Study Title: 6-Hz Primed Low and High-Frequency Repetitive Transcranial Magnetic Stimulation on Motor Function and Meta-Plasticity in Children with Cerebral Palsy: TMS-EEG Study

Principal Investigator: Christos Papadelis, PhD
Director of Research
Jane and John Justin Neurosciences Center
X53236
Christos.Papadelis@cookchilrens.org

Sponsoring Institution: Cook Children's Health Care System

Short Title: Primed rTMS in Children with Cerebral Palsy

Study Drug/Study Device:

IND/IDE Number:

IND/IDE Holder Name:

Funding Source:

Initial Protocol Version Date: 14 October 2024

Amendment Version Number & Date: Version 1.5, 26 March 2026

Project Summary

Cerebral Palsy (CP) is a neurodevelopmental condition marked by motor impairments that affect both upper and lower limbs. This project investigates the therapeutic benefits, safety, and tolerability of repetitive transcranial magnetic stimulation (rTMS) for improving motor function and meta-plasticity (re-organization) in children with CP. Specifically, the study investigates the effects of 6-Hz primed low and high-frequency rTMS on neural motor function and meta-plasticity, utilizing TMS-EEG as a core method for assessing motor evoked potentials (MEPs) and TMS-evoked potentials (TEPs). Through modulating cortical excitability, we expect that 6-Hz primed low and high-frequency rTMS will improve motor function and generate meta-plasticity in children with CP. This improvement in motor and induced meta-plasticity potentially leads to faster rehabilitation outcomes and reduced need for long-term care, which would benefit both patients and hospital systems by lowering treatment costs and improving resource allocation.

TABLE OF CONTENTS

1. BACKGROUND AND RATIONALE 4

1.1 Background on Condition 4
1.2 Device Background 6
1.3 Rationale 9

2. AIMS AND OBJECTIVES 9
2.1 Study Aim 9
2.2 Primary Objectives 9
2.2.1 To assess and compare the efficacy of 6-Hz primed low-frequency with 6-Hz primed high-frequency rTMS on motor function in children with CP. 9
2.2.2 To assess and compare the efficacy of 6-Hz primed low-frequency with 6-Hz primed high-frequency rTMS on a meta-plasticity mechanism using TEPs in children with CP. 10
2.3 Secondary Objectives 10
2.4 Endpoints 10

3. STUDY DESIGN 10

4. SELECTION AND ENROLLMENT OF SUBJECTS 15
4.1 Inclusion Criteria 15
4.2 Exclusion Criteria 15
4.3 Recruitment and Retention of Subjects 16
4.4 Consent Procedures 16

5. STUDY INTERVENTIONS 17
5.1 Interventions, Administration, and Duration 18

6. STUDY PROCEDURES 22
6.1 Behavioral Assessments 22

7. RESPONSE CRITERIA 28
7.1 Risks and Benefits 28

8. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS 32
8.1 Adverse Event Monitoring 32
8.2 Definitions 33
8.3 Steps to Determine If an Adverse Event Requires Expedited Reporting 34
8.4 Reporting Requirements for Adverse Events/Unanticipated Problems Involving Risks to Subjects or Others 35

9. INTERVENTION DISCONTINUATION 35
9.1 Removal of Patients from Protocol Therapy 35
9.2 Subject Withdrawals or Discontinuation of Study Intervention 36

9.3 Premature Termination or Suspension of Study 36

10. STATISTICAL CONSIDERATIONS 37
10.1 Treatment Assignment Procedures 38
10.1.1 Behavioral Scores Statistical Analysis 38
10.1.2 TMS-EEG data analysis 38
10.1.3 Visualization 39

10 DATA MANAGEMENT AND MONITORING/AUDITING 39
10.2 Data Collection 39
10.3 Data Management 40
10.4 Data Storage 40
10.5 Procedures to Maintain Confidentiality 40
10.6 Quality Assurance 41

11 STUDY MANAGEMENT 41
11.2 Conflict of Interest 41
11.3 Registration Procedures 42
11.4 Adherence to the Protocol 42
11.5 Emergency Modifications 42
11.6 Other Protocol Deviations/Exceptions/Violations 42
11.7 Amendments to the Protocol 43
11.8 Record Retention 44
11.9 Obligations of Investigators 44

12 REFERENCES 45

1. BACKGROUND AND RATIONALE

1.1 Background on Condition

Cerebral palsy (CP) is a category of persistent central motor and postural developmental disorders, along with activity limitation syndromes, caused by non-progressive brain damage occurring during fetal development or infancy (before, during, or shortly after birth)[1–4]. The clinical manifestations of CP resulting from different etiologies occurring before birth up to the neonatal period typically present within the first 18 months of life. In contrast, symptoms of CP associated with brain injuries—such as hypoxia, trauma, poisoning, central nervous system infections, and others—emerge based on the timing of the brain injury. CP is associated with pathological changes in the brain, including irregular brain development, damage resulting from brain hypoxia, and intracranial hemorrhage[5,6].

Clinically, CP is generally categorized into three types based on movement disorders: spasticity, dyskinesia, and ataxia[4,7,8]. Movement disorders typically characterize CP and may also be associated with impairments in sensation, perception, cognition, communication, and behavior[1,9]. CP is characterized by delayed gross motor responses and challenges in executing movements, attributed to dystonia, muscle weakness, and inadequate muscle coordination[10]. Spasms and abnormal motor postures increase energy expenditure and impede average muscle growth during development. This can result in secondary muscle and soft tissue contractures, as well as skeletal deformities[11]. Children with CP who experience these movement disorders often face functional impairments in daily activities, including self-care tasks (such as dressing and feeding) and mobility[12]. In the United States, the prevalence of CP is approximately 3 to 4 cases per 1,000 live births[13,14]. The goal for children with CP is to attain independence in self-care and mobility.

One movement pattern commonly observed in children with CP is mirror movements (MM), which are widely observed in early typical development and children with CP. These movements involve an involuntary replication of actions by one limb during voluntary movements of the opposite limb. Although the precise mechanisms underlying MM in atypical development are not fully understood and may vary depending on individual motor system pathology, two potential explanations have been proposed for children with early brain injury: (i) ipsilateral corticospinal tract (CST) projections from the contralesional motor cortex to both upper extremities[15] and/or (ii) coactivation of both motor cortices due to inadequate or altered inhibition between the hemispheres, potentially involving dysfunction or immaturity of the corpus callosum.

Interhemispheric inhibition (IHI) plays a critical role in motor control, particularly in coordinating movements between the two hemispheres of the brain[16]. In typical development, this inhibition ensures that each hemisphere can independently control movements on the opposite side of the body, preventing unwanted MM. However, in

children with CP, the mechanisms of IHI may be disrupted due to early brain injury or abnormal brain development. This disruption can lead to the coactivation of both motor cortices, contributing to the manifestation of MM[17]. The dysfunction or immaturity of the corpus callosum, a structure responsible for communication between the hemispheres, may exacerbate this lack of inhibition, resulting in less precise and more mirrored motor outputs[18]. Understanding the role of IHI in CP is crucial for developing targeted interventions that can improve motor function and reduce the occurrence of MM. To achieve a meaningful and lasting therapeutic effect, treatments must be designed to influence the brain's meta-plasticity over the long term[19].

CP is currently assessed and treated based on behavioral evaluations and interventions[20]. While behavioral data and outcome measures have enhanced intervention strategies, a deeper mechanistic understanding of the brain underlying these strategies offers significant potential but remains largely untapped. Recent research in pediatric neurology has concentrated on evaluating the efficacy of non-invasive brain stimulation (NIBS) for treating a range of pediatric neurological disorders[20], the very epitome of which is CP. As a result, it presents a non-pharmacological approach to managing pediatric movement disorders[21,22]. Transcranial magnetic stimulation (TMS), a type of NIBS, is distinguished by its non-invasive and painless nature[23], utilizing electromagnetic principles to target specific brain regions[24]. Repetitive Transcranial Magnetic Stimulation (rTMS) involves the application of TMS in a repeated, rhythmic manner, allowing for the modulation of brain activity over a longer duration. rTMS modulates the function of different regions of the cerebral cortex by altering neuronal excitability. It has demonstrated significant therapeutic benefits in treating neurological conditions such as stroke and has increasingly become an essential technique for managing these diseases[25].

The use of rTMS is increasingly prevalent in the treatment of children with CP[26], demonstrating its ability to enhance motor function[27], reduce spasticity[28], restore speech capabilities[29], and influence developmental plasticity[30]. The effects of rTMS on neural networks are complex, as it can induce changes in brain meta-plasticity[31], a phenomenon where prior synaptic activity impacts subsequent synaptic plasticity, leading to varying outcomes in the healing process. This concept of metaplasticity is crucial in understanding and modulating motor cortex functions. rTMS is also employed to investigate how different patterns of cortical stimulation might affect the brain's capacity to produce long-term potentiation (LTP) or long-term depression (LTD).

