrode optimisation. Several studies have utilised reverse dosing calculations to determine individualised tDCS doses[362,363,352] and electrode placement[352,362], and demonstrated more consistent and improved focal targeting of the designated stimulation region when individualised doses and montages are used[352]. A logical next progression would be to prospectively optimise tDCS dosage and montage, and determine if this reduces response variability.

5.5.4 Limitations

This study constitutes a small sample, and larger studies are needed to critically evaluate if there is a role for EF modelling in the prediction of treatment response to tDCS.

While there is clear evidence that anatomical variation leads to simulated electric field variability, we cannot be certain that this corresponds to in vivo variability. Electric field modelling is an estimate rather than a direct measure of how much stimulation reaches the cortex. In vivo validation remains challenging, but there is evidence that EF estimates and measured intracranial recordings

demonstrate a linear relationship[364,365], and therefore these models are being used with increasing confidence.

6

Discussion

Contents

This thesis makes several contributions to the field of brain stimulation intervention in children and adolescents with cerebral palsy. The clinical trial detailed in Chapter 3 is unique: it is the only trial to assess ipsilesional anodal tDCS, combined with clinically proven motor training, to target upper and lower limb function in children with cerebral palsy.

In Chapter 2, I showed the importance of ongoing physical activity and rehabilitation and assessed the impact of disruption of care during the unprecedented COVID-19 lockdown. In Chapter 3, I summarised the motor outcomes of the StimCP study, a randomised clinical trial of brain-stimulation-assisted rehabilitation in adolescents with cerebral palsy. Chapters 4 and 5 explored the use of individualised tractography and electric field modelling, respectively, to test whether variations in baseline motor

tract integrity and brain structure could help explain the motor outcomes in this study. In this chapter, I will review the strengths and limitations of the above work and the implications for future work on brain stimulation in cerebral palsy.

6.1 Transcranial Direct Current Stimulation to improve motor function in cerebral palsy

In Chapter 3, the results of a clinical trial of anodal tDCS are presented. While a pilot study, the sample size of 27 participants is comparatively large for trials in this population, and adherence to study protocol was good (20 participants [74%]) completed all 10 interventions, and all participants completed the primary endpoint).

Both upper and lower limb function improved post-intervention, and these improvements persisted at the 12-week follow-up. However, anodal stimulation had no significant additional effect on any objective metrics of upper and lower limb function.

Subjective measurements of function demonstrated participant-reported increases in the use of both hands for bimanual activities, but no additional effect of stimulation. Perceptions of lower limb function did not change significantly post-intervention, and there was no additional effect of stimulation.

This study has several important strengths. This tDCS intervention followed best practices in the field in several domains including: utilising sham stimulation in the control group, double (participant and researcher) blinding, and adhering to safety guidelines on dosage[146,366,112]. Participants performed motor tasks that were functionally relevant, involving repetition and increasing difficulty over time, which are recognised as important principles of motor learning[80,81,82,83]. Engaging participants in a related motor task during M1 stimulation with tDCS is based on evidence of preferential enhancement or consolidation of a specific pattern of concurrent neural activity through neuroplasticity[123,221,220].

It was important for our study to include a clinically relevant population of children with cerebral palsy. The majority of children with cerebral palsy will have more than one limb affected[52], but the majority of studies assessing tDCS in this population assess either upper or lower limb function in isolation, not both. Additionally, a significant proportion of the literature focuses on specific subtypes of CP, such as perinatal stroke, which limits wider clinical translation of these findings. As the majority of children with CP in the UK will not have access to neuroimaging after infancy, and typically do not receive a more granular diagnosis (such as periventricular leukomalacia or perinatal stroke), ensuring that eligibility for the study reflected current clinical diagnoses enabled more translatable findings.

However, this 'catch-all' approach has also presented challenges. Cerebral palsy theoretically has a common pathway that leads to non-progressive motor impairments, but the underlying pathology and timing of insult lead to significant variability in underlying pathophysiology. This variability may contribute to our finding of a lack of additional effect on motor outcome of anodal tDCS, and our sample size was not large enough to do effective sub-group analyses. Larger sample size may afford adequate power to run sub-group analyses, but is limited by resources. Multi-site studies with harmonised protocols, and publication of trial outcome data for meta-analysis may also address this limitation. Recruitment is a major barrier to achieving ideal study sample size. A UK-wide registry of children with CP would be hugely beneficial to assisting recruitment for studies, while also improving access to research for families.

There are many possible reasons for null results in this study. It might be that non-invasive brain stimulation does not improve motor function in CP. However, there may be sources of variability that have influenced the result. The presence of genetic polymorphisms (e.g. BDNF Val66Met), may reduce possible effects of tDCS[367,368,128]. Other sources of variability, such as biological sex and

hormonal cycles[177,178] and baseline neurochemistry are difficult to control for in small sample sizes.

Lastly, given that this was a pragmatic trial, it was not possible to perform baseline cortical excitability assessments using transcranial magnetic stimulation; nor to perform a baseline MRI for neuronavigation purposes. It is possible that individual cortical re-organisation from early injury resulted in atypical motor area and/or corticospinal tract organisation[282,109,107]. Future studies may benefit from utilising TMS guided motor mapping and neuronavigation to ensure electrodes are optimally placed and account for individual differences.

6.2 The potential utility of corticospinal tract integrity as a correlate of function

In Chapter 4, characterisation of corticospinal tract integrity using probabilistic tractography was performed, and metrics related to baseline function and treatment response.

We demonstrated the successful generation of corticospinal tracts in all included participants. This is a significant finding, as previous studies have relied on manual delineation or excluded participants with larger lesions. In a condition such as CP, which comprises patients with varied but significant lesions, it is extremely important for research to reflect clinical cohorts to ensure work is clinically relevant and reproducible. We hope that our study can be used as a proof of concept for future studies and that ongoing work will be as inclusive as possible.

Secondly, we demonstrated that corticospinal tract FA and volume correlate strongly with baseline hand function on the affected side, such that increased FA and volume relate to better hand function. While this is perhaps intuitive, this relationship has only previously been demonstrated in unilateral upper limb cerebral

palsy. We were unable to demonstrate a similar relationship between baseline lower limb function, and FA or volume. One possible explanation may be that TUG and 10m walk time may not be the most sensitive or specific markers of lower limb function. While these tests are commonly used in this population, gait sensors have been used to good effect in several studies, and may be a more sensitive correlate[203,202]. Additionally, we utilised a combined FA measurement of both the right and left corticospinal tracts, as standing up from seated and walking are tasks reliant on both lower limbs. However, the relative contributions of both tracts to the movement are unknown and it is possible that some participants had undergone early reorganisation of their corticospinal tracts to preference ipsilesional connections[282,109]. TMS motor mapping would be a useful modality to further investigate this.

Finally, we aimed to determine if baseline CST integrity is a sensitive metric for predicting response to treatment. In this cohort of patients, we did not demonstrate a correlation between changes in upper or lower limb function and metrics of corticospinal tract integrity. To our knowledge, this is the first study to assess this relationship in a general, undifferentiated cerebral palsy population.

We specifically focused on the corticospinal tract, given that it predominates skilled voluntary limb movements. CST integrity has been demonstrated to play a role in upper limb functional outcome in adult stroke[369]. However, given that children with cerebral palsy tend to have a more global brain injury, as well as subsequent cortical reorganisation, assessing the CST alone may not be sufficient to explain the underlying motor function and predict treatment response. Further assessment of complementary tracts involved in motor planning and movement, such as the corpus callosum and superior longitudinal fasciculus, may help to disentangle the role of white matter and rehabilitation potential.

A key strength of our study is the use of automated tractography in native space, which has two main advantages. Firstly, this technique is less amenable to biases than other methods, such as manual segmentation or atlas-based techniques, both of which

may be less robust in patient groups with variable anatomy and more subjective[321]. Secondly, data were analysed in native space and, therefore, retain their inherent structural variability and size, an important consideration in this population.

As mentioned above, our study is limited by the use of only one tract. One potential avenue for investigation moving forward is to utilise network-based analysis using individual connectome maps. Work by Ballester-Plane et al. (2017) in children with dyskinetic CP demonstrated reduced FA in bilateral pathways comprising sensorimotor, intraparietal and frontoparietal connections[307]. They were also able to demonstrate gross and fine motor function correlated with FA in a pathway comprising the sensorimotor system. Englander et al (2015) utilised a similar approach in a trial of cord blood transfusion for children with spastic cerebral palsy - and demonstrated a potential correlation between white matter connectivity and functional improvement, though this was not statistically significant[370]. This presents a promising future avenue of research.

6.3 Individualised electric field modelling to explain variability in tDCS effect

In Chapter 5, individualised simulated electric field models of current flow were generated for a subset of participants in the StimCP trial. Anatomical variability may be one potential contributor to inter-individual responses to tDCS, and the aim of this work was to characterise the induced electric field in our trial cohort, as well as determine if EF modelling can predict motor outcomes after tDCS.

We demonstrated variability in peak and mean electric field strength in regions of interest, in keeping with previous studies of healthy adults and children[342]. A unique contribution of this study is the inclusion of participants with large lesions, which have previously been excluded from similar studies due to difficulties in segmentation and tissue labelling[263]. The impact of such lesions on estimated

electric fields is likely large, given that CSF is an excellent electrical conductor and most lesions are CSF-filled areas.

Secondly, in our sample, mean electric field strength in M1 was lower than in the premotor cortex and supplementary motor cortex of the same side, which demonstrates the overall lack of specificity of tDCS. Future work to utilise computational modelling to estimate the optimal dose and electrode placement for individuals may assist in increasing overall specificity, and could potentially decrease variability in treatment response.

Finally, we aimed to determine if electric field parameters such as mean M1 electric field, peak electric field, and field focality would correlate to tDCS response. To our knowledge, this is the first study to investigate this relationship. Our sample size was small, and we found no correlations between EF measures and motor outcomes. As computational modelling becomes more utilised, we hope that future studies will investigate possible correlations between behavioural outcomes and electric field parameters, to further interrogate if inter-individual response may be mediated by tDCS dosage and/or electric field.

6.4 Overall reflections and implications for future work

There is an ongoing need for clinically relevant, time-efficient, and effective interventions to improve motor function in children with cerebral palsy. However, clinical research in this population is challenging. Despite pragmatism in study population inclusion criteria for this study, recruitment was extremely challenging. The lack of a centralised registry for CP in the United Kingdom remains a barrier for research, and many parents highlight difficulties in accessing clinical trials. Beyond recruitment, the heterogeneity of the group makes analysis of outcomes and exploration into mediating effects challenging. A significant proportion of research into neuroimaging,

neuromodulation, and electric field modelling addresses only one subtype of cerebral palsy. However, we strongly believe that, in order to ensure real world applicability of interventions, we must aim to have study populations mirror our clinical populations. An additional limitation of the current evidence in neuroimaging and modelling in CP is the exclusion of participants with large lesions, where segmentation or tractography fails, which again limits real-world translation of findings.

The utility of tDCS as a therapeutic adjunct remains unclear: our study of 27 children demonstrated no effect of anodal tDCS on outcome, yet other studies have demonstrated effects. In order to robustly answer the question of utility, we need multi-site studies with harmonised protocols, and publication of trial outcome data for meta-analysis. Collaboration will enable a better understanding of treatments for cerebral palsy as a heterogenous condition, as well as adequately power sub-group analysis.

Advances in electric field modelling may also enable us to optimise stimulation protocols for each individual through electrode placement and current strength, and therefore standardise the effective dose of tDCS. Additionally, improvements in segmentation tools may improve the quality of segmentations, require less manual cleanup, and enable greater inclusion of participants with lesions into neuroimaging studies.

There remains much to uncover in order to better understand the unique mechanisms at play in motor system plasticity during development, the role of neuroplasticity in relation to early brain injury, and possible therapeutic targets which could improve function.

Appendices

A

COVID-19 Impact Statement

Contents

A.1 Planned work before university buildings were closed to non-Covid-19 work

Prior to COVID-19 closures, we had submitted appropriate ethics applications, and developed our study protocol. We had planned to start recruiting for StimCP in April 2020, and had just received NRES approval when university buildings were closed to non-COVID-19 research. The original recruitment plan had been to run testing blocks in the school Easter, Summer, and Winter 2020 holidays, recruiting up to 15 children (half the sample size), in 2020. In the end, our recruitment ran from September 2021 and we completed the study in April 2024.

Prior to Covid closures, I had also hoped to visit the University of Calgary in

September 2020, as they have extensive experience in clinical trials in children with cerebral palsy/perinatal stroke, and I had hoped to learn from their experiences.

A.2 Return to clinical practice

In light of COVID-19, I returned to clinical practice in April 2020 to assist with training and education at the John Radcliffe Hospital and remained with them until August 2020. During this time, I helped to:

- Develop protocols and training materials on personal protective equipment use in the various clinical areas. We published a short letter on how we tried to disseminate and update the protocols as quickly and easily for staff as possible:

  Hong JS, Dwivedi K, Gavine B, Rughooputh N, Lee A, Salvagno C, Higham H. Improving staff confidence and morale through rapid, structured trust-wide technology-enhanced training in the use of COVID-19 personal protective equipment at Oxford University Hospitals. BMJ Simulation and Technology Enhanced Learning. 2020 Jul 14:bmjstel-2020

- Adapt and teach critical care procedures for COVID-19 amongst staff who were not ICU-trained.

- Develop resources for resuscitation protocols specific to COVID-19 including standard operating procedures for resuscitation in level 2 PPE and prone CPR for ICU patients with COVID-19.

- Design patient workflows for admission, theatre and ICU areas for COVID-19 patients.

- Create and train proning teams for ICU and critical care areas.

- Deliver skills training sessions for new Interim Foundation Doctors (who had graduated early as final year medical students to work in the hospital in May 2020).
- Deliver a simulated on call training session for Foundation Doctors who were starting at the John Radcliffe hospital from August 2020, and deliver virtual simulation based tutorials.
- Create and regularly update an online repository (https://www.oxstar.ox.ac.uk/covid-19) of all our teaching materials and protocols for public access across the world. The website was viewed across 105 countries.

A.3 Compensatory work

In August 2020, I recognised that the lockdown, with diminished access to medical and rehabilitative care, may impact our StimCP study population (children with CP) in terms of their motor function and ability to perform activities of daily living. As such, I conducted an opportunistic online survey of parents of and children with CP to try to understand their experience of lockdown and how this has affected their motor function. This forms the basis of Chapter 2.

Additionally, as an alternative to in-person experimental work, I began analysis on an existing data set of prism adaptation and tDCS. I had also began ethics application for a separate replication study based on this work, which I undertook from April 2021 - June 2022. Unfortunately, for non-COVID related reasons, continuation of these projects to completion was not feasible.

References

[1] W Little. "On the influence of abnormal parturition, difficult labours, premature birth, and asphyxia neonatorum, on the mental and physical condition of the child, especially in relation to deformities". In: *Trans Obstet Soc Lond* 3 (1862), pp. 293–344.