Motor priming, whether through unilateral or bilateral movements, is another important technique in neuro-pediatric rehabilitation, used to activate the motor cortex and promote neural plasticity. Unilateral motor priming can target either the affected or unaffected side, with the underlying theory suggesting that pre-activating the brain before a motor learning task heightens neural activity, increasing the brain's

responsiveness. This priming effect can modulate LTP and LTD and enhance motor learning, with changes in neuroplasticity linked to improved motor performance. Combining rTMS with motor priming offers a promising approach to exploring and optimizing the brain's capacity for meta-plasticity and rehabilitation in children with CP. However, the existing body of research on rTMS is characterized by varying sample sizes, leading to inconsistent findings. Furthermore, there is a lack of high-quality, evidence-based studies that systematically evaluate the efficacy of rTMS in treating CP.

Moreover, until recently, the widespread application, clinical translation, and generalization of findings have been constrained by the fact that TMS was predominantly focused on studying the brain's motor areas[32–34]. Integrating TMS with electroencephalography (EEG) has enabled non-invasive experiments to explore brain states[35] and the dynamics within both motor and non-motor cortical regions[36] as well as the mechanisms of normal and abnormal brain plasticity[36] along with the interactions between excitatory and inhibitory mechanisms[37]. Thus, TMS-EEG significantly broadens the neurophysiological insights from studies combining TMS with electromyography (EMG). It allows for examining brain function across nearly all regions of the cortical mantle and connected cortical networks, making it an increasingly powerful tool for studying clinical populations.

Our study will investigate the efficacy of rTMS in improving motor function and meta-plasticity in children with CP and focus on clinical outcomes and underlying neural mechanisms. We aim to bridge the gap between behavioral assessments and the mechanistic understanding of motor function and brain meta-plasticity in CP. Our study will employ a rigorous, evidence-based approach, utilizing a well-defined sample size and sham condition to evaluate the efficacy of rTMS. We will also explore the potential of rTMS to modulate developmental meta-plasticity, offering insights into how this intervention can be optimized for long-term therapeutic benefits. Ultimately, our study will contribute to the growing body of research on NIBS techniques, providing valuable data that could inform future treatment strategies and improve the quality of life for children living with CP. Our research could also have broader applications, potentially benefiting children with other neurological conditions such as pediatric stroke, epilepsy, and traumatic brain injury (TBI).

1.2 Device Background

The study by Gupta et al. (2023) evaluated the feasibility and efficacy of combining 6-Hz primed, low-frequency rTMS with modified CIMT (mCIMT). This randomized controlled trial included 46 children with unilateral CP who were assigned to either the intervention group receiving rTMS with mCIMT or a control group receiving sham rTMS with mCIMT. The results showed notable enhancements in upper limb function, as assessed by the Quality of Upper Extremity Skills Test (QUEST) and its subcategories, in the intervention group compared to the control group. Notably,

improvements in "weight bearing" and "protective extension" were sustained over 12 weeks, and quality of life scores also improved[38].

The study by C. Lee et al. (2022) indicates that priming the left dorsolateral prefrontal cortex (DLPFC) with intermittent theta-burst stimulation (iTBS) before rTMS sessions can enhance therapeutic effects, especially in patients who do not respond early to standard high-frequency left DLPFC stimulation. iTBS priming resulted in significantly greater improvements in depressive symptoms compared to continued high-frequency or bilateral stimulation. These findings support a measurement-based care approach, adjusting treatment strategies based on early response to optimize outcomes for Major Depressive Disorder (MDD)[39].

The study by Rajak et al. (2019) demonstrated that increasing the number of rTMS therapy sessions significantly improves motor development and reduces muscle spasticity in children with CP. Participants were divided into three groups, receiving 20, 30, or 40 rTMS sessions, followed by physical therapy. Results showed that motor function gains, measured by the Gross Motor Function Measure (GMFM), were highest in the group receiving 40 sessions, with a 4.27% improvement, compared to 3.12% and 2.36% in the 30- and 20-session groups, respectively. Additionally, all groups experienced a notable reduction in muscle spasticity, as assessed by the Modified Ashworth Scale (MAS). These findings suggest that a higher number of rTMS sessions may be more effective in enhancing motor function and reducing spasticity in CP children[40].

In the study conducted by Cassidy et al. (2015), the efficacy of different priming protocols for low-frequency rTMS was investigated in individuals with chronic stroke. Participants underwent three types of rTMS treatments: 6-Hz primed 1-Hz rTMS, 1-Hz primed 1-Hz rTMS, and sham 6-Hz primed active 1-Hz rTMS. The study aimed to compare changes in cortical excitability, mainly focusing on the ipsilesional cortical silent period (CSP) duration and short-interval intracortical inhibition (SICI), as well as functional outcomes related to paretic hand function. The results demonstrated that 6-Hz primed 1-Hz rTMS led to significant reductions in CSP duration and SICI compared to other conditions, indicating enhanced cortical inhibition. This suggests that 6-Hz priming effectively modulates homeostatic plasticity mechanisms in the stroke-affected brain. However, the study did not observe universal improvements across all measures of cortical excitability, such as intracortical facilitation or IHI. This variability highlights the complexity of neuroplastic responses to rTMS and underscores the need for further research to optimize stimulation protocols[41].

The study by Gillick et al. (2013) explores the combined effects of 6-Hz primed low-frequency rTMS with Constraint-induced movement therapy (CIMT) in children with congenital hemiparesis. The innovative use of primed rTMS involves an initial high-frequency stimulation (6-Hz) followed by low-frequency stimulation (1-Hz) aimed at modulating cortical excitability and enhancing motor recovery when paired with

intensive therapy like CIMT. The results indicated that the group receiving real rTMS combined with CIMT showed significant improvements in Assisting Hand Assessment (AHA) scores compared to the sham group, with most participants demonstrating improvements more significant than the smallest detectable difference (SDD). These findings suggest that combining rTMS with CIMT can effectively enhance motor function in children with hemiparesis without adverse effects[42].

In the study by Tallabs and Hammond-Tooke (2013), the authors investigated whether theta priming with 6-Hz theta-burst stimulation (TBS) could enhance the inhibitory effects of 1-Hz rTMS on the motor cortex in healthy volunteers. The study design compared three protocols: 10 minutes of 1-Hz rTMS alone, 5 minutes of 6-Hz TBS followed by 5 minutes of 1-Hz rTMS, and a sham condition using a sham coil. The primary outcome measures included changes in resting and active MEP amplitudes and unimanual reaction times. The results showed that 1-Hz rTMS alone significantly reduced resting and active MEP amplitudes, indicating effective corticospinal inhibition. However, when 1-Hz rTMS was preceded by theta priming, this inhibitory effect was abolished, suggesting that the priming protocol interfered with the subsequent inhibitory effects of 1-Hz rTMS. Interestingly, despite the lack of enhanced inhibition, both 1-Hz rTMS protocols significantly reduced reaction times compared to the sham condition with and without theta priming[43].

The study by Kakuda et al. (2011) investigates the safety, feasibility, and efficacy of combining 6-Hz primed low-frequency rTMS with intensive occupational therapy (OT) for improving upper limb function in stroke patients. Primed rTMS involves a brief period of high-frequency stimulation (6-Hz) followed by low-frequency rTMS (1-Hz) targeting the non-lesional hemisphere. This approach moderates cortical excitability, reduces IHI, and promotes motor recovery in the affected hemisphere[44].

Studies have demonstrated that 6-Hz primed low-frequency rTMS can modulate cortical excitability, potentially enhancing motor function in stroke patients[45]. Todd et al. (2009) demonstrated that while priming with 2 or 6-Hz rTMS did not significantly alter the inhibitory effects of continuous theta-burst stimulation (cTBS), priming with iTBS led to more significant suppression of MEPs compared to cTBS alone. This finding suggests that excitatory priming can enhance the inhibitory effects of subsequent cTBS, highlighting the complex interplay between different stimulation protocols in inducing metaplastic changes[46].

Overall, the reviewed studies underscore the potential of 6-Hz primed low-frequency rTMS in modulating cortical excitability and improving motor function. Integrating rTMS with rehabilitative therapies like CIMT or OT appears promising, particularly in enhancing motor recovery in individuals with motor impairments. To the best of our knowledge, while 6-Hz primed low-frequency rTMS on the contralesional hemisphere is effective in modulating cortical excitability and improving motor function, there is a notable absence of studies examining the effects of 6-Hz primed high-frequency rTMS

on the ipsilesional hemisphere specifically in CP. Future research should continue to explore the efficacy of 6-Hz primed high-frequency rTMS on the ipsilesional hemisphere and compare its efficacy with that of 6-Hz primed low-frequency rTMS on the contralesional hemisphere. Such studies could provide valuable insights into optimizing stimulation protocols for motor recovery and meta-plasticity in CP. Additionally, there is a lack of research on the effects of rTMS on MM, which are often observed in individuals with motor impairments. Future research should address the impact of rTMS on MM to better understand its potential benefits and limitations in clinical practice.

1.3 Rationale

Overall, the reviewed studies underscore the potential of 6-Hz primed low-frequency rTMS in modulating cortical excitability and improving motor function. Integrating rTMS with rehabilitative therapies like CIMT or OT appears promising, particularly in enhancing motor recovery in individuals with motor impairments. To the best of our knowledge, while 6-Hz primed low-frequency rTMS on the contralesional hemisphere is effective in modulating cortical excitability and improving motor function, there is a notable absence of studies examining the effects of 6-Hz primed high-frequency rTMS on the ipsilesional hemisphere specifically in CP. Future research should continue to explore the efficacy of 6-Hz primed high-frequency rTMS on the ipsilesional hemisphere and compare its efficacy with that of 6-Hz primed low-frequency rTMS on the contralesional hemisphere. Such studies could provide valuable insights into optimizing stimulation protocols for motor recovery and meta-plasticity in CP. Additionally, there is a lack of research on the effects of rTMS on MM, which are often observed in individuals with motor impairments. Future research should address the impact of rTMS on MM to better understand its potential benefits and limitations in clinical practice.

2. AIMS AND OBJECTIVES

2.1 Study Aim

The primary objective of this study is to assess the efficacy of 6-Hz primed low-frequency rTMS on the ipsilesional hemisphere and 6-Hz primed high-frequency on the contralesional hemisphere on improving motor function and meta-plasticity in children (ages 6-18) with CP. This evaluation will be conducted using standardized clinical scales and functional assessments.

2.2 Primary Objectives

2.2.1 To assess and compare the efficacy of 6-Hz primed low-frequency with 6-Hz primed high-frequency rTMS on motor function in children with CP.

2.2.2 To assess and compare the efficacy of 6-Hz primed low-frequency with 6-Hz primed high-frequency rTMS on a meta-plasticity mechanism using TEPs in children with CP.

2.3 Secondary Objectives

2.3.1 To examine changes in cortical excitability and motor learning during the rTMS intervention by analyzing MEPs.
2.3.2 To assess MM frequency and its modulation through IHI during the rTMS intervention.
2.3.3 To determine which rTMS protocols are most effective in reducing spasticity.
2.3.4 To compare TEPs of CP and typically developed (TD) kids

2.4 Endpoints

Data will be collected from MRI, behavioral assessment, HD-EEG, and rTMS.

3. STUDY DESIGN

3.1 Type/Design of Trial

This study will be a randomized, double-blind, sham-controlled trial designed to assess the efficacy of 6-Hz primed low-frequency rTMS applied to the ipsilesional hemisphere and 6-Hz primed high-frequency rTMS administered to the contralesional hemisphere on improving motor function and meta-plasticity in children with CP. We will recruit 45 children with CP (6-18 years old) to test this along with 15 TD kids to compare the TEPs of them with CP children (with no intervention). The CP children will be randomly assigned to experimental group 1, experimental group 2, and a sham group (15 children in each group). The study design is shown in Figure 1. TD children's participation is limited to non-interventional assessments for TEPs comparison purposes only (~3 hours). They will not receive any rTMS intervention. All the assessments and training will be done in the TMS room in the Dodson building. Behavioral assessment will be done at baseline, Day 12 post-intervention, and follow-up within one month. A session of the assessment will be completed in ~3 hours. Each assessment session can be separated into two successive days if a child and/or the parent/guardian do not want to complete the session one time.

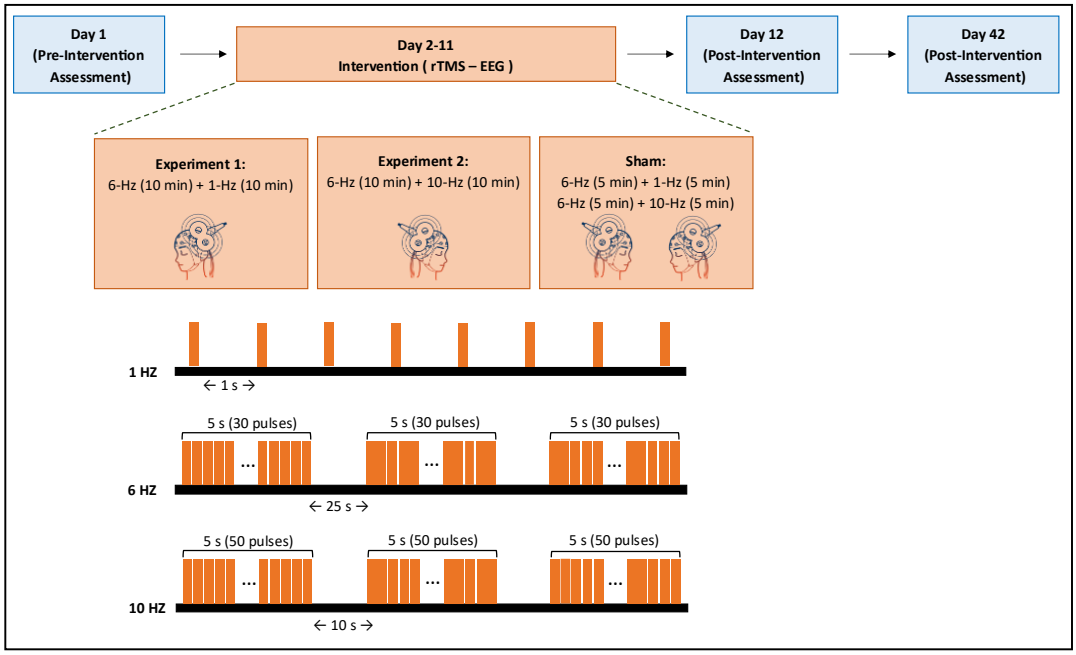

Figure 1: Flow chart diagram of the study's design.

3.2 Outcomes

3.2.1 Primary Outcomes Measures

Outcome Measure	Measure Description	Time Frame
Gross Motor Function Measure (GMFM-88)	It is a standardized observational tool specifically created and validated to assess changes in gross motor function over time in children with CP. The GMFM-88 allows for the summation of item scores to generate raw and percentage scores for each of the five GMFM dimensions, the selected goal areas, and an overall GMFM-88 score.	Within the end of 4 weeks and 1-month follow-up of sham/real rTMS
Change on TMS-evoked potentials (TEPs)	TEPs refer to the electrical responses in the brain elicited by rTMS. This technique involves applying magnetic pulses to specific brain areas, which induce electrical activity that can be recorded using EEG.	Within the end of 4 weeks and 1-month follow-up of sham/real rTMS

3.2.2 Secondary Outcomes Measures

Outcome Measure	Measure Description	Time Frame
Modified Ashworth Scale (MAS)	The Modified Ashworth Scale assesses spasticity in patients with central nervous system lesions or neurological disorders. It provides a rapid and straightforward method for clinicians to evaluate spasticity while performing passive soft-tissue stretches.	Within the end of 4 weeks and 1-month follow-up of sham/real rTMS
Change on cortical excitability - motor evoked potentials (MEPs)	MEPs are electrical signals generated by stimulating the brain's motor cortex and recorded from muscles.	Within the end of 4 weeks and 1-month follow-up of sham/real rTMS
Mirror Movement Assessment Scale (MMAS)	This scale evaluates the presence and severity of involuntary mirror movements, where movement in one limb is mirrored by involuntary movement in the opposite limb. It provides a structured approach to quantify and monitor these movements, helping to gauge the impact of therapeutic interventions and track changes over time.	Within the end of 4 weeks and 1-month follow-up of sham/real rTMS
Adverse effects	Adverse effects of TMS-EEG can include temporary headaches, scalp discomfort, and mild skin irritation from EEG electrodes. Rarely, individuals might experience transient cognitive effects or muscle twitching. There is also a minimal risk of inducing seizures, particularly in those with a history of epilepsy.	Within the end of 4 weeks and 1-month follow-up of sham/real rTMS

3.3 Study Population and Groups/Arms

This study will recruit children aged 6 to 18, with 45 participants (15 in each group) diagnosed with CP, along with 15 TD participants.

3.4 Study Location

MRI scan will be performed in the radiology of Cook Children's. Behavioral tests, HD-EEG recordings, and rTMS sessions will be performed in the TMS room in Dodson.

3.5 Approximate Duration of Enrollment Period and Follow-Up

The study will last for 4 years.