[2] S Freud. *Infantile Cerebral Paralysis. Original work published in 1897.* Florida,USA: University of Miami Press, Coral Gables, 1968.

[3] A. Kavčič and David B. Vodušek. "A historical perspective on cerebral palsy as a concept and a diagnosis". In: *European Journal of Neurology* 12.8 (Aug. 2005), pp. 582–587.

[4] Krzysztof Pietrzak, Andrzej Grzybowski, and Jacek Kaczmarczyk. "William John Little (1810-1894)." In: *Journal of neurology* 263.5 (May 2016), pp. 1047–1049. URL: http://www.ncbi.nlm.nih.gov/pubmed/26338817.

[5] Ronald C.Mac Keith, Ian C.K. Mackenzie, and Paul E. Polani. "The Little Club". In: *Developmental Medicine & Child Neurology* 1.5 (Sept. 1959), pp. 27–35. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8749.1959.tb08073.x.

[6] Martin Bax et al. "Proposed definition and classification of cerebral palsy, April 2005". In: *Developmental Medicine & Child Neurology* 47.8 (Feb. 2005), pp. 571–576. URL: http://www.journals.cambridge.org/abstract_S001216220500112X.

[7] Eve Blair and Sarah Love. "Definition and classification of cerebral palsy". In: *Developmental Medicine and Child Neurology* 47.8 (July 2005), pp. 510–510. URL: https://www.cambridge.org/core/journals/developmental-medicine-and-child-neurology/article/definition-and-classification-of-cerebral-palsy/74B920211DB6995BD64AED2EEE822BF3.

[8] Terence D. Sanger. "Is cerebral palsy a wastebasket diagnosis?" In: *Journal of Child Neurology* 23.7 (July 2008), pp. 726–728. URL: https://journals.sagepub.com/doi/10.1177/0883073808314963?icid=int.sj-full-text.similar-articles.1.

[9] P J Accardo. *Cupute and Accardo's Neurodevelopmental Disabilities in Infancy and Childhood.* 2008.

[10] Nigel Paneth and Marshalyn Yeargin-Allsopp. "Thinking about differences in the worldwide prevalence of cerebral palsy". In: *Developmental Medicine and Child Neurology* 64.12 (Dec. 2022), pp. 1436–1437.

[11] Sarah McIntyre et al. "Global prevalence of cerebral palsy: A systematic analysis". In: *Developmental Medicine and Child Neurology* 64.12 (Dec. 2022), pp. 1494–1506.

[12] Svetlana V. Glinianaia, Judith Rankin, and Allan Colver. "Cerebral palsy rates by birth weight, gestation and severity in North of England, 1991-2000 singleton births". In: *Archives of disease in childhood* 96.2 (Feb. 2011), pp. 180–185. URL: https://pubmed.ncbi.nlm.nih.gov/21068077/.

[13] R. Sundrum et al. "Cerebral palsy and socioeconomic status: a retrospective cohort study". In: *Archives of Disease in Childhood* 90.1 (Jan. 2005), pp. 15–18. URL: https://adc.bmj.com/content/90/1/15%20https://adc.bmj.com/content/90/1/15.abstract.

[14] Eve Blair et al. "Survival and mortality in cerebral palsy: observations to the sixth decade from a data linkage study of a total population register and National Death Index". In: *BMC Neurology* 19.1 (June 2019). URL: /pmc/articles/PMC6549269/.

[15] Mark Rosenthal. "Life expectancy and its adjustment in cerebral palsy with severe impairment: Are we doing this right?" In: *Developmental Medicine & Child Neurology* 64.6 (June 2022), pp. 709–714. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/dmcn.15120.

[16] Svetlana V. Glinianaia et al. "Predicting the prevalence of cerebral palsy by severity level in children aged 3 to 15 years across England and Wales by 2020". In: *Developmental Medicine and Child Neurology* 59.8 (Aug. 2017), pp. 864–870. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/dmcn.13475.

[17] Karl C.K. Kuban et al. "Cranial Ultrasound Lesions in the NICU Predict Cerebral Palsy at Age 2 Years in Children Born at Extremely Low Gestational Age". In: *Journal of child neurology* 24.1 (2009), p. 63. URL: /pmc/articles/PMC2814246/.

[18] Dag Moster et al. "The association of Apgar score with subsequent death and cerebral palsy: A population-based study in term infants". In: *The Journal of pediatrics* 138.6 (2001), pp. 798–803. URL: https://pubmed.ncbi.nlm.nih.gov/11391319/.

[19] Jonas H. Ellenberg and Karin B. Nelson. "Early recognition of infants at high risk for cerebral palsy: examination at age four months". In: *Developmental medicine and child neurology* 23.6 (1981), pp. 705–716. URL: https://pubmed.ncbi.nlm.nih.gov/7319139/.

[20] Jonas H. Ellenberg and Karin B. Nelson. "The association of cerebral palsy with birth asphyxia: a definitional quagmire". In: *Developmental Medicine & Child Neurology* 55.3 (Mar. 2013), pp. 210–216. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/dmcn.12016.

[21] Sarah McIntyre et al. "A systematic review of risk factors for cerebral palsy in children born at term in developed countries". In: *Developmental Medicine & Child Neurology* 55.6 (June 2013), pp. 499–508. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/dmcn.12017.

[22] Betty R. Vohr et al. "Maternal Age, Multiple Birth, and Extremely Low Birth Weight Infants". In: *The Journal of Pediatrics* 154.4 (Apr. 2009), pp. 498–503.

[23] Yoshie Yokoyama, Tadahiko Shimizu, and Kazuo Hayakawa. "Prevalence of Cerebral Palsy in Twins, Triplets and Quadruplets". In: *International Journal of Epidemiology* 24.5 (Oct. 1995), pp. 943–948. URL: https://dx.doi.org/10.1093/ije/24.5.943.

[24] Monica Topp et al. “Multiple birth and cerebral palsy in Europe: a multicenter study”. In: *Acta Obstetricia et Gynecologica Scandinavica* 83.6 (Jan. 2004), pp. 548–553. URL: https://www.tandfonline.com/doi/abs/10.1080/j.0001-6349.2004.00545.x.

[25] Stephen Jarvis et al. “Cerebral palsy and intrauterine growth in single births: European collaborative study”. In: *Lancet* 362.9390 (Oct. 2003), pp. 1106–1111. URL: http://www.thelancet.com/article/S0140673603144662/fulltext.

[26] D E Schendel. “Infection in pregnancy and cerebral palsy.” In: *Journal of the American Medical Women’s Association (1972)* 56.3 (Jan. 2001), pp. 105–108. URL: https://europepmc.org/article/med/11506145.

[27] Hayley Smithers-Sheedy et al. “Congenital Cytomegalovirus among Children with Cerebral Palsy”. In: *The Journal of Pediatrics* 181 (Feb. 2017), pp. 267–271.

[28] Elani Streja et al. “Congenital cerebral palsy and prenatal exposure to self-reported maternal infections, fever, or smoking”. In: *American Journal of Obstetrics and Gynecology* 209.4 (Oct. 2013), pp. 1–332.

[29] Steven J. Korzeniewski et al. *A systematic review of neuroimaging for cerebral palsy*. Feb. 2008.

[30] Yoshihiro Tsutsui, Masato Nagahama, and Akira Mizutani. “Neuronal migration disorders in cerebral palsy”. In: *Neuropathology* 19.1 (Jan. 1999), pp. 14–27. URL: https://onlinelibrary.wiley.com/doi/full/10.1046/j.1440-1789.1999.00197.x.

[31] R. Frank et al. “Clinical profile of children with cerebral palsy born term compared with late- and post-term: a retrospective cohort study”. In: *BJOG : an international journal of obstetrics and gynaecology* 124.11 (Oct. 2017), pp. 1738–1745. URL: https://pubmed.ncbi.nlm.nih.gov/27592548/.

[32] Adam Kirton et al. “Symptomatic Neonatal Arterial Ischemic Stroke: The International Pediatric Stroke Study”. In: *Pediatrics* 128.6 (Dec. 2011), e1402–e1410. URL: /pediatrics/article/128/6/e1402/31169/Symptomatic-Neonatal-Arterial-Ischemic-Stroke-The%20https://dx.doi.org/10.1542/peds.2011-1148.

[33] Yvonne W. Wu et al. “Perinatal Stroke in Children With Motor Impairment: A Population-Based Study”. In: *Pediatrics* 114.3 (Sept. 2004), pp. 612–619. URL: /pediatrics/article/114/3/612/67180/Perinatal-Stroke-in-Children-With-Motor-Impairment%20https://dx.doi.org/10.1542/peds.2004-0385.

[34] Steven J. Korzeniewski et al. “A "multi-hit" model of neonatal white matter injury: Cumulative contributions of chronic placental inflammation, acute fetal inflammation and postnatal inflammatory events”. In: *Journal of Perinatal Medicine* 42.6 (Nov. 2014), pp. 731–743. URL: https://www.degruyter.com/document/doi/10.1515/jpm-2014-0250/html.

[35] Omer Mor et al. “Early onset preeclampsia and cerebral palsy: a double hit model?” In: *American journal of obstetrics and gynecology* 214.1 (Jan. 2016), pp. 1–105. URL: https://pubmed.ncbi.nlm.nih.gov/26283455/.

[36] Rilla E. Schneider et al. “The Association Between Maternal Age and Cerebral Palsy Risk Factors”. In: *Pediatric Neurology* 82 (May 2018), pp. 25–28.

[37] Lan Wan Wang et al. “Hypoxic/Ischemic and Infectious Events Have Cumulative Effects on the Risk of Cerebral Palsy in Very-Low-Birth-Weight Preterm Infants”. In: *Neonatology* 106.3 (Oct. 2014), pp. 209–215. URL: https://dx.doi.org/10.1159/000362782.

[38] C. Kaur and E. A. Ling. “Periventricular white matter damage in the hypoxic neonatal brain: Role of microglial cells”. In: *Progress in Neurobiology* 87.4 (Apr. 2009), pp. 264–280.

[39] KCK Kuban et al. “The breadth and type of systemic inflammation and the risk of adverse neurological outcomes in extremely low gestation newborns”. In: *Elsevier* (). URL: https://www.sciencedirect.com/science/article/pii/S0887899414006031/.

[40] A Leviton et al. “Two-hit model of brain damage in the very preterm newborn: small for gestational age and postnatal systemic inflammation”. In: *nature.comA Leviton, RN Fichorova, TM O’Shea, K Kuban, N Paneth, O Dammann, EN AllredPediatric research, 2013 • nature.com* (). URL: https://www.nature.com/articles/pr2012188.

[41] Martin Bax et al. “Proposed definition and classification of cerebral palsy, April 2005”. In: *Developmental Medicine and Child Neurology* 47.8 (2005), p. 571.

[42] Steven J. Korzeniewski et al. “The complex aetiology of cerebral palsy”. In: *Nature Reviews Neurology 2018 14:9* 14.9 (Aug. 2018), pp. 528–543. URL: https://www.nature.com/articles/s41582-018-0043-6.

[43] Meera Shaunak and Veronica B Kelly. “Cerebral palsy in under 25 s: assessment and management (NICE Guideline NG62)”. In: *Archives of Disease in Childhood - Education and Practice* 103.4 (Aug. 2018), pp. 189–193. URL: https://ep.bmj.com/content/103/4/189%20https://ep.bmj.com/content/103/4/189.abstract.

[44] Wenbin Deng, Jeanette Pleasure, and David Pleasure. “Progress in Periventricular Leukomalacia”. In: *Archives of Neurology* 65.10 (Oct. 2008), pp. 1291–1295. URL: https://jamanetwork.com/journals/jamaneurology/fullarticle/796225.

[45] Joseph J. Volpe and Alvin Zipurksy. “Neurobiology of Periventricular Leukomalacia in the Premature Infant”. In: *Pediatric Research 2001 50:5* 50.5 (2001), pp. 553–562. URL: https://www.nature.com/articles/pr2001219.

[46] Janet S. Soul et al. “Fluctuating Pressure-Passivity Is Common in the Cerebral Circulation of Sick Premature Infants”. In: *Pediatric Research 2007 61:4* 61.4 (Apr. 2007), pp. 467–473. URL: https://www.nature.com/articles/pr200791.

[47] Susan M. Reid et al. “Population-based studies of brain imaging patterns in cerebral palsy”. In: *Developmental Medicine & Child Neurology* 56.3 (Mar. 2014), pp. 222–232. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/dmcn.12228.

[48] Laura Owens, Eileen Shieh, and Abigail Case. “Postnatal Causes of Cerebral Palsy”. In: *Cerebral Palsy: Second Edition* (Jan. 2020), pp. 77–83. URL: https://link.springer.com/referenceworkentry/10.1007/978-3-319-74558-9_7.

[49] Mary Dunbar and Adam Kirton. "Perinatal stroke: mechanisms, management, and outcomes of early cerebrovascular brain injury". In: *The Lancet. Child & adolescent health* 2.9 (Sept. 2018), pp. 666–676. URL: https://pubmed.ncbi.nlm.nih.gov/30119760/.

[50] H. Kerr Graham et al. "Cerebral palsy". In: *Nature Reviews Disease Primers 2016 2:1* 2.1 (Jan. 2016), pp. 1–25. URL: https://www.nature.com/articles/nrdp201582.

[51] Jan Willem Gorter et al. "Limb distribution, motor impairment, and functional classification of cerebral palsy". In: *Developmental Medicine and Child Neurology* 46.7 (July 2004), pp. 461–467. URL: https://www.cambridge.org/core/journals/developmental-medicine-and-child-neurology/article/abs/limb-distribution-motor-impairment-and-functional-classification-of-cerebral-palsy/F677F15F23F7C67BF162AB19128696CC.

[52] Marshalyn Yeargin-Allsopp et al. "Prevalence of Cerebral Palsy in 8-Year-Old Children in Three Areas of the United States in 2002: A Multisite Collaboration". In: *Pediatrics* 121.3 (Mar. 2008), pp. 547–554. URL: /pediatrics/article/121/3/547/72781/Prevalence-of-Cerebral-Palsy-in-8-Year-Old%20https://dx.doi.org/10.1542/peds.2007-1270.

[53] Robert Palisano et al. "Development and reliability of a system to classify gross motor function in children with cerebral palsy". In: *Developmental medicine and child neurology* 39.4 (1997), pp. 214–223. URL: https://pubmed.ncbi.nlm.nih.gov/9183258/.

[54] Ann-Christin Eliasson et al. "The Manual Ability Classification System (MACS) for children with cerebral palsy: scale development and evidence of validity and reliability". In: *Developmental Medicine & Child Neurology* 48.7 (July 2006), pp. 549–554. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8749.2006.tb01313.x.

[55] Liza B. Green and Edward A. Hurvitz. "Cerebral Palsy". In: *Physical Medicine and Rehabilitation Clinics of North America* 18.4 (Nov. 2007), pp. 859–882.