3.6 Overview of Intervention and Administration

3.6.1 MRI Acquisition

MRI scans are needed to accurately localize neural activities from HD-EEG data and navigate specific motor regions for rTMS. All participants (CP and TD) will undergo an MRI scan to acquire T1-weighted images before the intervention. See 5.1.1. for details of the MRI.

3.6.2 Behavioral Assessment

The study utilizes several behavioral assessment tools to determine inclusion criteria. The Gross Motor Function Classification System (GMFCS) classifies the severity of movement disabilities in individuals with CP, focusing on their ability to perform gross motor functions like sitting and walking. Moreover, tests outlined in section 6.1 will be selected by each child's age and skill level.

Similar to the CP cohort, TD children will undergo behavioral tests. Their involvement is one-time and focused solely on providing comparative TEPs data

3.6.3 HD-EEG Recording

The HD-EEG setup involves placing electrodes across the scalp to monitor brain responses while applying rTMS. We will use a 64-channel system (eego mylab and Waveguard™ cap, ANT Neuro, Enschede, The Netherlands). This allows for precise mapping of the brain's electrical activity, enabling the investigation of changes in motor function and meta-plasticity induced by different rTMS frequencies in children with CP. See 5.1.2 for details of the HD-EEG.

3.6.4 rTMS

CP Participants in Experiment Group 1 will use rTMS using a figure-of-eight-shaped coil and rTMS stimulator (Nexstim, Finland) targeting the contralesional primary motor cortex. The intervention will consist of 10 sessions, each lasting 20 minutes, spread over four weeks. Each session will begin with priming: 10 minutes of 6-Hz rTMS at 90% of the resting motor threshold, delivered in two trains per minute (5 seconds per train with 25-second intervals between trains), totaling 600 priming pulses. This will be followed immediately by 10 minutes of 1-Hz rTMS at 90% of the resting motor threshold, delivered continuously without interruption, totaling 600 low-frequency pulses[47].

CP Participants in Experiment Group 2 will receive rTMS targeting the ipsilesional primary motor cortex. The intervention will consist of 10 sessions, each lasting 20 minutes, spread over four weeks. Each session will start with priming: 10 minutes of 6-Hz rTMS at 90% of the resting motor threshold, delivered in two trains per minute (5 seconds per train with 25-second intervals between trains), totaling 600 priming pulses[47]. This will be followed immediately by 10 minutes of 10 Hz rTMS at 90% of the resting motor threshold, delivered continuously without interruption, totaling 2000 high-frequency pulses[48].

CP Participants in the Sham Group will receive rTMS by positioning the coil perpendicular to the scalp without delivering active stimulation, targeting both the contralesional and ipsilesional primary motor cortex. The intervention will consist of 10 sessions, each lasting 20 minutes, spread over four weeks. Each session will be divided into two parts:

a. Part 1: 5 minutes of 6 Hz rTMS at 90% of the resting motor threshold, delivered in two trains per minute (5 seconds per train with 25-second intervals between trains), resulting in a total of 300 priming pulses on the contralesional primary motor cortex. This will be followed immediately by 5 minutes of 1 Hz rTMS at 90% of the resting motor threshold, delivered continuously without interruption, totaling 300 low-frequency pulses.

b. Part 2: 5 minutes of 6 Hz rTMS at 90% of the resting motor threshold, delivered in two trains per minute (5 seconds per train with 25-second intervals between trains), resulting in a total of 300 priming pulses on the ipsilesional primary motor cortex. This will be followed immediately by 5 minutes of 10 Hz rTMS at 90% of the resting motor threshold, delivered continuously without interruption, totaling 1000 high-frequency pulses. See 5.1.3 for details of the rTMS.

TD children will complete a single baseline TMS-EEG session to measure TEPs (similar to the pre-intervention TMS-EEG in the CP cohort). This involves high-density EEG recording during single-pulse TMS to assess cortical excitability. The session will last approximately 60-90 minutes.

3.7 Randomization, Blinding and Any Stratification

After consent and assent are signed, only CP participants will be randomly assigned to the low-frequency, high-frequency, and sham groups using a computer-generated random sequence to ensure an even distribution of confounding factors. Blinding will be employed, with participants unaware of their group assignment to minimize bias.
At the conclusion of their participation in the study, CP subjects and their families will be informed whether they were assigned to the active or sham rTMS group.

4. SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria for CP

Individuals eligible to participate in this study must meet all of the following inclusion criteria to be registered in the study. Study treatment may not begin until a subject is registered.

4.1.1 Aged 6 – 18 years,
4.1.2 A confirmed diagnosis of CP by a specialized professional (pediatric neurologist, PM&R physician, neonatal developmental specialist, or neonatologist) is a prerequisite for participation,
4.1.3 Classified as high functioning (Level I, II, or III) according to the Gross Motor Function Classification System (GMFCS),
4.1.4 Age-appropriate ability to understand and comply with study procedure throughout the entire duration of the study,
4.1.5 Preserved vision and hearing (with or without correction).

4.2 Exclusion Criteria for CP

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

4.2.1 Syndromic or genetic associations,
4.2.2 History of trauma or brain surgery,
4.2.3 Inability to remain still,
4.2.4 History of Epilepsy,
4.2.5 Severe coexisting sickness or illness unrelated to CP or unstable medical conditions such as pneumonia,
4.2.6 Modified Ashworth Scale: Shoulder, elbow, and wrist scores more than 3,
4.2.7 Limb contractures caused by injury,
4.2.8 Severe movement disorders that prevent intentional limb movements, such as choreoathetosis, or ballismus,
4.2.9 Any congenital brain abnormality shown on a traditional brain MRI,
4.2.10 A cast, splint, or recent surgery on the affected limb,

4.2.11 Botulinum toxin/phenol block applied to the afflicted limb within the last six months or anticipated during the research period,

4.2.12 Contraindications for rTMS include non-removable metallic objects close to a coil and implanted electronic devices, such as cochlear implants and pacemakers.

4.3 Inclusion Criteria for TD

4.3.1 Aged 6 – 18 years,

4.3.2 Age-appropriate ability to understand and comply with study procedure throughout the entire duration of the study,

4.3.3 Preserved vision and hearing (with or without correction).

4.4 Exclusion Criteria for TD

Individuals who meet any of the following exclusion criteria will not be eligible to participate in the study:

4.4.1 Syndromic or genetic associations,

4.4.2 History of trauma or brain surgery,

4.4.3 Inability to remain still,

4.4.4 History of Epilepsy,

4.4.5 Severe coexisting sickness or illness unrelated to CP or unstable medical conditions such as pneumonia,

4.4.6 Limb contractures caused by injury,

4.4.7 Severe movement disorders that prevent intentional limb movements, such as choreoathetosis, or ballismus,

4.4.8 Any congenital brain abnormality shown on a traditional brain MRI,

4.4.9 Contraindications for rTMS include non-removable metallic objects close to a coil and implanted electronic devices, such as cochlear implants and pacemakers.

4.5 Recruitment and Retention of Subjects

Children with CP will be recruited through neurology, NEST, stroke, neuropsychology, and physical therapy clinics. Participants from other studies who have consented to be contacted for future research will also be considered potential candidates.

Recruitment of TD children will be done via social media, our website (Research Center pages are being built), and tri-fold brochures at pediatric offices/neighborhood clinics.

Participants will be compensated for their participation in the study. They will earn $50 for each assessment session, and $25 for every intervention session.

Diversity: The study will be open to all individuals who meet the inclusion criteria, regardless of race, ethnicity, or gender.

4.6 Consent Procedures

The patient will receive a thorough explanation of the study and have a chance to examine the permission form before enrolling in the research. The consent form must

include all currently required elements stipulated by the FDA, DHHS, and Cook Children's Institutional Review Board. The principal investigator (or a study team member) will acquire informed consent once this crucial information has been given to the patient and/or their parents/legally authorized representatives (LAR) and interest in participation has been verified. Following an informed consent conference, where the principal investigator (or a member of the study team leading the conference) will go over the goals of the study, the protocols to be followed, the length of participation, and the benefits and risks of participation as outlined in the consent form with the patient and/or their LAR, informed consent (and assent, if necessary) will be obtained. Once all of the patient's and/or parents/LAR's questions have been addressed and the person leading the consent conference is certain that the patient and/or parents'/LARs understand the ramifications of participation in the study, only then will the consent form be signed.

The subject and/or LAR will be asked to sign a consent document. In accordance with Cook Children's IRB policy, consent will be sought verbally from minors ages 8 to 12 and in writing from minors ages 13 to 17. Take note of the following: The IRB's standardized research permission and authorization template must be used to write consent, and the assent form must be written on the assent template. To comply with policy requirements, a script summarizing the proposed assent conference must be supplied (in language appropriate for the subjects' age range) if the assent is verbally acquired from subjects between the ages of 8 and 12. Additionally, the script must be developed using the IRB's assent template. Written copies of the informed consent and assent forms will be given to the patient and LAR. Copies of the original, signed consent and assent forms will be sent to the subject and/or LAR and kept in the patient's permanent medical record. To properly document consent that has been gained, a consent note will be filled out and placed with the completely executed consent document (or in the subject's study chart).