[56] Marina B Brandão, Rachel HS Oliveira, and Marisa C Mancini. "Functional priorities reported by parents of children with cerebral palsy: contribution to the pediatric rehabilitation process". In: *Brazilian Journal of Physical Therapy* 18 (2015), pp. 563–571.

[57] Lisa A. Chiarello et al. "Family Priorities for Activity and Participation of Children and Youth With Cerebral Palsy". In: *Physical Therapy* 90.9 (Sept. 2010), pp. 1254–1264. URL: https://dx.doi.org/10.2522/ptj.20090388.

[58] Rimke C. Vos et al. "Developmental trajectories of daily activities in children and adolescents with cerebral palsy". In: *Pediatrics* 132.4 (Oct. 2013), e915–e923. URL: www.aappublications.org/news.

[59] Steven E. Hanna et al. "Reference Curves for the Gross Motor Function Measure: Percentiles for Clinical Description and Tracking Over Time Among Children With Cerebral Palsy". In: *Physical Therapy* 88.5 (May 2008), pp. 596–607. URL: https://dx.doi.org/10.2522/ptj.20070314.

[60] Steve E. Hanna et al. “Stability and decline in gross motor function among children and youth with cerebral palsy aged 2 to 21 years”. In: *Developmental Medicine and Child Neurology* 51.4 (Apr. 2009), pp. 295–302. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8749.2008.03196.x.

[61] Norihiko Ando and Satoshi Ueda. “Functional deterioration in adults with cerebral palsy”. In: *http://dx.doi.org/10.1191/026921500672826716* 14.3 (June 2000), pp. 300–306. URL: https://journals.sagepub.com/doi/abs/10.1191/026921500672826716.

[62] Koyo Usuba et al. “Changes in Gross Motor Function and Health-Related Quality of Life in Adults With Cerebral Palsy: An 8-Year Follow-Up Study”. In: *Archives of Physical Medicine and Rehabilitation* 95.11 (Nov. 2014), pp. 2071–2077.

[63] L. Van Der Dussen et al. “Functional level of young adults with cerebral palsy”. In: *Clinical Rehabilitation* 15.1 (Jan. 2001), pp. 84–91. URL: http://journals.sagepub.com/doi/10.1191/026921501670159475.

[64] J. L. Durstine et al. *Physical activity for the chronically Ill and disabled*. Sept. 2000. URL: https://link.springer.com/article/10.2165/00007256-200030030-00005.

[65] Karen J. Dodd, Nicholas F. Taylor, and Diane L. Damiano. “A systematic review of the effectiveness of strength-training programs for people with cerebral palsy”. In: *Archives of Physical Medicine and Rehabilitation* 83.8 (2002), pp. 1157–1164. URL: https://pubmed.ncbi.nlm.nih.gov/12161840/.

[66] Liz Martin, Richard Baker, and Adrienne Harvey. “A Systematic Review of Common Physiotherapy Interventions in School-Aged Children with Cerebral Palsy”. In: *Physical & Occupational Therapy In Pediatrics* 30.4 (Oct. 2010), pp. 294–312. URL: http://www.tandfonline.com/doi/full/10.3109/01942638.2010.500581.

[67] Neil Wimalasundera and Valerie L Stevenson. “Cerebral palsy”. In: *Practical Neurology* 16.3 (June 2016), 184 LP –194. URL: http://pn.bmj.com/content/16/3/184.abstract.

[68] Iona Novak et al. “State of the Evidence Traffic Lights 2019: Systematic Review of Interventions for Preventing and Treating Children with Cerebral Palsy”. In: *Current Neurology and Neuroscience Reports 2020 20:2* 20.2 (Feb. 2020), pp. 1–21. URL: http://link.springer.com/10.1007/s11910-020-1022-z%20https://link.springer.com/article/10.1007/s11910-020-1022-z?shared-article-renderer.

[69] Steven L. Wolf et al. “Effect of Constraint-Induced Movement Therapy on Upper Extremity Function 3 to 9 Months After Stroke: The EXCITE Randomized Clinical Trial”. In: *JAMA* 296.17 (Nov. 2006), pp. 2095–2104. URL: https://jamanetwork.com/journals/jama/fullarticle/203876.

[70] SL Wolf et al. “Repetitive task practice: a critical review of constraint-induced movement therapy in stroke”. In: *journals.lww.com* (). URL: https://journals.lww.com/theneurologist/fulltext/2002/11000/Plasticity_in_the_motor_system_related_to/.

[71] B. J. Hoare et al. "Constraint-induced movement therapy in the treatment of the upper limb in children with hemiplegic cerebral palsy". In: *Cochrane Database of Systematic Reviews* 2 (2007).

[72] H Huang et al. "Bound for success: a systematic review of constraint-induced movement therapy in children with cerebral palsy supports improved arm and hand use". In: *academic.oup.comH Huang, L Fetters, J Hale, A McBridePhysical therapy, 2009 • academic.oup.com* (2009). URL: https://academic.oup.com/ptj/article-abstract/89/11/1126/2737694.

[73] LR Nascimento et al. "Effects of constraint-induced movement therapy as a rehabilitation strategy for the affected upper limb of children with hemiparesis: systematic review of the". In: *SciELO BrasilLR Nascimento, AE Gloria, ES HabibBrazilian Journal of Physical Therapy, 2009 • SciELO Brasil* (). URL: https://www.scielo.br/j/rbfis/a/q4TBS4DPhvdMYGkhGQVgbYy/?lang=en&format=html.

[74] Annika Sköld, Staffan Josephsson, and Ann Christin Eliasson. "Performing Bimanual Activities: The Experiences of Young Persons With Hemiplegic Cerebral Palsy". In: *The American Journal of Occupational Therapy* 58.4 (July 2004), pp. 416–425. URL: /ajot/article/58/4/416/4845/Performing-Bimanual-Activities-The-Experiences-of%20https://dx.doi.org/10.5014/ajot.58.4.416.

[75] AM Gordon et al. "Bimanual training and constraint-induced movement therapy in children with hemiplegic cerebral palsy: a randomized trial". In: *journals.sagepub.comAM Gordon, YC Hung, M Brandao, CL Ferre, HC Kuo, K Friel, E Petra, A ChinnanNeurorehabilitation and neural repair, 2011 • journals.sagepub.com* 25.8 (Oct. 2011), pp. 692–702. URL: https://journals.sagepub.com/doi/abs/10.1177/1545968311402508.

[76] L Sakzewski et al. "Systematic review and meta-analysis of therapeutic management of upper-limb dysfunction in children with congenital hemiplegia". In: *publications.aap.org* 123.6 (2009). URL: https://publications.aap.org/pediatrics/article-abstract/123/6/e1111/71610.

[77] Rang Ge Ouyang et al. "Effectiveness of hand-arm bimanual intensive training on upper extremity function in children with cerebral palsy: A systematic review". In: *European Journal of Paediatric Neurology* 25 (Mar. 2020), pp. 17–28.

[78] Véronique F.P. Plasschaert et al. "Interventions to improve upper limb function for children with bilateral cerebral palsy: a systematic review". In: *Developmental Medicine and Child Neurology* 61.8 (Aug. 2019), pp. 899–907. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/dmcn.14141.

[79] Yannick Bleyenheuft and Andrew M. Gordon. "Hand-Arm Bimanual Intensive Therapy Including Lower Extremities (HABIT-ILE) for Children with Cerebral Palsy". In: *http://dx.doi.org/10.3109/01942638.2014.932884* 34.4 (Nov. 2014), pp. 390–403. URL: https://www.tandfonline.com/doi/abs/10.3109/01942638.2014.932884.

[80] Jeffrey A. Kleim and Theresa A. Jones. "Principles of experience-dependent neural plasticity: implications for rehabilitation after brain damage". In: *Journal of speech, language, and hearing research : JSLHR* 51.1 (Feb. 2008). URL: https://pubmed.ncbi.nlm.nih.gov/18230848/.

[81] Janne Marieke Veerbeek et al. "What Is the Evidence for Physical Therapy Poststroke? A Systematic Review and Meta-Analysis". In: *PLOS ONE* 9.2 (Feb. 2014), e87987. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0087987.

[82] D. Michele Basso and Catherine E. Lang. "Consideration of Dose and Timing When Applying Interventions after Stroke and Spinal Cord Injury". In: *Journal of Neurologic Physical Therapy* 41 (July 2017), S24–S31. URL: https://journals.lww.com/jnpt/fulltext/2017/07001/consideration_of_dose_and_timing_when_applying.5.aspx.

[83] Jessica Livingston-Thomas et al. "Exercise and Environmental Enrichment as Enablers of Task-Specific Neuroplasticity and Stroke Recovery". In: *Neurotherapeutics* 13.2 (Apr. 2016), pp. 395–402.

[84] Andrew M. Gordon et al. "Efficacy of a hand-arm bimanual intensive therapy (HABIT) in children with hemiplegic cerebral palsy: a randomized control trial". In: *Developmental medicine and child neurology* 49.11 (Nov. 2007), pp. 830–838. URL: https://pubmed.ncbi.nlm.nih.gov/17979861/.

[85] Susan L. Andersen. "Trajectories of brain development: point of vulnerability or window of opportunity?" In: *Neuroscience & Biobehavioral Reviews* 27.1-2 (Jan. 2003), pp. 3–18.

[86] Lee B. Reid, Stephen E. Rose, and Roslyn N. Boyd. "Rehabilitation and neuroplasticity in children with unilateral cerebral palsy". In: *Nature Reviews Neurology 2015 11:7* 11.7 (June 2015), pp. 390–400. URL: https://www.nature.com/articles/nrneurol.2015.97.

[87] Michael M. Merzenich, Thomas M. Van Vleet, and Mor Nahum. "Brain plasticity-based therapeutics". In: *Frontiers in Human Neuroscience* 8.JUNE (June 2014), p. 74680. URL: www.frontiersin.org.

[88] N Murase et al. "Influence of interhemispheric interactions on motor function in chronic stroke". In: *Wiley Online LibraryN Murase, J Duque, R Mazzocchio, LG CohenAnnals of Neurology: Official Journal of the American Neurological, 2004 • Wiley Online Library* 55.3 (Mar. 2004), pp. 400–409. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/.

[89] Steven C. Cramer et al. *Harnessing neuroplasticity for clinical applications*. June 2011. URL: https://academic.oup.com/brain/article/134/6/1591/369496.

[90] Charles D. Gilbert, Wu Li, and Valentin Piech. "Perceptual learning and adult cortical plasticity". In: *The Journal of Physiology* 587.12 (June 2009), pp. 2743–2751. URL: https://onlinelibrary.wiley.com/doi/full/10.1113/jphysiol.2009.171488.

[91] Fatima Yousif Ismail, Ali Fatemi, and Michael V. Johnston. "Cerebral plasticity: Windows of opportunity in the developing brain". In: *European Journal of Paediatric Neurology* 21.1 (Jan. 2017), pp. 23–48. URL: https://www.sciencedirect.com/science/article/pii/S1090379816300964#bbib104.

[92] Michael V. Johnston et al. "Plasticity and injury in the developing brain". In: *Brain and Development* 31.1 (Jan. 2009), pp. 1–10.

[93] D. O. Hebb. *The Organization of Behaviour*. 1949.

[94] T V Bliss and T Lomo. "Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path." In: *The Journal of physiology* 232.2 (July 1973), pp. 331–56. URL: http://www.ncbi.nlm.nih.gov/pubmed/4727084.

[95] J C Magee and D Johnston. "A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons." In: *Science (New York, N.Y.)* 275.5297 (Jan. 1997), pp. 209–13. URL: http://www.ncbi.nlm.nih.gov/pubmed/8985013.

[96] Giacomo Koch et al. "Hebbian and anti-Hebbian spike-timing-dependent plasticity of human cortico-cortical connections." In: *The Journal of neuroscience : the official journal of the Society for Neuroscience* 33.23 (June 2013), pp. 9725–33. URL: http://www.ncbi.nlm.nih.gov/pubmed/23739969.

[97] T. V.P. Bliss and T. Lømo. "Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path". In: *The Journal of physiology* 232.2 (July 1973), pp. 331–356. URL: https://pubmed.ncbi.nlm.nih.gov/4727084/.

[98] S. Maren and M. Baudry. "Properties and Mechanisms of Long-Term Synaptic Plasticity in the Mammalian Brain: Relationships to Learning and Memory". In: *Neurobiology of Learning and Memory* 63.1 (Jan. 1995), pp. 1–18. URL: https://www.sciencedirect.com/science/article/pii/S1074742785710015.

[99] T. Lømo. "Discovering long-term potentiation (LTP) – recollections and reflections on what came after". In: *Acta Physiologica* 222.2 (Feb. 2018), e12921. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/apha.12921.

[100] John E. Lisman and Anthony A. Grace. "The Hippocampal-VTA Loop: Controlling the Entry of Information into Long-Term Memory". In: *Neuron* 46.5 (June 2005), pp. 703–713. URL: http://www.cell.com/article/S0896627305003971/fulltext.

[101] S. R. Kelso, A. H. Ganong, and T. H. Brown. "Hebbian synapses in hippocampus." In: *Proceedings of the National Academy of Sciences* 83.14 (July 1986), pp. 5326–5330. URL: https://www.pnas.org/doi/abs/10.1073/pnas.83.14.5326.

[102] A. Baranyi and O. Fehér. "Conditioned changes of synaptic transmission in the motor cortex of the cat". In: *Experimental brain research* 33.2 (Oct. 1978), pp. 283–298. URL: https://pubmed.ncbi.nlm.nih.gov/212285/.

[103] A. Baranyi, M. B. Szente, and C. D. Woody. "Properties of associative long-lasting potentiation induced by cellular conditioning in the motor cortex of conscious cats". In: *Neuroscience* 42.2 (1991), pp. 321–334. URL: https://pubmed.ncbi.nlm.nih.gov/1896132/.

[104] G. Hess and J. P. Donoghue. "Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps". In: *Journal of neurophysiology* 71.6 (1994), pp. 2543–2547. URL: https://pubmed.ncbi.nlm.nih.gov/7931533/.

[105] Jerome N. Sanes, Jing Wang, and John P. Donoghue. "Immediate and delayed changes of rat motor cortical output representation with new forelimb configurations". In: *Cerebral cortex (New York, N.Y. : 1991)* 2.2 (1992), pp. 141–152. URL: https://pubmed.ncbi.nlm.nih.gov/1633412/.

[106] Kathleen Friel et al. "Using motor behavior during an early critical period to restore skilled limb movement after damage to the corticospinal system during development". In: *Soc NeuroscienceK Friel, S Chakrabarty, HC Kuo, J MartinJournal of Neuroscience, 2012 • Soc Neuroscience* (2012). URL: https://www.jneurosci.org/content/32/27/9265.short.

[107] JA Eyre. "Corticospinal tract development and its plasticity after perinatal injury". In: *Neuroscience & Biobehavioral Reviews* (2007), pp. 1136–1149. URL: https://www.sciencedirect.com/science/article/pii/S0149763407000693.

[108] M Staudt et al. "Two types of ipsilateral reorganization in congenital hemiparesis: a TMS and fMRI study". In: *Brain* 10.125 (Oct. 2002), pp. 2222–2237. URL: https://academic.oup.com/brain/article-abstract/125/10/2222/300438.

[109] Martin Staudt et al. "Reorganization in congenital hemiparesis acquired at different gestational ages". In: *Annals of Neurology* 56.6 (Dec. 2004), pp. 854–863. URL: https://onlinelibrary.wiley.com/doi/full/10.1002/ana.20297.

[110] A Kirton et al. "Cortical excitability and interhemispheric inhibition after subcortical pediatric stroke: plastic organization and effects of rTMS". In: *Elsevier* (). URL: https://www.sciencedirect.com/science/article/pii/S1388245710003743.

[111] Eran Dayan et al. *Noninvasive brain stimulation: From physiology to network dynamics and back*. July 2013. URL: /pmc/articles/PMC4876726/%20/pmc/articles/PMC4876726/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4876726/.

[112] M Bikson and A Datta. "Guidelines for precise and accurate computational models of tDCS". In: *Brain Stimulation* 5.3 (2012), p. 430. URL: https://www.researchgate.net/profile/Marom_Bikson/publication/51514874_Guidelines_for_precise_and_accurate_computational_models_of_tDCS/links/09e4150afe18ecc833000000.

[113] M. A. Nitsche and W. Paulus. "Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation". In: *Journal of Physiology* 527.3 (Sept. 2000), pp. 633–639. URL: http://doi.wiley.com/10.1111/j.1469-7793.2000.t01-1-00633.x.

[114] Michael A. Nitsche et al. "Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human". In: *Journal of Cognitive Neuroscience* 15.4 (May 2003), pp. 619–626. URL: http://direct.mit.edu/jocn/article-pdf/15/4/619/1757873/089892903321662994.pdf.

[115] Thomas Radman et al. "Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro". In: *Brain Stimulation* 2.4 (Oct. 2009), pp. 215–228.

[116] Zeinab Esmaeilpour et al. “Incomplete evidence that increasing current intensity of tDCS boosts outcomes”. In: *Brain Stimulation* 11.2 (Mar. 2018), pp. 310–321.

[117] Vera Moliadze et al. “Stimulation intensities of transcranial direct current stimulation have to be adjusted in children and adolescents”. In: *Clinical Neurophysiology* 126.7 (2015), pp. 1392–1399. URL: http://dx.doi.org/10.1016/j.clinph.2014.10.142.

[118] G. Batsikadze et al. “Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans”. In: *The Journal of physiology* 591.7 (Apr. 2013), pp. 1987–2000. URL: https://pubmed.ncbi.nlm.nih.gov/23339180/.

[119] Asif Rahman et al. “Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects”. In: *The Journal of Physiology* 591.10 (May 2013), pp. 2563–2578. URL: https://onlinelibrary.wiley.com/doi/full/10.1113/jphysiol.2012.247171.

[120] Andre Russowsky Brunoni et al. “Clinical research with transcranial direct current stimulation (tDCS): Challenges and future directions”. In: *Brain Stimulation* 5.3 (July 2012), pp. 175–195. URL: https://www.sciencedirect.com/science/article/pii/S1935861X1100026X.

[121] Alberto Priori. “Brain polarization in humans: a reappraisal of an old tool for prolonged non-invasive modulation of brain excitability”. In: *Clinical Neurophysiology* 114.4 (Apr. 2003), pp. 589–595. URL: https://www.sciencedirect.com/science/article/pii/S1388245702004376.

[122] C. J. Stagg et al. “Polarity-Sensitive Modulation of Cortical Neurotransmitters by Transcranial Stimulation”. In: *Journal of Neuroscience* 29.16 (Apr. 2009), pp. 5202–5206. URL: http://www.ncbi.nlm.nih.gov/pubmed/19386916%20http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.4432-08.2009.

[123] C. J. Stagg et al. “Polarity and timing-dependent effects of transcranial direct current stimulation in explicit motor learning”. In: *Neuropsychologia* 49.5 (Apr. 2011), pp. 800–804.

[124] Velicia Bachtiar et al. “Modulation of GABA and resting state functional connectivity by transcranial direct current stimulation”. In: *eLife* 4.September 2015 (Sept. 2015).

[125] Vincent P. Clark et al. “Transcranial direct current stimulation (tDCS) produces localized and specific alterations in neurochemistry: A 1H magnetic resonance spectroscopy study”. In: *Neuroscience Letters* 500.1 (Aug. 2011), pp. 67–71.

[126] Michael A Nitsche et al. “Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human”. In: *Journal of Cognitive Neuroscience* 15.4 (2003), pp. 619–626. URL: https://ezproxy-prd.bodleian.ox.ac.uk:3938/doi/pdf/10.1162/089892903321662994%20http://www.mitpressjournals.org/doi/pdf/10.1162/089892903321662994.

[127] Michael A. Nitsche et al. “Consolidation of human motor cortical neuroplasticity by D-cycloserine”. In: *Neuropsychopharmacology* 29.8 (Aug. 2004), pp. 1573–1578. URL: http://www.acnp.org/citations/.

[128] Binith Cheeran et al. “A common polymorphism in the brain-derived neurotrophic factor gene (BDNF) modulates human cortical plasticity and the response to rTMS”. In: *Journal of Physiology* 586.23 (Dec. 2008), pp. 5717–5725. URL: https://physoc.onlinelibrary.wiley.com/doi/full/10.1113/jphysiol.2008.159905.

[129] T. V.P. Bliss and G. L. Collingridge. “A synaptic model of memory: long-term potentiation in the hippocampus”. In: *Nature* 361.6407 (1993), pp. 31–39. URL: https://pubmed.ncbi.nlm.nih.gov/8421494/.

[130] M. S. Rioult-Pedotti, D. Friedman, and J. P. Donoghue. “Learning-induced LTP in neocortex”. In: *Science (New York, N.Y.)* 290.5491 (2000), pp. 533–536. URL: https://pubmed.ncbi.nlm.nih.gov/11039938/.

[131] Ivor B. Gartside. “Mechanisms of Sustained Increases of Firing Rate of Neurones in the Rat Cerebral Cortex after Polarization: Role of Protein Synthesis”. In: *Nature* 220.5165 (Oct. 1968), pp. 383–384. URL: http://www.nature.com/articles/220383a0.

[132] Brita Fritsch et al. “Direct Current Stimulation Promotes BDNF-Dependent Synaptic Plasticity: Potential Implications for Motor Learning”. In: *Neuron* 66.2 (Apr. 2010), pp. 198–204. URL: http://www.ncbi.nlm.nih.gov/pubmed/20434997.

[133] Y Hattori, A Moriwaki, and Y Hori. “Biphasic effects of polarizing current on adenosine-sensitive generation of cyclic AMP in rat cerebral cortex.” In: *Neuroscience letters* 116.3 (Aug. 1990), pp. 320–4. URL: http://www.ncbi.nlm.nih.gov/pubmed/2173816.

[134] Nadira Islam et al. “Increase in the calcium level following anodal polarization in the rat brain”. In: *Brain Research* 684.2 (July 1995), pp. 206–208. URL: https://ezproxy-prd.bodleian.ox.ac.uk:2073/science/article/pii/000689939500434R.

[135] L J Bindman, O C Lippold, and J W Redfearn. “The action of brief polarizing currents on the cerebral cortex of the rat (1) during current flow and (2) in the production of long lasting after effects”. In: *The Journal of physiology* 172 (Aug. 1964), pp. 369–82. URL: http://www.ncbi.nlm.nih.gov/pubmed/14199369%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1368854.

[136] L J Bindman, O C Lippold, and J W Redfearn. “Long-lasting changes in the level of the electrical activity of the cerebral cortex produced bypolarizing currents.” In: *Nature* 196 (Nov. 1962), pp. 584–5. URL: http://www.ncbi.nlm.nih.gov/pubmed/13968314.

[137] Otto D. Creutzfeldt, Gerhard H. Fromm, and Hermann Kapp. “Influence of transcortical d-c currents on cortical neuronal activity”. In: *Experimental neurology* 5.6 (1962), pp. 436–452. URL: https://pubmed.ncbi.nlm.nih.gov/13882165/.

[138] Marom Bikson et al. “Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro”. In: *The Journal of Physiology* 557.1 (May 2004), pp. 175–190. URL: https://onlinelibrary.wiley.com/doi/full/10.1113/jphysiol.2003.055772.

[139] Dorothee Wachter et al. "Transcranial direct current stimulation induces polarity-specific changes of cortical blood perfusion in the rat". In: *Experimental Neurology* 227.2 (Feb. 2011), pp. 322–327.

[140] Marco Cambiaghi et al. "Brain transcranial direct current stimulation modulates motor excitability in mice". In: *The European journal of neuroscience* 31.4 (Feb. 2010), pp. 704–709. URL: https://pubmed.ncbi.nlm.nih.gov/20141528/.

[141] L. Schweid, R. J. Rushmore, and A. Valero-Cabré. "Cathodal transcranial direct current stimulation on posterior parietal cortex disrupts visuo-spatial processing in the contralateral visual field". In: *Experimental brain research* 186.3 (Apr. 2008), pp. 409–417. URL: https://pubmed.ncbi.nlm.nih.gov/18196224/.

[142] Dae Yul Kim et al. "Effect of transcranial direct current stimulation on motor recovery in patients with subacute stroke". In: *American journal of physical medicine & rehabilitation* 89.11 (Nov. 2010), pp. 879–886. URL: https://pubmed.ncbi.nlm.nih.gov/20962598/.

[143] David Liebetanz et al. "Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy". In: *Epilepsia* 47.7 (July 2006), pp. 1216–1224. URL: https://pubmed.ncbi.nlm.nih.gov/16886986/.

[144] Tanat Peanlikhit et al. "The antidepressant-like effect of tDCS in mice: A behavioral and neurobiological characterization". In: *Brain Stimulation* 10.4 (July 2017), pp. 748–756.

[145] Andressa Souza et al. "Neurobiological mechanisms of antiallodynic effect of transcranial direct current stimulation (tDCS) in a mice model of neuropathic pain". In: *Brain Research* 1682 (Mar. 2018), pp. 14–23.

[146] Hayley Thair et al. "Transcranial direct current stimulation (tDCS): A Beginner's guide for design and implementation". In: *Frontiers in Neuroscience* 11.NOV (Nov. 2017), p. 276151. URL: www.frontiersin.org.

[147] Michael A. Nitsche and Walter Paulus. "Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans". In: *Neurology* 57.10 (Nov. 2001), pp. 1899–1901. URL: http://n.neurology.org/content/57/10/1899.full#otherarticles.

[148] BW Vines, DG Nair, and G Schlaug. "Contralateral and ipsilateral motor effects after transcranial direct current stimulation". In: *Neuroreport* 17.6 (2006), pp. 671–674. URL: https://journals.lww.com/neuroreport/fulltext/2006/04240/contralateral_and_ipsilateral_motor_effects_after/.

[149] Andrea Antal et al. "Towards unravelling task-related modulations of neuroplastic changes induced in the human motor cortex". In: *European Journal of Neuroscience* 26.9 (Oct. 2007), pp. 2687–2691. URL: http://doi.wiley.com/10.1111/j.1460-9568.2007.05896.x.

[150] Paulo S. Boggio et al. "Enhancement of non-dominant hand motor function by anodal transcranial direct current stimulation". In: *Neuroscience Letters* 404.1-2 (Aug. 2006), pp. 232–236.

[151] Janine Reis et al. *Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation.* Tech. rep. 5. 2009, pp. 1590–1595. URL: www.pnas.org/cgi/content/full/0805413106/DCSupplemental.www.pnas.orgcgidoi10.1073pnas.0805413106.

[152] Claudia Ammann, Danny Spampinato, and Javier Márquez-Ruiz. "Modulating motor learning through transcranial direct-current stimulation: An integrative view". In: *Frontiers in Psychology* 7.DEC (Dec. 2016), p. 1981. URL: www.frontiersin.org.

[153] Min Fang Kuo et al. "Limited impact of homeostatic plasticity on motor learning in humans". In: *Neuropsychologia* 46.8 (July 2008), pp. 2122–2128.

[154] Kaoru Matsunaga et al. "Effect of transcranial DC sensorimotor cortex stimulation on somatosensory evoked potentials in humans". In: *Clinical Neurophysiology* 115.2 (Feb. 2004), pp. 456–460.

[155] Neri Accornero et al. "Visual evoked potentials modulation during direct current cortical polarization". In: *Experimental Brain Research* 178.2 (Apr. 2007), pp. 261–266. URL: https://link.springer.com/article/10.1007/s00221-006-0733-y.

[156] Jean Pascal Lefaucheur. "A comprehensive database of published tDCS clinical trials (2005–2016)". In: *Neurophysiologie Clinique/Clinical Neurophysiology* 46.6 (Dec. 2016), pp. 319–398.

[157] Myoung Hwan Ko et al. "Improvement of visual scanning after DC brain polarization of parietal cortex in stroke patients with spatial neglect". In: *Neuroscience Letters* 448.2 (Dec. 2008), pp. 171–174.

[158] Elisabetta Làdavas et al. "A-tDCS on the ipsilesional parietal cortex boosts the effects of prism adaptation treatment in neglect". In: *Restorative Neurology and Neuroscience* 33.5 (Oct. 2015), pp. 647–662.

[159] A. Monti et al. "Improved naming after transcranial direct current stimulation in aphasia". In: *Journal of Neurology, Neurosurgery & Psychiatry* 79.4 (Apr. 2008), pp. 451–453. URL: https://jnnp.bmj.com/content/79/4/451%20https://jnnp.bmj.com/content/79/4/451.abstract.

[160] Sandeep Kumar et al. "Noninvasive brain stimulation may improve stroke-related dysphagia: A pilot study". In: *Stroke* 42.4 (Apr. 2011), pp. 1035–1040. URL: https://www.ahajournals.org/doi/10.1161/STROKEAHA.110.602128.

[161] Takashi Shigematsu, Ichiro Fujishima, and Kikuo Ohno. "Transcranial direct current stimulation improves swallowing function in stroke patients". In: *Neurorehabilitation and Neural Repair* 27.4 (May 2013), pp. 363–369. URL: https://journals.sagepub.com/doi/full/10.1177/1545968312474116.

[162] Andre Russowsky Brunoni et al. "Enhancement of Affective Processing Induced by Bifrontal Transcranial Direct Current Stimulation in Patients With Major Depression". In: *Neuromodulation: Technology at the Neural Interface* 17.2 (Feb. 2014), pp. 138–142.

[163] MA Vanderhasselt et al. "Transcranial electric stimulation and neurocognitive training in clinically depressed patients: a pilot study of the effects on rumination". In: *ElsevierMA Vanderhasselt, R De Raedt, V Namur, PA Lotufo, IM Bensenor, PS Boggio, AR BrunoniProgress in Neuro-Psychopharmacology and Biological Psychiatry, 2015 • Elsevier* (). URL: https://www.sciencedirect.com/science/article/pii/S027858461400195X?casa_token=JrscyqdoI1gAAAAA:joDucyMziyxudmit33Tap4v8qtX6wzFiEHQhx41OS5Sfor-U5ximL3m46AS4_4mtuqxoWuqt.