The investigator will adhere to Good Clinical Practice (GCP), ethical principles derived from the Declaration of Helsinki, and applicable regulatory requirements when obtaining and documenting informed consent. This study will also follow all applicable federal, state, and municipal laws, rules, and guidelines for conducting research on a vulnerable population.

5. STUDY INTERVENTIONS

The Principal Investigator will collect research material exclusively for research purposes, including demographic information, age, height, handedness, MR images, behavioral test scores, HD-EEG recordings, TMS data, and videos. No genetic testing will be conducted. With the consent of the participant's parent or guardian, video recordings will also be made during the assessments of MM. These recordings will be used strictly for educational purposes, research, and/or professional presentations and publications, separate from videos captured for research purposes. The faces of the

participants will appear in all video recordings. Videos utilized for research will have IDs to connect them to other data; optional videos used for publications, presentations, research, and/or teaching will not have identifiers except for displaying the participants' faces. Fourteen (14) visits will be required to collect the data: one for obtaining MRI, one for pre-intervention assessment, 10 for intervention, and two for conducting post-intervention and follow-up assessments, respectively. Figure 1 shows the investigation workflow. The subject's medical record will only contain the results of the MRI and behavioral tests; all other testing is done only for research. When feasible, consent conferences will take place at routine clinic visits; if not, we will give our consent over the phone. There will not be a separate visit planned for the consent conference.

5.1 Interventions, Administration, and Duration

5.1.1 MRI

All participants (CP and TD) will undergo an MRI scan to acquire T1-weighted images before the intervention. This makes it possible for us to precisely pinpoint activity detected by the HD-EEG and to correlate the functional and anatomical characteristics of the brain more closely. The Cook Children's Radiology Department will provide the MRI data. In addition to being given the chance to see the scanner, participants who might find the close quarters within the machine uncomfortable will also be told where to press a button to halt the scan process. Younger children may sit inside the scanning room with a parent or guardian if they wear non-metallic clothing. To ensure that the scans obtained have minimal motion artifact, the researchers will advise parents/guardians to bring younger children during their regular naps, even if no sedation procedures will be utilized. Participants may also be permitted to watch a movie while the scan is taking place, and they will also receive earplugs to lessen scanner noise. Before entering the scan room, every participant will be inspected for metallic things, including jewelry, metallic implants, and anything considered dangerous near the MR scanner. Since an MRI scan doesn't use radiation, it has no known long-term negative effects. No medical diagnosis will be made using the MRI scan; it is being done solely for scientific reasons. If the participant's structural MRI is abnormal, either the attending radiologist or we will contact the child's Primary Care Physician (PCP) to discuss our findings. Every MRI will be included in the participant's medical file.

The MRI scan of any child who had an MRI before the intervention session will be obtained via the PACS system. We shall use template MRIs for data analysis if there isn't an available MRI and we cannot obtain MRI data from the child. This template MRI will come from an MRI database with templates of different age ranges. This MRI is required for two reasons: (i) HD-EEG data must be placed on the participant's 3D brain image to interpret the data, and (ii) MR images will be used for neuronavigation. Coregistration of HD-EEG data with structural MRI data is needed to pinpoint the origins of neural activity from HD-EEG recordings. Structural MRI (T1) will be included in

the imaging protocol. Cook Children's will use a 3T Siemens Tim Trio (Siemens Healthcare, USA) for scanning. The entire time spent gathering data will be about thirty minutes. The proposed structural sequence involves volumetric EPI navigators for real-time motion correction in a T1-weighted high-resolution magnetization-prepared rapid-acquisition gradient-echo (MPRAGE) acquisition. Additionally, we want to look into the quickest way to obtain various b-value data.

5.1.2 HD-EEG Recording

High-density EEG (HD-EEG) is a safe and non-invasive brain imaging technique. It involves placing a cap with small sensors on the child's head to measure the brain's electrical activity. This technique does not send any energy into the brain. We will use the HD-EEG to measure motor-evoked potentials (MEPs) and TMS-evoked potentials (TEPs). The MEPs will map the M1 cortex fully and assess cortical excitability, while the TEPs will assess meta-plasticity.

Upon arrival at the facility, the participant and their parents will receive an overview of the procedure and any questions they may have will be addressed. Children will have time to get comfortable in the room before the recordings start, with toys to play with. The child's parent or guardian can accompany the child into the room during the intervention to help make the child feel more at ease with the procedure. Once consent for the procedure is obtained, initial preparations for the recording session will commence. This includes changing the child's clothing into non-metallic garments provided by the hospital and recording the child's head coordinates and scalp shape information.

Afterward, we measure the child's maximum head circumference to select the appropriate EEG net. The HD-EEG net is then positioned on the child's head and connected to the EEG recording device, which records the electrical potential generated by the child's brain on the scalp. This device is passive and does not apply any energy to the brain. The net accommodates 64 electrodes. The lab provides various cap sizes to fit different head circumferences and is designed to minimize discomfort for young children. Two types of EEG nets are available: one requiring conductive gel for optimal conductivity between the scalp and electrodes and another with sponges on the electrodes that do not require conductive gel. The choice of EEG net for this study will depend on the child's cooperation during the preparation process.

Once the net is positioned on the child's head, additional leads will be attached to measure electromyography (EMG). Precisely, three leads will be placed on the wrist and hand to record EMG activity.

5.1.3 rTMS

rTMS is a noninvasive technique that maps the motor cortex by stimulating brain nerve cells with magnetic fields and excites or suppresses brain activity. Near the forehead, an electromagnetic coil is pressed up to the scalp. The brain area responsible for motor control is stimulated by a magnetic pulse painlessly delivered by the electromagnet. Numerous rTMS studies have demonstrated its safety and efficacy in investigating a range of motor cortex excitability measures.

We will use the Nexstim NBS 5 with a cooled pulse coil, the Nexspeech 1.1.2 software, and the analyzer 1.1.2 for motor mapping. Before the mapping session, the patient's MRI DICOM file will create a 3-D reconstruction of the child's cortical surface. The EMG electrodes will be placed on three right and three left bodily sides, with the locations identified by channels 1-6 (unless otherwise specified): (i) right thumb, (ii) right shoulder, (iii) right tibialis anterior, (iv) left thumb, (v) left shoulder, and (vi) left tibialis anterior. A second grounding electrode will be placed on the inside of the right forearm.

Throughout the rTMS session, the child will lie in an armchair designed for them. The operator will initially wrap a band around the child's head to coregister the child's anatomy with respect to the location of the TMS coil. Next, the operator will use the TMS coil to provide non-invasive, painless magnetic stimulation to the child's brain to identify their motor threshold. Operationally, a motor threshold is the lowest machine output necessary to elicit a 50 microvolt response from the contralateral stimulated hemisphere's right abductor pollicis brevis (APB) in more than 50% of trials.

Upper limb motor mapping uses the abductor pollicis brevis motor threshold, or 100% machine output (MO), in each hemisphere. Lower limb motor mapping is performed with a fixed machine output equal to the amount needed to elicit tibialis anterior (TA) motor consistently MEPs. Single pulse TMS is performed while bilateral motor evoked potentials from the TA, deltoid, and APB are recorded using surface EMG.

The Nexstim system utilizes a combination of MRI-based neuronavigation (Figure 2) and an integrated head tracker (Figure 3) to map the brain's anatomy and guide the rTMS coil to the exact locations required for effective intervention. The head tracker, a critical component of the Nexstim system, continuously monitors the position and orientation of the TMS coil relative to the child's head, ensuring that the stimulation is delivered consistently and accurately according to the pre-defined parameters.

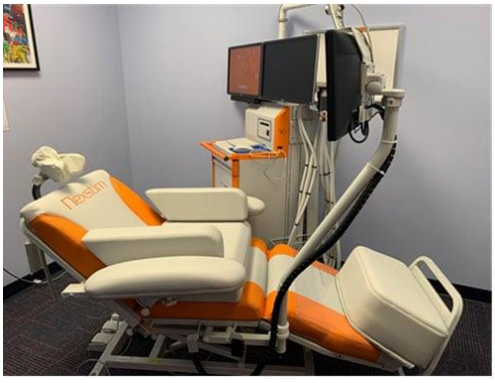

Figure 2: Real-time neuro-navigation using the Nexstim system ensuring accurate coil positioning

The Nexstim NBS 5 system integrates a single pulse coil with the Nexspeech 1.1.2 software for real-time adjustments and monitoring during stimulation. The analyzer 1.1.2 will be

employed to assess the effectiveness of the rTMS and adjust stimulation protocols as needed based on the ongoing data collected from both the rTMS and HD-EEG recordings.