[164] Sri Mahavir Agarwal et al. "Impact of antipsychotic medication on transcranial direct current stimulation (tDCS) effects in schizophrenia patients". In: *Psychiatry Research* 235 (Jan. 2016), pp. 97–103.

[165] Paulo S. Boggio et al. "Prefrontal cortex modulation using transcranial DC stimulation reduces alcohol craving: A double-blind, sham-controlled study". In: *Drug and Alcohol Dependence* 92.1-3 (Jan. 2008), pp. 55–60.

[166] F Fregni et al. "Cortical stimulation of the prefrontal cortex with transcranial direct current stimulation reduces cue-provoked smoking craving: a randomized, sham-controlled study." In: *psychiatrist.comF Fregni, P Liguori, S Fecteau, MA Nitsche, A Pascual-Leone, PS BoggioJournal of Clinical Psychiatry, 2008 • psychiatrist.com* (). URL: https://www.psychiatrist.com/read-pdf/3829/.

[167] Anuwat Amatachaya et al. "Effect of Anodal Transcranial Direct Current Stimulation on Autism: A Randomized Double-Blind Crossover Trial". In: *Behavioural Neurology* 2014.1 (Jan. 2014), p. 173073. URL: https://onlinelibrary.wiley.com/doi/full/10.1155/2014/173073%20https://onlinelibrary.wiley.com/doi/abs/10.1155/2014/173073%20https://onlinelibrary.wiley.com/doi/10.1155/2014/173073.

[168] Camila Cosmo et al. "A Randomized, Double-Blind, Sham-Controlled Trial of Transcranial Direct Current Stimulation in Attention-Deficit/Hyperactivity Disorder". In: *PLOS ONE* 10.8 (Aug. 2015), e0135371. URL: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0135371.

[169] Nadia Bolognini et al. "Motor and parietal cortex stimulation for phantom limb pain and sensations". In: *PAIN®* 154.8 (Aug. 2013), pp. 1274–1280.

[170] Felipe Fregni et al. "A randomized, sham-controlled, proof of principle study of transcranial direct current stimulation for the treatment of pain in fibromyalgia". In: *Arthritis & Rheumatism* 54.12 (Dec. 2006), pp. 3988–3998. URL: https://onlinelibrary.wiley.com/doi/full/10.1002/art.22195%20https://onlinelibrary.wiley.com/doi/abs/10.1002/art.22195%20https://onlinelibrary.wiley.com/doi/10.1002/art.22195.

[171] Liron Jacobson, Meni Koslowsky, and Michal Lavidor. "tDCS polarity effects in motor and cognitive domains: a meta-analytical review". In: *Experimental brain research* (2012). URL: https://link.springer.com/content/pdf/10.1007/s00221-011-2891-9.pdf.

[172] JC Horvath et al. "Evidence that transcranial direct current stimulation (tDCS) generates little-to-no reliable neurophysiologic effect beyond MEP amplitude modulation in healthy". In: *Elsevier* (). URL: https://www.sciencedirect.com/science/article/pii/S0028393214004394.

[173] Virginia López-Alonso et al. “Inter-individual variability in response to non-invasive brain stimulation paradigms”. In: *Brain Stimulation* 7.3 (May 2014), pp. 372–380.

[174] Sarah Wiethoff, Masashi Hamada, and John C. Rothwell. “Variability in response to transcranial direct current stimulation of the motor cortex”. In: *Brain Stimulation* 7.3 (May 2014), pp. 468–475.

[175] Taariq Chew, Kerrie Anne Ho, and Colleen K. Loo. “Inter- and intra-individual variability in response to transcranial direct current stimulation (tDCS) at varying current intensities”. In: *Brain Stimulation* 8.6 (Nov. 2015), pp. 1130–1137.

[176] Lucia M. Li et al. “Brain state and polarity dependent modulation of brain networks by transcranial direct current stimulation”. In: *Human Brain Mapping* 40.3 (Feb. 2019), pp. 904–915. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/hbm.24420.

[177] Leila Chaieb, Andrea Antal, and Walter Paulus. “Gender-specific modulation of short-term neuroplasticity in the visual cortex induced by transcranial direct current stimulation”. In: *Visual Neuroscience* 25.1 (Jan. 2008), pp. 77–81. URL: https://www.cambridge.org/core/product/identifier/S0952523808080097/type/journal_article.

[178] Min-Fang Kuo, Walter Paulus, and Michael A. Nitsche. “Sex differences in cortical neuroplasticity in humans”. In: *NeuroReport* 17.16 (Nov. 2006), pp. 1703–1707. URL: https://journals.lww.com/00001756-200611060-00009.

[179] Hakuei Fujiyama et al. “Delayed plastic responses to anodal tDCS in older adults”. In: *Frontiers in Aging Neuroscience* 6.JUN (June 2014), p. 115. URL: http://journal.frontiersin.org/article/10.3389/fnagi.2014.00115/abstract.

[180] Jung Hoon Kim et al. “Inconsistent outcomes of transcranial direct current stimulation may originate from anatomical differences among individuals: Electric field simulation using individual MRI data”. In: *Neuroscience Letters* 564 (Apr. 2014), pp. 6–10.

[181] Andrea Antal et al. “Brain-derived neurotrophic factor (BDNF) gene polymorphisms shape cortical plasticity in humans”. In: *Brain Stimulation* 3.4 (Oct. 2010), pp. 230–237.

[182] James T.H. Teo et al. “Late cortical plasticity in motor and auditory cortex: role of met-allele in BDNF Val66Met polymorphism”. In: *The international journal of neuropsychopharmacology* 17.5 (2014), pp. 705–713. URL: https://pubmed.ncbi.nlm.nih.gov/24405657/.

[183] Charlotte Rosso et al. “Connectivity between right inferior frontal gyrus and supplementary motor area predicts after-effects of right frontal cathodal tDCS on picture naming speed”. In: *Brain stimulation* 7.1 (Jan. 2014), pp. 122–129. URL: https://pubmed.ncbi.nlm.nih.gov/24099835/.

[184] Abhishek Datta et al. “Individualized model predicts brain current flow during transcranial direct-current stimulation treatment in responsive stroke patient”. In: *Brain Stimulation* 4.3 (July 2011), pp. 169–174.

[185] Abhishek Datta et al. "Inter-Individual Variation during Transcranial Direct Current Stimulation and Normalization of Dose Using MRI-Derived Computational Models". In: *Frontiers in Psychiatry* 3.OCT (Oct. 2012), p. 91. URL: http://journal.frontiersin.org/article/10.3389/fpsyt.2012.00091/abstract.

[186] Nick S. Ward and Leonardo G. Cohen. "Mechanisms Underlying Recovery of Motor Function After Stroke". In: *Archives of neurology* 61.12 (Dec. 2004), p. 1844. URL: /pmc/articles/PMC3713312/%20/pmc/articles/PMC3713312/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3713312/.

[187] Friedhelm Hummel et al. "Effects of non-invasive cortical stimulation on skilled motor function in chronic stroke". In: *Brain* 128.3 (Mar. 2005), pp. 490–499. URL: https://dx.doi.org/10.1093/brain/awh369.

[188] Jing Xu et al. "Rethinking interhemispheric imbalance as a target for stroke neurorehabilitation". In: *Annals of neurology* 85.4 (Apr. 2019), pp. 502–513. URL: https://pubmed.ncbi.nlm.nih.gov/30805956/.

[189] Nick S. Ward et al. "Motor system activation after subcortical stroke depends on corticospinal system integrity". In: *Brain* 129.3 (Mar. 2006), pp. 809–819. URL: https://dx.doi.org/10.1093/brain/awl002.

[190] Lynley V. Bradnam, Cathy M. Stinear, and Winston D. Byblow. "Ipsilateral motor pathways after stroke: Implications for noninvasive brain stimulation". In: *Frontiers in Human Neuroscience* 7.APR 2013 (Apr. 2013), p. 45172. URL: www.frontiersin.org.

[191] Heidi Johansen-Berg et al. "The role of ipsilateral premotor cortex in hand movement after stroke". In: *Proceedings of the National Academy of Sciences* 99.22 (Oct. 2002), pp. 14518–14523. URL: https://www.pnas.org/doi/abs/10.1073/pnas.222536799.

[192] Martin Lotze et al. "The Role of Multiple Contralesional Motor Areas for Complex Hand Movements after Internal Capsular Lesion". In: *Journal of Neuroscience* 26.22 (May 2006), pp. 6096–6102. URL: https://www.jneurosci.org/content/26/22/6096%20https://www.jneurosci.org/content/26/22/6096.abstract.

[193] Michelle N. McDonnell and Cathy M. Stinear. "TMS measures of motor cortex function after stroke: A meta-analysis". In: *Brain Stimulation* 10.4 (July 2017), pp. 721–734.

[194] Andrew J. Butler et al. "A meta-analysis of the efficacy of anodal transcranial direct current stimulation for upper limb motor recovery in stroke survivors". In: *Journal of Hand Therapy* 26.2 (Apr. 2013), pp. 162–171.

[195] Eman M. Khedr et al. "Effect of anodal versus cathodal transcranial direct current stimulation on stroke rehabilitation: a pilot randomized controlled trial". In: *Neurorehabilitation and neural repair* 27.7 (Sept. 2013), pp. 592–601. URL: https://pubmed.ncbi.nlm.nih.gov/23609526/.

[196] Jodie Marquez et al. "Transcranial Direct Current Stimulation (tDCS): Does it Have Merit in Stroke Rehabilitation? A Systematic Review". In: *International Journal of Stroke* 10.3 (Apr. 2015), pp. 306–316. URL: http://journals.sagepub.com/doi/full/10.1111/ijs.12169.

[197] Bernhard Elsner et al. “Transcranial direct current stimulation (tDCS) for improving activities of daily living, and physical and cognitive functioning, in people after stroke”. In: *Cochrane Database of Systematic Reviews* 2020.11 (Nov. 2020). URL: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD009645.pub4/full.

[198] Lucia M. Li, Kazumasa Uehara, and Takashi Hanakawa. “The contribution of interindividual factors to variability of response in transcranial direct current stimulation studies”. In: *Frontiers in Cellular Neuroscience* 9.MAY (May 2015), p. 181. URL: www.frontiersin.org.

[199] Chandramouli Krishnan et al. *Safety of noninvasive brain stimulation in children and adolescents*. 2015. URL: http://dx.doi.org/10.1016/j.brs.2014.10.012.

[200] E. Zewdie et al. “Safety and tolerability of non-invasive neurostimulation in children”. In: *Brain Stimulation* 12.2 (Mar. 2019), p. 550.

[201] Luanda A C Grecco et al. “Effect of a single session of transcranial direct-current stimulation on balance and spatiotemporal gait variables in children with cerebral palsy: A randomized sham-controlled study”. In: *Braz J Phys Ther* 18.5 (2014), pp. 419–427. URL: http://dx.doi.org/10.1590/bjpt-rbf.2014.0053.

[202] Luanda André Collange Grecco et al. “Effects of anodal transcranial direct current stimulation combined with virtual reality for improving gait in children with spastic diparetic cerebral palsy: a pilot, randomized, controlled, double-blind, clinical trial”. In: *Clinical Rehabilitation* 29.12 (2015), pp. 1212–1223. URL: https://pubmed.ncbi.nlm.nih.gov/25604912/.

[203] Roberta Delasta Lazzari et al. “Effect of a single session of transcranial direct-current stimulation combined with virtual reality training on the balance of children with cerebral palsy: a randomized, controlled, double-blind trial”. In: *Journal of Physical Therapy Science* 27.3 (Mar. 2015), pp. 763–768. URL: http://www.ncbi.nlm.nih.gov/pubmed/25931726.

[204] Roberta Delasta Lazzari et al. “Effect of Transcranial Direct Current Stimulation Combined With Virtual Reality Training on Balance in Children With Cerebral Palsy: A Randomized, Controlled, Double-Blind, Clinical Trial”. In: *Journal of Motor Behavior* 49.3 (2017), pp. 329–336. URL: http://www.tandfonline.com/action/journalInformation?journalCode=vjmb20.

[205] Benchaporn Aree-uea et al. “Reduction of spasticity in cerebral palsy by anodal transcranial direct current stimulation.” In: *Journal of the Medical Association of Thailand* 97.9 (Sept. 2014), pp. 954–62. URL: http://www.ncbi.nlm.nih.gov/pubmed/25536713.

[206] Paradee Auvichayapat et al. “Transient Changes in Brain Metabolites after Transcranial Direct Current Stimulation in Spastic Cerebral Palsy: A Pilot Study”. In: *Frontiers in Neurology* 8 (July 2017), p. 366. URL: http://journal.frontiersin.org/article/10.3389/fneur.2017.00366/full.

[207] Adam Kirton et al. "Transcranial direct current stimulation for children with perinatal stroke and hemiparesis". In: *Neurology* 88.3 (Jan. 2017), pp. 259–267. URL: http://ezproxy-prd.bodleian.ox.ac.uk:6451/content/neurology/88/3/259.full.pdf%20http://cochranelibrary-wiley.com/o/cochrane/clcentral/articles/733/CN-01297733/frame.html%20http://www.neurology.org/lookup/doi/10.1212/WNL.0000000000003518%20https://n.neurology.or.

[208] Renata Calhes Franco Moura et al. "Effects of a single session of transcranial direct current stimulation on upper limb movements in children with cerebral palsy: A randomized, sham-controlled study". In: *Developmental Neurorehabilitation* 20.6 (Aug. 2017), pp. 368–375. URL: https://www.tandfonline.com/doi/full/10.1080/17518423.2017.1282050%20http://www.tandfonline.com/action/journalInformation?journalCode=ipdr20.

[209] Bernadette Gillick et al. "Transcranial direct current stimulation and constraint-induced therapy in cerebral palsy: A randomized, blinded, sham-controlled clinical trial". In: *European Journal of Paediatric Neurology* 22.3 (2018), pp. 358–368. URL: https://doi.org/10.1016/j.ejpn.2018.02.001.

[210] N Duarte et al. "P 082 – Effect of bilateral tDCS on functional balance and the Gait Profile score in a child with hemiparetic spastic cerebral palsy". In: *Gait and Posture* 65 (2018), pp. 365–366. URL: https://doi.org/10.1016/j.gaitpost.2018.07.017.

[211] Emanuela Inguaggiato et al. "Transcranial Direct Current Stimulation (tDCS) in Unilateral Cerebral Palsy: A Pilot Study of Motor Effect". In: *Neural Plasticity* 2019 (Jan. 2019), pp. 1–10. URL: https://www.hindawi.com/journals/np/2019/2184398/.

[212] Wenjie He et al. "Safety and effects of transcranial direct current stimulation on hand function in preschool children with hemiplegic cerebral palsy: A pilot study". In: *Frontiers in Behavioral Neuroscience* 16 (Sept. 2022), p. 925122.