Figure 3: Head tracker on child's head to coregister anatomy with the TMS coil location.

By leveraging these advanced technologies, we aim to enhance the precision of our rTMS interventions and improve the reliability of our findings to motor cortex excitability and brain network modulation.

After the brain mapping is completed, the intervention will use rTMS while the HD-EEG cap remains on the child's head to record TEPs (Figure 4). This approach allows for the simultaneous recording of both the cortical responses to rTMS and the ongoing brain activity captured by the HD-EEG, providing a comprehensive view of the neurophysiological effects of the stimulation.

Figure 4: The child sits comfortably in an armchair during the rTMS session. The operator places the electromagnetic coil next to the child's head to stimulate the brain.

A combination of special earplugs and soundproof headphones will be used to attenuate the acoustic click produced by the coil. To minimize the auditory responses evoked by the click sound of the rTMS pulse, subjects will be presented with auditory masking via earbuds that contain white noise and randomly jittering click noise from a recorded coil click (https://github.com/iTCf/TAAC). Besides, The TMS coil holder will be used in TMS-EEG procedures. Its main application is to accurately position and stabilize the TMS coil over specific regions of the brain to deliver targeted magnetic pulses. This precise positioning is crucial for effectively stimulating neural pathways. The holder ensures consistency and safety, reducing variability and enhancing the reliability of the stimulation (Figure 5).

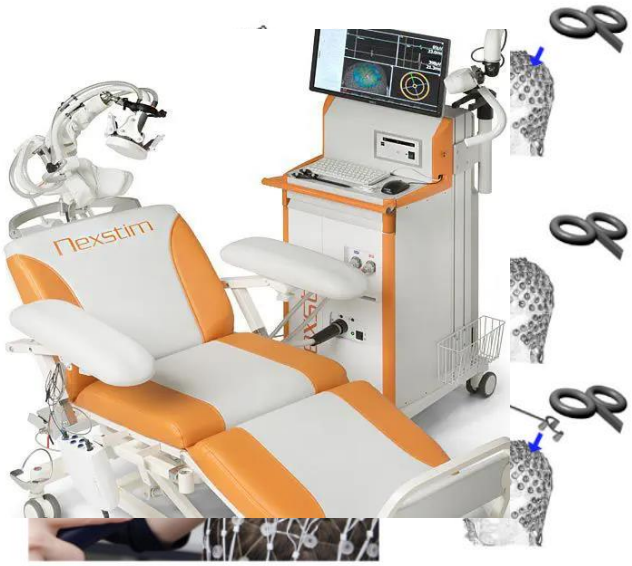

Figure 5: The TMS coil holder is depicted as a robust, adjustable arm designed to securely hold the TMS coil in place during TMS sessions.

Moreover, a coil spacer (Figure 6) is a 3D-printed plastic circular tripod, standing 1.1 cm tall with a 12.5 cm handle will be used. The online

design (Ultimaker2, https://3dprint.nih.gov/discover/3dpx-007789) can be customized to fit nearly any EEG cap and TMS coil. The spacer features three conical feet wider at the base to evenly distribute pressure on the scalp, minimizing discomfort. The circular ring is hollow, allowing for clear visibility when positioning the ring's center over the marked hotspot. For precise coil placement, a red line on the spacer handle should be aligned with the top edge of the TMS coil, ensuring that the coil's center—where the magnetic pulse is strongest—aligns directly over the hotspot at the ring's center. The primary function of the coil spacer is to maintain a consistent and precise distance between the TMS coil and the scalp. This distance helps in reducing the direct magnetic artifacts on the EEG signals, thereby improving data quality. The spacer also helps to evenly distribute the pressure of the TMS coil on the scalp, providing greater comfort to the participant during the study. In addition to artifact reduction, the coil spacer is

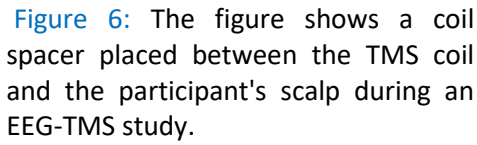

Figure 6: The figure shows a coil spacer placed between the TMS coil and the participant's scalp during an EEG-TMS study.

designed to accommodate the EEG cap and electrodes, ensuring they remain in stable contact with the scalp throughout the experiment. This stability is crucial for maintaining HD-EEG recordings and accurate localization of the stimulation site. Some coil spacers may have customizable thicknesses or adjustable components to suit different experimental needs, allowing researchers to fine-tune the stimulation parameters and EEG recording conditions.

6. STUDY PROCEDURES

6.1 Behavioral Assessments

6.1.1 Primary Behavioral Assessments

6.1.1.1 Gross Motor Function Classification System (GMFCS)

The Gross Motor Function Classification System (GMFCS)[49] is a standardized tool used to assess and classify the motor function of children with CP and other motor impairments. It provides a framework for evaluating gross motor abilities and determining the level of functional limitation based on observed performance and capability.

The GMFCS divides gross motor function into five distinct levels, each reflecting a different range of abilities and limitations:

- **Level I: Walks without limitations**

Children at this level can walk indoors and outdoors and climb stairs without any limitations. They may have slight difficulties with more advanced motor skills, but their mobility is not significantly affected.

- **Level II: Walks with limitations**

Children in this category walk outdoors and in most settings, but they have limitations in their ability to walk long distances and climb stairs. They may require some assistive devices or adaptations for mobility in certain situations.

- **Level III: Walks using a hand-held mobility device**

Children at this level walk using a hand-held mobility device, such as a walker, to help them move about in various settings. They may use wheelchairs for longer distances or when fatigued**.**

- **Level IV: Self-mobility with limitations; may use powered mobility**

Children at this level cannot walk and may require a wheelchair or powered mobility device for self-mobility. They can operate their wheelchair or powered mobility device independently or with minimal assistance.

- **Level V: Transported in a wheelchair**

Children at this level have severe limitations in their ability to move independently and are generally transported in a wheelchair. They require full assistance with all mobility-related activities and cannot perform gross motor skills effectively.

6.1.1.2 Gross Motor Function Measure (GMFM-88)

The Gross Motor Function Measure (GMFM-88) is a comprehensive assessment tool designed to evaluate and track gross motor function in children with CP. It comprises 88 items divided into five dimensions: lying and rolling, sitting, crawling and kneeling, standing and walking, running and jumping. Each item assesses specific motor tasks, and scores can be used to calculate raw and percentage scores for each dimension and an overall GMFM-88 score. This measure helps clinicians and researchers monitor motor development, assess the effectiveness of interventions, and guide treatment planning[50].

6.1.1.3 Modified Ashworth Scale (MAS)

The Modified Ashworth Scale (MAS)[51] is a clinical assessment tool used to measure muscle spasticity, particularly in individuals with neurological conditions such as CP. It evaluates the resistance of a muscle to passive stretch, which provides insight into the severity of spasticity. The MAS quantifies muscle spasticity by assessing the resistance when a muscle is passively stretched. This assessment helps understand the degree of spasticity and its impact on the individual's motor function.

6.1.1.4 Mirror Movement Assessment Scale (MMAS)

The Mirror Movement Assessment Scale (MMAS) is designed to evaluate MM, where involuntary movements in one limb mirror those in the opposite limb. It assesses these movements' frequency, amplitude, and impact on functional tasks. The MMAS helps understand the extent of mirror movements in individuals with neurological conditions such as CP, monitor changes over time, and evaluate the effectiveness of therapeutic interventions.

6.1.2 Secondary Behavioral Assessments

The following tests will not be administered for every subject; rather, they will be selected in accordance with each child's age and skill level. The person conducting these assessments will be a licensed occupational therapist.

6.1.2.1 Assisting Hand Assessment (AHA)

An assessment instrument called the AHA[52] gauges and characterizes how kids with upper limb disabilities work together with their non-affected hand when using their afflicted hand or aiding hand. Together with the other exams (behavioral assessment and somatosensory evaluation), the test will be administered to children between the ages of 18 months and 12 years. The AHA evaluates how naturally and spontaneously the youngster handles objects when they are playing. Each kid will play for fifteen minutes, handling and investigating items from the Small Kids AHA test kit, either before or after the scanning process. A member of the study team will administer the AHA and score it afterward. The AHA score is a number between 22 and 88, indicating a complete lack of use of the hand or use of the hand as efficiently as one would expect. After that, a Rasch-analysis will convert the AHA scores to equal interval unit logics.