[213] Adam Kirton et al. "Transcranial direct current stimulation for children with perinatal stroke and hemiparesis". In: *Neurology* 88.3 (Jan. 2017), pp. 259–267. URL: https://n.neurology.org/content/88/3/259.

[214] Jhosedyn Carolaym Salazar Fajardo et al. "The Effects of tDCS with NDT on the Improvement of Motor Development in Cerebral Palsy". In: *Journal of Motor Behavior* 54.4 (2022), pp. 480–489. URL: https://www.tandfonline.com/doi/abs/10.1080/00222895.2021.2016572.

[215] Samuel T. Nemanich et al. "Bimanual Skill Learning after Transcranial Direct Current Stimulation in Children with Unilateral Cerebral Palsy: A Brief Report". In: *Developmental Neurorehabilitation* 22.7 (Oct. 2019), pp. 504–508. URL: https://www.tandfonline.com/doi/abs/10.1080/17518423.2019.1600065.

[216] Asmaa Radwan et al. "Effect of Transcranial Direct Current Stimulation versus Virtual Reality on Gait for Children with Bilateral Spastic Cerebral Palsy: A Randomized Clinical Trial". In: *Children 2023, Vol. 10, Page 222* 10.2 (Jan. 2023), p. 222. URL: https://www.mdpi.com/2227-9067/10/2/222/htm%20https://www.mdpi.com/2227-9067/10/2/222.

[217] PC Gandiga et al. "Transcranial DC stimulation (tDCS): a tool for double-blind sham-controlled clinical studies in brain stimulation". In: *ElsevierPC Gandiga, FC Hummel, LG CohenClinical neurophysiology, 2006 • Elsevier* (). URL: https://www.sciencedirect.com/science/article/pii/S1388245705005079?casa_token=lZN4CvZtmtMAAAAA:GwXkShoBn5d4pB0iCbSlqdAsgvvhVgEka2JnDpPhhIh4aLI3UdDCcdkz7Lbqw3z3mETNUaZo.

[218] Géza Gergely Ambrus et al. "The fade-in - Short stimulation - Fade out approach to sham tDCS - Reliable at 1 mA for naïve and experienced subjects, but not investigators". In: *Brain Stimulation* 5.4 (Oct. 2012), pp. 499–504. URL: http://www.brainstimjrnl.com/article/S1935861X11001707/fulltext.

[219] GG Ambrus et al. "Cutaneous perception thresholds of electrical stimulation methods: comparison of tDCS and tRNS". In: *ElsevierGG Ambrus, W Paulus, A AntalClinical Neurophysiology, 2010 • Elsevier* (). URL: https://www.sciencedirect.com/science/article/pii/S1388245710003731/.

[220] Marom Bikson and Asif Rahman. "Origins of specificity during tDCS: Anatomical, activity-selective, and input-bias mechanisms". In: *Frontiers in Human Neuroscience* 7.OCT (Oct. 2013), p. 62035. URL: www.frontiersin.org.

[221] Olivia Morgan Lapenta et al. "Jepense donc je fais: Transcranial direct current stimulation modulates brain oscillations associated with motor imagery and movement observation". In: *Frontiers in Human Neuroscience* 7.MAY (May 2013), p. 54253. URL: www.frontiersin.org.

[222] Eun Young Park. "Gross Motor Function and Activities of Daily Living in Children and Adolescents with Cerebral Palsy: a Longitudinal Study". In: *Journal of Developmental and Physical Disabilities* 30.2 (Apr. 2018), pp. 189–203. URL: https://doi.org/10.1007/s10882-017-9579-4.

[223] Lynn M. Jeffries et al. "Developmental Trajectories and Reference Percentiles for Range of Motion, Endurance, and Muscle Strength of Children with Cerebral Palsy". In: *Physical Therapy* 99.3 (Mar. 2019), pp. 329–338. URL: https://academic.oup.com/ptj.

[224] Ofsted. *COVID-19 series: briefing on local areas' special educational needs and disabilities provision*. Tech. rep. Office for Standards in Education, Children's Services and Skills, Nov. 2020. URL: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/933499/SEND_COVID-19_briefing_October_2020.pdf.

[225] Pam Thomason et al. "The Gait Outcomes Assessment List (GOAL): validation of a new assessment of gait function for children with cerebral palsy". In: *Developmental Medicine & Child Neurology* 60.6 (June 2018), pp. 618–623. URL: http://doi.wiley.com/10.1111/dmcn.13722.

[226] Eoin McElroy et al. "Demographic and health factors associated with pandemic anxiety in the context of COVID-19". In: *British Journal of Health Psychology* 25.4 (Nov. 2020), pp. 934–944. URL: https://onlinelibrary.wiley.com/doi/10.1111/bjhp.12470.

[227] Disabled Children's Partnership. *#LeftInLockdown-Parent carers' experiences of lockdown*. Tech. rep. 2020. URL: https://disabledchildrenspartnership.org.uk/wp-content/uploads/2020/06/LeftInLockdown-Parent-carers%E2%80%99-experiences-of-lockdown-June-2020.pdf.

[228] Ashley Murphy et al. "The Impact of the Novel Coronavirus Disease 2019 on Therapy Service Delivery for Children with Disabilities". In: *Journal of Pediatrics* (Dec. 2021).

[229] Nicola Theis et al. "The effects of COVID-19 restrictions on physical activity and mental health of children and young adults with physical and/or intellectual disabilities". In: *Disability and Health Journal* (Jan. 2021), p. 101064.

[230] Stefania Maria Bova et al. "Impact of COVID-19 lockdown in children with neurological disorders in Italy". In: *Disability and Health Journal* (Dec. 2020), p. 101053.

[231] Marine Cacioppo et al. "Emerging health challenges for children with physical disabilities and their parents during the COVID-19 pandemic: The ECHO French survey". In: *Annals of Physical and Rehabilitation Medicine* (Aug. 2020).

[232] Tania Tirraoro, Renata Blower, and Matt Keer. *COVID-19 & SEND Education Survey*. Tech. rep. Special Needs Jungle, 2020. URL: https://www.specialneedsjungle.com/.

[233] Olaf Verschuren et al. "Exercise and physical activity recommendations for people with cerebral palsy". In: *Developmental Medicine & Child Neurology* 58.8 (Aug. 2016), pp. 798–808. URL: http://doi.wiley.com/10.1111/dmcn.13053.

[234] Adam Kirton. *Modeling developmental plasticity after perinatal stroke: Defining central therapeutic targets in cerebral palsy*. Feb. 2013.

[235] J Armand et al. *Postnatal Development of Corticospinal Projections from Motor Cortex to the Cervical Enlargement in the Macaque Monkey*. Tech. rep. 1996. URL: https://www.jneurosci.org/content/17/1/251.short.

[236] Adam Kirton et al. "Cortical excitability and interhemispheric inhibition after subcortical pediatric stroke: Plastic organization and effects of rTMS". In: *Clinical Neurophysiology* 121.11 (Nov. 2010), pp. 1922–1929.

[237] Melissa G. Chung and Warren D. Lo. "Noninvasive brain stimulation: The potential for use in the rehabilitation of pediatric acquired brain injury". In: *Archives of Physical Medicine and Rehabilitation* 96.4 (Apr. 2015), S129–S137. URL: https://www.sciencedirect.com/science/article/pii/S0003999314012143%20http://www.ncbi.nlm.nih.gov/pubmed/25448248%20https://linkinghub.elsevier.com/retrieve/pii/S0003999314012143.

[238] A. T. O'Brien et al. "Non-invasive brain stimulation for fine motor improvement after stroke: a meta-analysis". In: *European journal of neurology* 25.8 (Aug. 2018), pp. 1017–1026. URL: https://pubmed.ncbi.nlm.nih.gov/29744999/.

[239] Bernhard Elsner et al. "Transcranial direct current stimulation (tDCS) for improving capacity in activities and arm function after stroke: a network meta-analysis of randomised controlled trials". In: *Journal of neuroengineering and rehabilitation* 14.1 (Sept. 2017). URL: https://pubmed.ncbi.nlm.nih.gov/28903772/.

[240] Heather O Dickinson et al. "Self-reported quality of life of 8-12-year-old children with cerebral palsy: a cross-sectional European study". In: *Lancet* 369.9580 (2007), pp. 2171–2178. URL: http://kidscreen..

[241] Gregory S Liptak et al. "Health status of children with moderate to severe cerebral palsy". In: *Developmental Medicine & Child Neurology* 43.6 (Mar. 2007), pp. 364–370. URL: http://doi.wiley.com/10.1111/j.1469-8749.2001.tb00223.x.

[242] Jeanne R. Charles et al. "Efficacy of a child-friendly form of constraint-induced movement therapy in hemiplegic cerebral palsy: a randomized control trial". In: *Developmental Medicine and Child Neurology* 48.8 (Aug. 2006), pp. 635–642. URL: https://www.cambridge.org/core/journals/developmental-medicine-and-child-neurology/article/abs/efficacy-of-a-childfriendly-form-of-constraintinduced-movement-therapy-in-hemiplegic-cerebral-palsy-a-randomized-control-trial/E608A707D93C07F2E51AB06EA8598016.

[243] Martina Mancini et al. "Postural sway as a marker of progression in Parkinson's disease: A pilot longitudinal study". In: *Gait & Posture* 36.3 (July 2012), pp. 471–476. URL: http://www.ncbi.nlm.nih.gov/pubmed/22750016.

[244] Ann-Christin Eliasson et al. "Effects of constraint-induced movement therapy in young children with hemiplegic cerebral palsy: an adapted model". In: *Developmental Medicine and Child Neurology* 47.4 (Apr. 2005), pp. 266–275. URL: https://www.cambridge.org/core/journals/developmental-medicine-and-child-neurology/article/abs/effects-of-constraintinduced-movement-therapy-in-young-children-with-hemiplegic-cerebral-palsy-an-adapted-model/344CDB699EA4CE7786FB8DA2D0342DB2.

[245] Wolfgang Deppe[1] et al. "Modified constraint-induced movement therapy versus intensive bimanual training for children with hemiplegia–a randomized controlled trial". In: *Clinical Rehabilitation* 27.10 (Oct. 2013), pp. 909–920. URL: https://journals.sagepub.com/doi/pdf/10.1177/0269215513483764.

[246] Yannick Bleyenheuft et al. "Hand and Arm Bimanual Intensive Therapy Including Lower Extremity (HABIT-ILE) in Children With Unilateral Spastic Cerebral Palsy". In: *Neurorehabilitation and Neural Repair* 29.7 (Aug. 2015), pp. 645–657. URL: http://www.ncbi.nlm.nih.gov/pubmed/25527487%20http://journals.sagepub.com/doi/10.1177/1545968314562109.

[247] Anthony Demont et al. "Evidence-Based, Implementable Motor Rehabilitation Guidelines for Individuals with Cerebral Palsy". In: *Neurology* 99.7 (Aug. 2022), pp. 283–297.

[248] Brian J. Hoare et al. "Constraint-induced movement therapy in children with unilateral cerebral palsy". In: *The Cochrane Database of Systematic Reviews* 2019.4 (Apr. 2019). URL: /pmc/articles/PMC6442500/%20/pmc/articles/PMC6442500/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6442500/.

[249] Maykel Orozco-Monteagudo et al. “Combined hierarchical watershed segmentation and SVM classification for pap smear cell nucleus extraction”. In: *Computacion y Sistemas*. Vol. 16. 2. Society for Neuroscience, Jan. 2012, pp. 133–145. URL: www.jneurosci.org%20http://www.ncbi.nlm.nih.gov/pubmed/10627619.

[250] Charlotte J. Stagg, Velicia Bachtiar, and Heidi Johansen-Berg. “The role of GABA in human motor learning”. In: *Current Biology* 21.6 (Mar. 2011), pp. 480–484.

[251] A Bastani and S Jaberzadeh. “Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: A systematic review and meta-analysis”. In: *Clinical Neurophysiology* 123 (2012), pp. 644–657. URL: https://ezproxy-prd.bodleian.ox.ac.uk:6335/S1388245711006687/1-s2.0-S1388245711006687-main.pdf?_tid=84da7d99-9a72-4786-8108-f9d96919ae05&acdnat=1546436057_78f02e41b1ad8dd8f85848ba418d2d2c.

[252] Claire Allman et al. “Ipsilesional anodal tDCS enhances the functional benefits of rehabilitation in patients after stroke”. In: *Science translational medicine* 8.330 (Mar. 2016). URL: https://pubmed.ncbi.nlm.nih.gov/27089207/.

[253] Melanie K. Fleming et al. “Transcranial direct current stimulation for promoting motor function in cerebral palsy: A review”. In: *Journal of NeuroEngineering and Rehabilitation* 15.1 (Dec. 2018), pp. 1–8. URL: https://link.springer.com/articles/10.1186/s12984-018-0476-6%20https://link.springer.com/article/10.1186/s12984-018-0476-6.

[254] R H Jebsen et al. “An objective and standardized test of hand function.” In: *Archives of physical medicine and rehabilitation* 50.6 (June 1969), pp. 311–9. URL: http://www.ncbi.nlm.nih.gov/pubmed/5788487.

[255] Sanjivani Dhote, Prema Khatri, and Suvarna Ganvir. “Reliability of "Modified timed up and go" test in children with cerebral palsy”. In: *Journal of Pediatric Neurosciences* 7.2 (May 2012), p. 96. URL: http://www.ncbi.nlm.nih.gov/pubmed/23248683.

[256] Lavan Sivarajah et al. “The Feasibility and Validity of Body-Worn Sensors to Supplement Timed Walking Tests for Children with Neurological Conditions”. In: *Physical and Occupational Therapy in Pediatrics* 38.3 (May 2018), pp. 280–290.

[257] Annika Sköld et al. “Development and evidence of validity for the Children’s Hand-use Experience Questionnaire (CHEQ)”. In: *Developmental Medicine and Child Neurology* 53.5 (May 2011), pp. 436–442. URL: http://doi.wiley.com/10.1111/j.1469-8749.2010.03896.x.

[258] Ana Belén Meseguer-Henarejos et al. *Inter-and intra-rater reliability of the Modified Ashworth Scale: A systematic review and meta-analysis*. Aug. 2018.

[259] Marjorie A. Garvey et al. “Subjective reactions of children to single-pulse transcranial magnetic stimulation”. In: *Journal of Child Neurology* 16.12 (2001), pp. 891–894.

[260] Jeanne Charles and Andrew M. Gordon. "Development of hand–arm bimanual intensive training (HABIT) for improving bimanual coordination in children with hemiplegic cerebral palsy". In: *Developmental Medicine and Child Neurology* 48.11 (Nov. 2006), pp. 931–936. URL: https://www.cambridge.org/core/journals/developmental-medicine-and-child-neurology/article/abs/development-of-handarm-bimanual-intensive-training-habit-for-improving-bimanual-coordination-in-children-with-hemiplegic-cerebral-palsy/D06ABAB2295B96A8FD92FE96EEB1836B.

[261] Dido Green et al. "A multi-site study of functional outcomes following a themed approach to hand-arm bimanual intensive therapy for children with hemiplegia". In: *Developmental Medicine & Child Neurology* 55.6 (June 2013), pp. 527–533. URL: http://www.ncbi.nlm.nih.gov/pubmed/23458353%20http://doi.wiley.com/10.1111/dmcn.12113.

[262] Kyra J. Kane et al. "Preliminary study of novel, timed walking tests for children with spina bifida or cerebral palsy". In: *SAGE Open Medicine* 4 (2016). URL: /pmc/articles/PMC4959299/%20/pmc/articles/PMC4959299/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4959299/.

[263] Helen L Carlson et al. "Electric field simulations of transcranial direct current stimulation in children with perinatal stroke". In: *Front. Hum. Neurosci* 17 (2023), p. 1075741. URL: http://www.neuro.uni-jena.de/cat.

[264] Yannick Bleyenheuft et al. "Capturing neuroplastic changes after bimanual intensive rehabilitation in children with unilateral spastic cerebral palsy: A combined DTI, TMS and fMRI pilot study". In: *Research in Developmental Disabilities* 43-44 (Aug. 2015), pp. 136–149.

[265] Yannick Bleyenheuft et al. "Motor Skill Training May Restore Impaired Corticospinal Tract Fibers in Children With Cerebral Palsy". In: *Neurorehabilitation and Neural Repair* 34.6 (June 2020), pp. 533–546. URL: https://journals.sagepub.com/doi/full/10.1177/1545968320918841.

[266] Alberto Lazari et al. "Hebbian activity-dependent plasticity in white matter". In: *Cell Reports* 39.11 (June 2022), p. 110951. URL: http://www.cell.com/article/S2211124722007331/fulltext.

[267] Tomoko Ishibashi et al. "Astrocytes promote myelination in response to electrical impulses". In: *Neuron* 49.6 (Mar. 2006), pp. 823–832. URL: http://www.cell.com/article/S0896627306000961/fulltext.

[268] Beth Stevens, Sandra Tanner, and R. Douglas Fields. "Control of Myelination by Specific Patterns of Neural Impulses". In: *Journal of Neuroscience* 18.22 (Nov. 1998), pp. 9303–9311. URL: https://www.jneurosci.org/content/18/22/9303%20https://www.jneurosci.org/content/18/22/9303.abstract.

[269] C. Demerens et al. "Induction of myelination in the central nervous system by electrical activity." In: *Proceedings of the National Academy of Sciences* 93.18 (Sept. 1996), pp. 9887–9892. URL: https://www.pnas.org/doi/abs/10.1073/pnas.93.18.9887.

[270] Jacob H Hines et al. "Neuronal activity biases axon selection for myelination in vivo". In: *Nature Neuroscience* 18.5 (May 2015), pp. 683–689. URL: http://www.ncbi.nlm.nih.gov/pubmed/25849987%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4414883%20http://www.nature.com/articles/nn.3992.

[271] Ian A. McKenzie et al. "Motor skill learning requires active central myelination". In: *Science* 346.6207 (Oct. 2014), pp. 318–322. URL: http://www.ncbi.nlm.nih.gov/pubmed/25324381.

[272] Jan Scholz et al. "Training induces changes in white-matter architecture". In: *Nature neuroscience* 12.11 (2009), pp. 1370–1. URL: https://www.nature.com/articles/nn.2412%20http://www.ncbi.nlm.nih.gov/pubmed/19820707.

[273] Marco Taubert et al. "Long-term effects of motor training on resting-state networks and underlying brain structure". In: *NeuroImage* 57.4 (Aug. 2011), pp. 1492–1498.

[274] Lee B. Reid et al. "Brain changes following four weeks of unimanual motor training: Evidence from fMRI-guided diffusion MRI tractography". In: *Human Brain Mapping* 38.9 (Sept. 2017), pp. 4302–4312. URL: https://onlinelibrary.wiley.com/doi/full/10.1002/hbm.23514.

[275] Bernhard Weber et al. "Learning Unicycling Evokes Manifold Changes in Gray and White Matter Networks Related to Motor and Cognitive Functions". In: *Scientific Reports 2019 9:1* 9.1 (Mar. 2019), pp. 1–11. URL: https://www.nature.com/articles/s41598-019-40533-6.

[276] Xue Wang et al. "White matter microstructure changes induced by motor skill learning utilizing a body machine interface". In: *NeuroImage* 88 (Mar. 2014), pp. 32–40. URL: https://www.sciencedirect.com/science/article/pii/S1053811913010896.

[277] Simon M. Scheck, Roslyn N. Boyd, and Stephen E. Rose. "New insights into the pathology of white matter tracts in cerebral palsy from diffusion magnetic resonance imaging: a systematic review". In: *Developmental Medicine & Child Neurology* 54.8 (Aug. 2012), pp. 684–696. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8749.2012.04332.x.

[278] Christian Hagel. "Neuropathology of cerebral palsy". In: *Cerebral Palsy: A Multidisciplinary Approach, Third Edition* (Jan. 2018), pp. 35–47. URL: https://link.springer.com/chapter/10.1007/978-3-319-67858-0_5.

[279] Inge Franki et al. "The relationship between neuroimaging and motor outcome in children with cerebral palsy: A systematic review – Part A. Structural imaging". In: *Research in Developmental Disabilities* 100 (May 2020), p. 103606.

[280] Ingeborg Krägeloh-Mann and Veronka Horber. "The role of magnetic resonance imaging in elucidating the pathogenesis of cerebral palsy: a systematic review". In: *Developmental Medicine & Child Neurology* 49.2 (Feb. 2007), pp. 144–151. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8749.2007.00144.x.

[281] Kate Himmelmann and Paul Uvebrant. "Function and neuroimaging in cerebral palsy: a population-based study". In: *Developmental Medicine & Child Neurology* 53.6 (June 2011), pp. 516–521. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8749.2011.03932.x.

[282] Martin Staudt et al. "Functional topography of early periventricular brain lesions in relation to cytoarchitectonic probabilistic maps". In: *Brain and Language* 106.3 (Sept. 2008), pp. 177–183.

[283] P E Grant and A J Barkovich. "NEUROIMAGING IN CP: ISSUES IN PATHOGENESIS AND DIAGNOSIS". In: *Inc. MRDD Research Reviews* 3 (1997), pp. 118–128. URL: https://onlinelibrary.wiley.com/doi/10.1002/.

[284] Vinit Baliyan et al. "Diffusion weighted imaging: Technique and applications". In: *World Journal of Radiology* 8.9 (Sept. 2016), p. 785. URL: /pmc/articles/PMC5039674/%20/pmc/articles/PMC5039674/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5039674/.

[285] Lauren J. O'Donnell and Carl Fredrik Westin. "An introduction to diffusion tensor image analysis". In: *Neurosurgery clinics of North America* 22.2 (Apr. 2011), p. 185. URL: /pmc/articles/PMC3163395/%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3163395/.

[286] Christian Beaulieu and Peter S. Allen. "Determinants of anisotropic water diffusion in nerves". In: *Magnetic Resonance in Medicine* 31.4 (Apr. 1994), pp. 394–400. URL: https://onlinelibrary.wiley.com/doi/full/10.1002/mrm.1910310408.

[287] Dmitry S. Novikov. "The present and the future of microstructure MRI: From a paradigm shift to normal science". In: *Journal of Neuroscience Methods* 351 (Mar. 2021), p. 108947.

[288] Carlo Pierpaoli et al. "Diffusion tensor MR imaging of the human brain." In: *https://doi.org/10.1148/radiology.201.3.8939209* 201.3 (Dec. 1996), pp. 637–648. URL: https://pubs.rsna.org/doi/10.1148/radiology.201.3.8939209.

[289] Thomas L. Chenevert, James A. Brunberg, and James G. Pipe. "Anisotropic diffusion in human white matter: demonstration with MR techniques in vivo." In: *https://doi.org/10.1148/radiology.177.2.2217776* 177.2 (Nov. 1990), pp. 401–405. URL: https://pubs.rsna.org/doi/10.1148/radiology.177.2.2217776.

[290] M E Moseley et al. "Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system." In: *Radiology* 176.2 (Aug. 1990), pp. 439–445. URL: http://www.ncbi.nlm.nih.gov/pubmed/2367658.

[291] Alberto Lazari and Ilona Lipp. "Can MRI measure myelin? Systematic review, qualitative assessment, and meta-analysis of studies validating microstructural imaging with myelin histology". In: *NeuroImage* 230 (Apr. 2021), p. 117744.

[292] Peter J Basser, James Mattiello, and Denis Lebihan. "MR Diffusion Tensor Spectroscopy and Imaging". In: *Biophysical Journal* 66 (1994), pp. 259–267.

[293] Peter J. Basser, James Mattiello, and Denis Lebihan. "Estimation of the Effective Self-Diffusion Tensor from the NMR Spin Echo". In: *Journal of Magnetic Resonance, Series B* 103.3 (Mar. 1994), pp. 247–254.

[294] Susumu Mori and Jiangyang Zhang. "Principles of diffusion tensor imaging and its applications to basic neuroscience research". In: *Neuron* 51.5 (Sept. 2006), pp. 527–539. URL: https://pubmed.ncbi.nlm.nih.gov/16950152/.

[295] C. Lebel et al. "Microstructural maturation of the human brain from childhood to adulthood". In: *NeuroImage* 40.3 (Apr. 2008), pp. 1044–1055.

[296] Masaya Takahashi et al. "Magnetic resonance microimaging of intraaxonal water diffusion in live excised lamprey spinal cord". In: *Proceedings of the National Academy of Sciences of the United States of America* 99.25 (Dec. 2002), p. 16192. URL: /pmc/articles/PMC138587/%20/pmc/articles/PMC138587/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC138587/.

[297] Robert J. Zatorre, R. Douglas Fields, and Heidi Johansen-Berg. "Plasticity in gray and white: neuroimaging changes in brain structure during learning". In: *Nature neuroscience* 15.4 (Apr. 2012), pp. 528–536. URL: https://pubmed.ncbi.nlm.nih.gov/22426254/.

[298] V. Gulani et al. "Apparent diffusion tensor measurements in myelin-deficient rat spinal cords". In: *Magnetic Resonance in Medicine* 45.2 (Feb. 2001), pp. 191–195. URL: http://doi.wiley.com/10.1002/1522-2594%28200102%2945%3A2%3C191%3A%3AAID-MRM1025%3E3.0.CO%3B2-9.

[299] Gregor Kasprian et al. "In utero tractography of fetal white matter development". In: *NeuroImage* 43.2 (Nov. 2008), pp. 213–224.

[300] Hao Huang et al. "White and gray matter development in human fetal, newborn and pediatric brains". In: *NeuroImage* 33.1 (Oct. 2006), pp. 27–38.

[301] Naama Barnea-Goraly et al. "White Matter Development During Childhood and Adolescence: A Cross-sectional Diffusion Tensor Imaging Study". In: *Cerebral Cortex* 15.12 (Dec. 2005), pp. 1848–1854. URL: https://dx.doi.org/10.1093/cercor/bhi062.

[302] Heidi M. Feldman et al. "Diffusion Tensor Imaging: A Review for Pediatric Researchers and Clinicians". In: *Journal of developmental and behavioral pediatrics : JDBP* 31.4 (May 2010), p. 346. URL: /pmc/articles/PMC4245082/%20/pmc/articles/PMC4245082/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4245082/.

[303] Anna Mackey et al. "Upper limb function and cortical organization in youth with unilateral cerebral palsy". In: *Frontiers in Neurology* 5 JUL (July 2014), p. 92083. URL: http://www.fmrib.ox.ac.uk/fsl/.

[304] Maya Weinstein et al. "Brain Plasticity following Intensive Bimanual Therapy in Children with Hemiparesis: Preliminary Evidence". In: *Neural Plasticity* 2015 (2015).

[305] Jacquie Hodge et al. "Segmental Diffusion Properties of the Corticospinal Tract and Motor Outcome in Hemiparetic Children With Perinatal Stroke". In: *Journal of Child Neurology* 32.6 (2017), pp. 550–559.

[306] F Arrigoni et al. “Whole-Brain DTI Assessment of White Matter Damage in Children with Bilateral Cerebral Palsy: Evidence of Involvement beyond the Primary Target of the Anoxic Insult”. In: *American Journal of Neuroradiology* 37.7 (July 2016), pp. 1347–1353. URL: https://www.ajnr.org/content/37/7/1347%20https://www.ajnr.org/content/37/7/1347.abstract.

[307] Julia Ballester-Plane et al. “Whole-brain structural connectivity in dyskinetic cerebral palsy and its association with motor and cognitive function”. In: *Human Brain Mapping* 38.9 (Sept. 2017), pp. 4594–4612. URL: https://onlinelibrary.wiley.com/doi/full/10.1002/hbm.23686.

[308] Meg Morris et al. “Abnormalities in the stride length-cadence relation in parkinsonian gait”. In: *Movement Disorders* 13.1 (Jan. 1998), pp. 61–69. URL: http://doi.wiley.com/10.1002/mds.870130115.

[309] Xin Zheng and Gottfried Schlaug. “Structural white matter changes in descending motor tracts correlate with improvements in motor impairment after undergoing a treatment course of tDCS and physical therapy”. In: *Frontiers in Human Neuroscience* 9.APR (Apr. 2015), p. 136990.

[310] Robert Palisano et al. “Development and reliability of a system to classify gross motor function in children with cerebral palsy”. In: *Developmental Medicine and Child Neurology* 39.4 (Apr. 1997), pp. 214–223. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8749.1997.tb07414.x.

[311] John P. Mugler and James R. Brookeman. “Three-dimensional magnetization-prepared rapid gradient-echo imaging (3D MP RAGE)”. In: *Magnetic Resonance in Medicine* 15.1 (July 1990), pp. 152–157. URL: https://onlinelibrary.wiley.com/doi/full/10.1002/mrm.1910150117.

[312] Mark Jenkinson et al. “FSL”. In: *NeuroImage* 62.2 (Aug. 2012), pp. 782–790. URL: http://www.ncbi.nlm.nih.gov/pubmed/21979382%20https://linkinghub.elsevier.com/retrieve/pii/S1053811911010603.

[313] Yongyue Zhang, Michael Brady, and Stephen Smith. “Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm”. In: *IEEE Transactions on Medical Imaging* 20.1 (Jan. 2001), pp. 45–57.

[314] Stephen M. Smith. “Fast robust automated brain extraction”. In: *Human Brain Mapping* 17.3 (Nov. 2002), pp. 143–155. URL: https://onlinelibrary.wiley.com/doi/full/10.1002/hbm.10062.

[315] Mark Jenkinson et al. “Improved optimization for the robust and accurate linear registration and motion correction of brain images”. In: *NeuroImage* 17.2 (2002), pp. 825–841. URL: https://pubmed.ncbi.nlm.nih.gov/12377157/.