6.1.2.2 Manual Ability Classification System (MACS)

The children between the ages of four and eighteen will be administered the MACS[53] test. The MACS is designed to gauge how well children with cerebral palsy typically perform manual tasks during everyday encounters. The MACS outlines five levels based on the child's independence in handling things, and whether or not they require assistance to carry out particular tasks. Items utilized in the exam include eating utensils, clothing, and writing pens. All of the items are age- and situationally-appropriate for children. Given that every etiology can typically be categorized into one of the five stages, the test covers a wide range of CP types. The five levels comprise an ordinal scale from I (handles items successfully and readily) to V (does not handle objects and has severely limited ability to do even simple activities). A

study team member qualified to administer the MACS will do so, and another member who was blind to the participant's diagnoses will grade the results afterward.

6.1.2.3 Assessment of Motor and Process Skills (AMPS)

A person's performance quality in instrumental or personal daily life activities is assessed using the AMPS[54] test. While completing these tasks, subjects are tested in situations that are familiar or relevant to them. The test gauges how well the person can complete these actions independently, without needing assistance, with greater physical effort, at a lower efficiency, or clumsiness. Subjects who struggle with everyday tasks and are three years of age or older will take the test. A study team member qualified to administer the AMPS will do so, and another member who was blind to the participant's diagnoses will grade the results afterward.

6.1.2.4 Melbourne Assessment of Unilateral Upper Limb Function (MUUL)

Children with CP can have their unilateral upper-extremity function evaluated with the MUUL[55]. It evaluates the range, accuracy, dexterity, and fluency of movements to determine the quality of upper limb movement. Children are given a variety of objects to manipulate, and each movement element is assigned a distinct score. Thirty item scores are used in total. A research team member qualified to administer the MUUL will do so, and another person who was blind to the participant's diagnoses will grade the results afterward—the children who will take the test range in age from five to fifteen.

6.1.2.5 Jebsen-Taylor Hand Function Test (JHFT)

Weighted and non-weighted hand function tasks are used in the JHFT[56] to evaluate fine motor abilities. The seven items on the exam assess everyday life skills like writing a brief phrase, stacking objects, and picking up objects of different sizes and weights. The participant's time to complete each item on the exam determines its score, which is then added up to get the final result. Six to eighteen-year-old subjects will use the assessment. A study team member qualified to administer the JHFT will do so, and a member who was blind to the participant's diagnosis will score the test afterward.

6.1.2.6 Canadian Occupations Performance Measure (COPM)

Using the COPM[57] interview technique, both the subject and a research team member can candidly identify areas of their daily routines that they would like to change. After ranking the significance of these activities on a 10-point rating scale, the participant chooses which two to address using intervening techniques. After

that, they graded how well they performed on each problem, with a certified research team member to conduct the interview, assigning a score of 1 to 10. It is advised that the participants be mature enough—typically older than six—to comprehend their motor and sensory limitations.

6.1.2.7 Goal Attainment Scale (GAS)

The GAS results in choosing a standard goal (eating with a spoon, for example) and standardizing the goal scaling (e.g., spoon does not shake). This makes it possible to calculate the degree to which the subject's goals are achieved. The priority (0 – not important, 3 – very important) and complexity (0 – not tough, 3 – very difficult) of the goals determine their value. Each patient will receive a customized assessment to see how well they are doing in reaching their objectives.

6.1.2.8 Besta Scale

Reaching is measured with the Besta scale to evaluate upper limb function. Hemiplegic patients are used in this way to compare the affected and unaffected sides of their limbs. Transitioning from rest to reaching toward an object or from rest to reaching from hand to mouth are examples of reaching tasks. Each arm completes each activity three times. Attached to the upper extremities, 3D retroreflective markers track joint and segment motion when paired with high-speed infrared cameras to create a three-dimensional patient reconstruction for in-depth motion analysis. Accuracy of reach, upper extremity range of motion, compensatory patterns, and motion symmetry relative to the unaffected limb will all be objectively determined by analysis.

6.1.2.9 Burke Fahn Marsden Dystonia Rating Scale (BFMDRS)

The BFMDRS comprises two distinct subscales: the movement subscale and the disability subscale. It is a dystonia rating scale. The Burke Fahn Marsden Movement Scale (BFMMS), a movement subscale, rates nine body regions from 0 to 4. The degree of dystonia symptoms in each location is assessed, and the areas are then weighted by a factor of 0.5 to 1.0 (the arms and legs being heavier than the neck, for example). The sum of the products from every category results in a maximum rating of 120. Burke Fahn Marsden Disability Scale (BFMDS), a disability subscale, is based on functional markers that are reported on daily activities by the patient and/or caregiver. The scores are assigned from 0 (totally dependent) to 30 (independent). When combined, these two subscales provide a rating that indicates the severity of dystonia.

6.1.2.10 Barry Albright Dystonia Scale (BADS)

BADS evaluates secondary dystonia using a 5-point rating system across eight body areas. It was created using the BFMMS as a model and administered similarly to provide patients with secondary dystonia with a global dystonia score. Since it fills in the gaps left by the BFMMS, it can measure posturing and involuntary movements that may not be represented in function but rather in the patient's comfort. Since it doesn't weigh sections like the BFMMS, 32 is the highest possible score.

6.1.2.11 Finger Tapping Task

A keyboard tapping task measuring low-level motor ability and psychomotor speed. Modeled after the Finger Tapping or Oscillation task from the Reitan Test Battery. Participants tap a key as quickly as possible for multiple trials, testing dominant and/or non-dominant hand.

6.1.2.12 Task Fitt's Law Task

Classic motor control task measuring the speed-accuracy tradeoff in rapid aimed movements. Tests the relationship between movement time, distance, and target size, following Fitts' Law (MT = a + b*log2(2D/W)). Participants rapidly move the mouse cursor from a home position to rectangular targets of varying sizes and distances. Each trial begins at the home position on the left side of the screen. When ready, participants move to the target rectangle as quickly as possible. Movement time and accuracy are recorded for each trial.

6.1.2.13 Pursuit Rotor

Motor tracking task requiring continuous tracking of a moving target with mouse or touch input. Measures motor coordination and learning.

6.1.2.14 Time Tapping Task

Motor timing task measuring self-paced tapping consistency across multiple trials. Participants tap at a self-paced even rate following a visual entrainment period. After seeing a flashing cross that demonstrates the target tapping rate, participants must maintain that rhythm for a sustained period (default 180 seconds per trial). The task assesses low-level motor timing ability and may be sensitive to fatigue, sleep deprivation, and motor control deficits.

6.1.2.15 Simple Reaction Time

A simple reaction time task where a single stimulus (an 'X' or mouse button prompt) appears at a specifiable delay from the previous response. Measures basic alertness and motor response speed across multiple blocks with breaks.

7. RESPONSE CRITERIA

7.1 Risks and Benefits

7.1.1 Risks

The participants in this study are not at all at risk. There are 3 types of possible dangers related to taking part in these studies: (i) discomfort from the MRI's magnetic field exposure, (ii) discomfort from the HD-EEG, and (iii) discomfort from rTMS.

Use of 3 Tesla (T) MRI: Every participant will use a Siemens Tim 3T Trio, an FDA-approved device. The 3T magnet has a bigger bore opening than the present system, which should lessen patient claustrophobia because gradient coil design has improved over the past few years. The FDA published guidelines on September 29, 1997, classifying these devices as "Non-significant risk devices." Since 1990, studies have safely used four Tesla (and higher field) scanners on human beings. While assessing the safety of an MR device, there are four main factors to consider. These include acoustic noise, dB/dt (time rate of change of magnetic fields), specific absorption rate (SAR) of radiofrequency (RF) energy, and static magnetic field strength. These are covered in more detail down below.

Static magnetic field strength: The strength of the static magnetic field distinguishes the three Tesla magnet systems. According to FDA rules, "significant risk devices" do not include field strengths up to 4 Tesla.

“The original MRDD Guidance stated a level of concern for static magnetic field strength of 2T. For the above 2T, a manufacturer was to provide evidence of safety. Clause 6.8.2 (jj) of the IEC 60601-2-33 states that examination of the whole body above 2T, or locally set limit, should be performed under an approved investigational human studies protocol, and vital body functions should be monitored. However, this requirement was published in July 1995. Since then, several research sites have been operating at field strengths up to 4T with no reported occurrence of adverse events. Consequently, operation up to 4T is not considered a significant risk.”