[316] Mark Jenkinson et al. “FSL”. In: *NeuroImage* 62.2 (Aug. 2012), pp. 782–790. URL: https://linkinghub.elsevier.com/retrieve/pii/S1053811911010603.

[317] Stephen M. Smith et al. "Advances in functional and structural MR image analysis and implementation as FSL". In: *NeuroImage*. Vol. 23. SUPPL. 1. Jan. 2004, S208–S219. URL: http://www.ncbi.nlm.nih.gov/pubmed/15501092%20https://linkinghub.elsevier.com/retrieve/pii/S1053811904003933.

[318] Jesper L.R. Andersson, Stefan Skare, and John Ashburner. "How to correct susceptibility distortions in spin-echo echo-planar images: Application to diffusion tensor imaging". In: *NeuroImage* 20.2 (Oct. 2003), pp. 870–888. URL: https://pubmed.ncbi.nlm.nih.gov/14568458/.

[319] Stephen M. Smith et al. "Advances in functional and structural MR image analysis and implementation as FSL". In: *NeuroImage* 23 Suppl 1.SUPPL. 1 (2004). URL: https://pubmed.ncbi.nlm.nih.gov/15501092/.

[320] Jesper L.R. Andersson and Stamatios N. Sotiropoulos. "An integrated approach to correction for off-resonance effects and subject movement in diffusion MR imaging". In: *NeuroImage* 125 (Jan. 2016), pp. 1063–1078.

[321] Shaun Warrington et al. "XTRACT - Standardised protocols for automated tractography in the human and macaque brain". In: *Neuroimage* 217 (Aug. 2020). URL: /pmc/articles/PMC7260058/%20/pmc/articles/PMC7260058/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7260058/.

[322] Aki Murakami et al. "Fiber-Tracking Techniques Can Predict the Degree of Neurologic Impairment for Periventricular Leukomalacia". In: *Pediatrics* 122.3 (Sept. 2008), pp. 500–506. URL: /pediatrics/article/122/3/500/72321/Fiber-Tracking-Techniques-Can-Predict-the-Degree%20https://dx.doi.org/10.1542/peds.2007-2816.

[323] Bejoy Thomas et al. "Quantitative diffusion tensor imaging in cerebral palsy due to periventricular white matter injury". In: *Brain* 128.11 (Nov. 2005), pp. 2562–2577. URL: https://dx.doi.org/10.1093/brain/awh600.

[324] Shoko Yoshida et al. "Quantitative diffusion tensor tractography of the motor and sensory tract in children with cerebral palsy". In: *Developmental medicine and child neurology* 52.10 (Oct. 2010), pp. 935–940. URL: https://pubmed.ncbi.nlm.nih.gov/20412261/.

[325] Julia Jaatela et al. "Limb-specific thalamocortical tracts are impaired differently in hemiplegic and diplegic subtypes of cerebral palsy". In: *Cerebral Cortex* 33.19 (Sept. 2023), pp. 10245–10257. URL: https://dx.doi.org/10.1093/cercor/bhad279.

[326] Ophélie Martinie et al. "The Challenge of Diffusion Magnetic Resonance Imaging in Cerebral Palsy: A Proposed Method to Identify White Matter Pathways". In: *Brain Sciences* 13.10 (Oct. 2023), p. 1386. URL: https://www.mdpi.com/2076-3425/13/10/1386/htm%20https://www.mdpi.com/2076-3425/13/10/1386.

[327] Lisa Mailleux et al. "White matter characteristics of motor, sensory and interhemispheric tracts underlying impaired upper limb function in children with unilateral cerebral palsy". In: *Brain Structure and Function* 225.5 (June 2020), pp. 1495–1509. URL: https://link.springer.com/article/10.1007/s00429-020-02070-1.

[328] Andrea M. Kuczynski et al. “Corticospinal tract diffusion properties and robotic visually guided reaching in children with hemiparetic cerebral palsy”. In: *Human Brain Mapping* 39.3 (Mar. 2018), pp. 1130–1144.

[329] Catherine Lebel and Christian Beaulieu. “Longitudinal Development of Human Brain Wiring Continues from Childhood into Adulthood”. In: *Journal of Neuroscience* 31.30 (July 2011), pp. 10937–10947. URL: https://www.jneurosci.org/content/31/30/10937.

[330] Xiaofu He et al. “Altered White Matter Microstructure in Adolescents and Adults with Bulimia Nervosa”. In: *Neuropsychopharmacology 2016 41:7* 41.7 (Dec. 2015), pp. 1841–1848. URL: https://www.nature.com/articles/npp2015354.

[331] Steffen Angstmann et al. “Microstructural asymmetry of the corticospinal tracts predicts right–left differences in circle drawing skill in right-handed adolescents”. In: *Brain Structure & Function* 221.9 (Dec. 2016), p. 4475. URL: /pmc/articles/PMC5102955/%20/pmc/articles/PMC5102955/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5102955/.

[332] Cassandra Sampaio-Baptista and Heidi Johansen-Berg. “White Matter Plasticity in the Adult Brain.” In: *Neuron* 96.6 (Dec. 2017), pp. 1239–1251. URL: http://www.ncbi.nlm.nih.gov/pubmed/29268094%20http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5766826.

[333] Hui Zhang et al. “NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain”. In: *NeuroImage* 61.4 (July 2012), pp. 1000–1016. URL: https://pubmed.ncbi.nlm.nih.gov/22484410/.

[334] SM Gan et al. “Psychometric properties of functional balance assessment in children with cerebral palsy”. In: *journals.sagepub.comSM Gan, LC Tung, YH Tang, CH WangNeurorehabilitation and neural repair, 2008 • journals.sagepub.com* 22.6 (Nov. 2008), pp. 745–753. URL: https://journals.sagepub.com/doi/abs/10.1177/1545968308316474.

[335] Ana Carolina De Campos, Carolina S N Da Costa, and Nelci A C F Rocha. “Developmental Neurorehabilitation Measuring changes in functional mobility in children with mild cerebral palsy”. In: (). URL: https://www.tandfonline.com/action/journalInformation?journalCode=ipdr20.

[336] Michal Katz-Leurer et al. “Developmental Neurorehabilitation Balance abilities and gait characteristics in post-traumatic brain injury, cerebral palsy and typically developed children Balance abilities and gait characteristics in post-traumatic brain injury, cerebral palsy and typically developed children”. In: *Developmental Neurorehabilitation* 12.2 (2009), pp. 100–105. URL: https://www.tandfonline.com/action/journalInformation?journalCode=ipdr20.

[337] Y Salem, EM Godwin - NeuroRehabilitation, and undefined 2009. “Effects of task-oriented training on mobility function in children with cerebral palsy”. In: *content.iospress.comY Salem, EM GodwinNeuroRehabilitation, 2009 • content.iospress.com* (2009). URL: https://content.iospress.com/articles/neurorehabilitation/nre00483.

[338] Tyler J. Richards, Keri L. Anderson, and Jeffrey S. Anderson. ""Fully automated segmentation of the corticospinal tract using the TractSeg algorithm in patients with brain tumors"". In: *Clinical Neurology and Neurosurgery* 210 (Nov. 2021), p. 107001.

[339] Stephen Rose et al. "MRI structural connectivity, disruption of primary sensorimotor pathways, and hand function in cerebral palsy". In: *Brain connectivity* 1.4 (Oct. 2011), pp. 309–316. URL: https://pubmed.ncbi.nlm.nih.gov/22432420/.

[340] Henry Tsao et al. "Reduced integrity of sensorimotor projections traversing the posterior limb of the internal capsule in children with congenital hemiparesis". In: *Research in developmental disabilities* 35.2 (Feb. 2014), pp. 250–260. URL: https://pubmed.ncbi.nlm.nih.gov/24291822/.

[341] Marom Bikson, Abhishek Datta, and Maged Elwassif. "Establishing safety limits for transcranial direct current stimulation". In: *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* 120.6 (June 2009), p. 1033. URL: /pmc/articles/PMC2754807/%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2754807/.

[342] Patrick Ciechanski et al. "Modeling Transcranial Direct-Current Stimulation-Induced Electric Fields in Children and Adults". In: *Frontiers in Human Neuroscience* 12 (July 2018), p. 268. URL: https://www.frontiersin.org/article/10.3389/fnhum.2018.00268/full.

[343] Zhigang Li et al. "A Statistical Skull Geometry Model for Children 0-3 Years Old". In: *PLOS ONE* 10.5 (May 2015). Ed. by James Cray Jr., e0127322. URL: https://dx.plos.org/10.1371/journal.pone.0127322.

[344] Michele Walsh. "Paediatric trauma". In: *Cleveland Clinic Intensive Review of Pediatrics: Fourth Edition* (Jan. 2014), pp. 1133–1139.

[345] Sudha Kilaru Kessler et al. "Dosage Considerations for Transcranial Direct Current Stimulation in Children: A Computational Modeling Study". In: *PLoS ONE* 8.9 (Sept. 2013). Ed. by Chris Chambers, e76112. URL: https://dx.plos.org/10.1371/journal.pone.0076112.

[346] Eric Courchesne et al. *Normal Brain Development and Aging: Quantitative Analysis at in Vivo MR Imaging in Healthy Volunteers 1.* Tech. rep. 2000, pp. 672–682.

[347] Marom Bikson, Asif Rahman, and Abhishek Datta. "Computational models of transcranial direct current stimulation". In: *Clinical EEG and Neuroscience* 43.3 (July 2012), pp. 176–183. URL: http://journals.sagepub.com/doi/10.1177/1550059412445138.

[348] Bernadette T. Gillick et al. "Pediatric stroke and transcranial direct current stimulation: methods for rational individualized dose optimization". In: *Frontiers in Human Neuroscience* 8 (Sept. 2014), p. 739. URL: http://journal.frontiersin.org/article/10.3389/fnhum.2014.00739/abstract.

[349] Preet Minhas et al. "Transcranial direct current stimulation in pediatric brain: A computational modeling study". In: *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS.* 2012, pp. 859–862.

[350] Paulo J.C. Suen et al. “Association between tDCS computational modeling and clinical outcomes in depression: data from the ELECT-TDCS trial”. In: *European Archives of Psychiatry and Clinical Neuroscience* 271.1 (Feb. 2021), pp. 101–110. URL: https://link.springer.com/article/10.1007/s00406-020-01127-w.

[351] Alejandro Albizu et al. “Machine learning and individual variability in electric field characteristics predict tDCS treatment response”. In: *Brain Stimulation* 13.6 (Nov. 2020), pp. 1753–1764.

[352] Kevin A. Caulfield et al. “Electric Field Strength From Prefrontal Transcranial Direct Current Stimulation Determines Degree of Working Memory Response: A Potential Application of Reverse-Calculation Modeling?” In: *Neuromodulation: Technology at the Neural Interface* 25.4 (June 2022), pp. 578–587.

[353] Kai Yuan et al. “Individual electric field predicts functional connectivity changes after anodal transcranial direct-current stimulation in chronic stroke”. In: *Neuroscience Research* 186 (Jan. 2023), pp. 21–32.

[354] Guilherme B Saturnino et al. “SimNIBS 2.1: A Comprehensive Pipeline for Individualized Electric Field Modelling for Transcranial Brain Stimulation”. In: *bioRxiv* (Dec. 2018), p. 500314.

[355] Oula Puonti et al. “Value and limitations of intracranial recordings for validating electric field modeling for transcranial brain stimulation”. In: *NeuroImage* 208 (Mar. 2020), p. 116431.

[356] Paul McCarthy. “FSLeyes”. In: (Apr. 2021). URL: https://zenodo.org/record/4701233.

[357] Christophe Geuzaine and Jean-François Remacle. “Gmsh: A 3-D finite element mesh generator with built-in pre-and post-processing facilities”. In: *INTERNATIONAL JOURNAL FOR NUMERICAL METHODS IN ENGINEERING Int. J. Numer. Meth. Engng* 79 (2009), pp. 1309–1331. URL: www.interscience.wiley.com.

[358] Matthew F. Glasser et al. “A multi-modal parcellation of human cerebral cortex”. In: *Nature* 536.7615 (Aug. 2016), p. 171. URL: /pmc/articles/PMC4990127/%20/pmc/articles/PMC4990127/?report=abstract%20https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4990127/.

[359] Ilkka Laakso et al. “Inter-subject variability in electric fields of motor cortical tDCS”. In: *Brain Stimulation* 8.5 (Sept. 2015), pp. 906–913.

[360] Stephanie Lefebvre et al. “Differences in high-definition transcranial direct current stimulation over the motor hotspot versus the premotor cortex on motor network excitability”. In: *Scientific Reports 2019 9:1* 9.1 (Nov. 2019), pp. 1–15. URL: https://www.nature.com/articles/s41598-019-53985-7.

[361] Tulika Nandi et al. “tDCS induced GABA change is associated with the simulated electric field in M1, an effect mediated by grey matter volume in the MRS voxel”. In: *Brain stimulation* 15.5 (Sept. 2022), pp. 1153–1162.

[362] Guilherme B. Saturnino et al. “SimNIBS 2.1: A Comprehensive Pipeline for Individualized Electric Field Modelling for Transcranial Brain Stimulation”. In: *Brain and Human Body Modeling*. Springer International Publishing, 2019, pp. 3–25. URL: https://doi.org/10.1007/978-3-030-21293-3_1.

[363] Carys Evans et al. “Inter-individual variability in current direction for common tDCS montages”. In: *NeuroImage* 260 (Oct. 2022), p. 119501.

[364] Oula Puonti et al. “Accurate and robust whole-head segmentation from magnetic resonance images for individualized head modeling”. In: *NeuroImage* 219 (Oct. 2020), p. 117044.

[365] Yu Huang et al. “Measurements and models of electric fields in the in vivo human brain during transcranial electric stimulation”. In: *eLife* 6 (Feb. 2017).

[366] Marom Bikson et al. “Safety of Transcranial Direct Current Stimulation: Evidence Based Update 2016”. In: *Brain Stimulation* 9 (2016), pp. 641–661. URL: http://dx.doi.org/10.1016/j.brs.2016.06.004.

[367] Rohan Puri et al. “Duration-dependent effects of the BDNF Val66Met polymorphism on anodal tDCS induced motor cortex plasticity in older adults: A group and individual perspective”. In: *Frontiers in Aging Neuroscience* 7.JUN (June 2015), p. 136519. URL: www.frontiersin.org.

[368] Julius Fridriksson et al. “BDNF genotype and tDCS interaction in aphasia treatment”. In: *Brain Stimulation* 11.6 (Nov. 2018), pp. 1276–1281.

[369] Cathy M. Stinear et al. “Functional potential in chronic stroke patients depends on corticospinal tract integrity”. In: *Brain* 130.1 (Jan. 2007), pp. 170–180. URL: https://dx.doi.org/10.1093/brain/awl333.

[370] Zoë A. Englander et al. “Brain structural connectivity increases concurrent with functional improvement: Evidence from diffusion tensor MRI in children with cerebral palsy during therapy”. In: *NeuroImage: Clinical* 7 (Jan. 2015), pp. 315–324.