Guidance for Submission of pre-market notifications for Magnetic Resonance Diagnostic Devices, Issued November 14, 1998
Food and Drug Administration

Center for Devices and Radiological Health

High static magnetic field strengths do not appear to have any known direct effects, but there are secondary safety concerns about the static magnet field to take into account. These concerns include the potential for medical devices, like pacemakers, to malfunction in the magnetic field or ferromagnetic materials (either implanted or external to the body) to move due to the static field's magnetic force. These are common problems with MR imaging, and protocols have previously been established to deal with them while imaging on the 1.5 Tesla clinical system. The 3T scanner will follow the same protocols. Specifically, in the consent form and throughout the examination, the parent/guardian of each subject will be questioned about potential metallic and electronic implants. If there is any uncertainty about a specific device's safety (or existence), no scan is carried out. We have a "zero-tolerance" policy regarding ferromagnetic items in the scan room during patient scans to reduce the possibility of harm from outside objects. Before entering the magnet room, the subject's parent or guardian must remove any ferromagnetic materials from their possession, such as jewelry, pocket contents, belt buckles, shoes with steel nails or toe coverings, etc. Only non-magnetic equipment is permitted within the scan room. Before entering the scanning suite, each individual is put through a magnetic wand sweep, akin to what happens at airport security booths. These procedures follow the Guidelines for Submission of Premarket Notifications for Magnetic Resonance Diagnostic Devices.

Specific absorption rate (SAR) of radiofrequency (RF) energy: The FDA establishes RF energy deposition limits to ensure that heat absorbed radioactively and deposited in tissue does not exceed the body's safe removal capacity. The requirements are expressed in Watts of deposited power per kilogram of tissue throughout predetermined intervals. There is no clear mention of radiofrequency field frequency in these requirements. The energy absorption rate of biological tissues is a complex function of frequency; in the frequency range of interest for MR imaging, the absorption rate increases with increasing frequency. On the other hand, there is a linear relationship between the static magnetic field strength and the RF resonance frequency of an MR sample. As a result, SAR considerations are crucial to prevent potentially dangerous tissue heating at high fields. The Siemens Trio system is equipped with various redundant safety mechanisms to safeguard subjects against excessive RF power deposition and ensure that the deposited power remains well within FDA restrictions.

dB/dt (time rate of magnetic fields): There must be a way to locally change the magnetic field inside the magnet hole to create a magnetic resonance image. Gradient coils with a magnetic field are used for this. These large amplitude gradient fields can vary quickly over time to images with high spatial resolution. Suddenly, changing magnetic fields have the ability to directly activate peripheral nerves and create voltages in tissue, both of which can be uncomfortable. To

prevent this, dB/dt limitations have been set by the FDA. Adhering to these restrictions dictates the time and geographic boundaries of echoplanar image investigations. The study's required image parameters are within the FDA's dB/dt maximums. The console software will also notify the experimenter and request acknowledgment before allowing the experiment to continue if a sequence requires a dB/dt level higher than the FDA's "normal mode" restrictions.

Acoustic noise: The gradient field coils carry large, time-varying electrical currents during regular operation. The gradient coil in the static magnetic field is subject to force from these currents. The gradient coils produce an audible sound similar to a loudspeaker since they are not infinitely stiff and move slightly (about 100 microns) in response to these forces. This sound can be loud for some imaging sequences, such as echoplanar imaging, which this technique will use. The FDA has determined that a peak, unweighted sound pressure level of 140 dB represents a severe risk for acoustic noise in an MR system. This is not the sound level; the scan room is engineered to minimize acoustic reflections. For every MR scan, the MIC gives subjects headphones and earplugs, regardless of the system's sound intensity.

HD-EEG: HD-EEG has no known risks and is widely used in clinical and research settings. The non-invasive HD-EEG method is painless and uncomfortable. For all skin-contact physiological recordings, we shall use a hypoallergenic gel.

rTMS: Since TMS was first introduced in 1984, a number of side effects have been reported. The type of stimulation employed greatly impacts the likelihood of these side effects. To reduce the hazards as much as possible, the researchers will adhere scrupulously to all currently suggested safety precautions. When one pulse is administered at a time, as suggested in this study, there are no known negative effects or hazards associated with rTMS techniques. Hundreds of children have previously received a TMS or rTMS[58]. Using this magnet can put a person in danger of having a seizure since it sends electromagnetic pulses through the brain. However, in the past, seizures have only happened in adult humans and animals when the magnet was employed for consecutive high-frequency impulses at a sustained level[59].

No children previously administered rTMS by the scientists experienced seizures or other notable neurologic problems. Numerous studies have shown that most children tolerate rTMS well[60–62]. The various stimuli that can be used fall well within the boundaries of the most recent globally recognized safety standards. Risks, however, could include:

a. **Headache and neck pain:** It is estimated that up to 20 out of every 100 TMS patients have
Tightness in the neck muscles can cause headaches or neck pain. Acetaminophen (Tylenol®) quickly relieves discomfort in such situations.

b. **Ringing in the ears:** When the stimulation coil's current is run through it, the TMS makes a loud clicking sound. This loud click may cause ear ringing and temporary hearing loss if no protection is used. Earplugs are used in the laboratory to prevent this possible negative effect. Research on humans and animals has shown that earplugs can significantly reduce the chance of experiencing hearing loss due to TMS.

c. **Seizure:** TMS can induce a seizure even in the absence of epilepsy, brain abnormalities, or other seizure risk factors. It is estimated that the total probability of this consequence is less than 1% for patients without any neurologic condition and between 1% and 2% for patients who have epilepsy. Nevertheless, the International Society for Transcranial Stimulation has identified this as a very worrisome consequence, and the professionals will make all necessary efforts to minimize the danger. It's crucial to remember that having a seizure brought on by rTMS has never increased the likelihood of epilepsy or caused seizures to follow. Seizures brought on by rTMS would happen either during or after the rTMS. It is not anticipated that seizures brought on by rTMS will happen hours or days later.

d. **Cognitive alteration:** Transient modifications in TMS may modify memory, attention, and other cognitive processes. This is a theoretical concern since no such adverse effects have been reported in any of the safety studies.

e. **Unexpected problems:** Although TMS has been utilized for more than 20 years in numerous laboratories worldwide, it is still an experimental therapy for the treatment of epilepsy, so unanticipated issues could arise.

7.1.2 Protection Against Risks

Several safety measures are included in the rTMS procedures to reduce danger for children. We will implement pre-session screening to assess the risk of adverse effects, including any history of seizures or neurological conditions that may contraindicate rTMS, and ensure continuous monitoring of the participant during the procedure, both in terms of physical well-being and the rTMS system's performance. Emergency first aid protocols are in place in the improbable event that a seizure happens, to stop it if needed. Moreover, in case of an emergency, the facility's medical alert protocol will be activated. A seizure brought on by an experimental method does not imply epilepsy in the person being studied. To accomplish this, a letter certifying that the seizure was caused experimentally would be supplied for any reason relevant to driving, insurance, or employment. Since surface electrodes will be placed over the muscle to capture the EMG, there won't be any problems with pain, infection, or bleeding. For TD participants, all

procedures follow the same safety protocols as for CP children including pre-screening for TMS contraindications and real-time monitoring for discomfort.

7.1.3 Benefits

The study offers several potential benefits for the subjects involved:

- **Improvement in Motor Function:** This study's primary aim is likely to explore the efficacy of different rTMS protocols on motor function. Participants may experience improvements in motor control, coordination, and overall physical abilities, which could enhance their daily activities and quality of life.

- **Meta-Plasticity Enhancements:** The study may induce beneficial changes in the brain's ability to reorganize and adapt, potentially leading to long-term improvements in neural connectivity and motor function. These changes can be significant for children with CP, who often face challenges related to motor function.

- **Access to Cutting-Edge Therapies:** Participants in the study may receive access to innovative and advanced therapeutic interventions that are not yet widely available. This could provide them with unique treatment options that positively impact their condition.
- **Contribution to Scientific Knowledge:** By participating in this study, subjects and their families contribute to research that could benefit future children with CP. The findings from this study may help develop more effective treatments and interventions for others with similar conditions.

- **Monitoring and Support:** Medical professionals often closely monitor participants throughout the study, which can lead to early detection of any issues and provide additional support to the child and their family.

While these benefits are potential outcomes, it's important to note that they may vary depending on the individual response to the intervention.

8. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.1 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient

safety and care. Adverse events will be coded and reported using the MedDRA dictionary.

All patients experiencing an adverse event, regardless of its relationship to study drug/device, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

8.2 Definitions

8.2.1 Definition of Adverse Event

An adverse event (AE) is generally defined as any unfavorable and unintended diagnosis, symptom, sign (including an abnormal laboratory finding), syndrome or disease which either occurs during the study, having been absent at baseline, or if present at baseline, appears to worsen. Adverse events are to be recording regardless of their relationship to the study intervention.

8.2.2 Severity of Adverse Events

The severity of an AE is graded as follows:

Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
Moderate (grade 2): the event causes discomfort that affects normal daily activities.
Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
Life-threatening (grade 4): the patient was at risk of death at the time of the event.
Fatal (grade 5): the event caused death.

8.2.3 Serious Adverse Events (SAE)

A “serious” adverse event is defined in regulatory terminology as any untoward medical occurrence that:

- Results in death.

If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

- Is life-threatening.
  (The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).

- Requires in-patient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.

- Results in persistent or significant disability or incapacity.

- Is a congenital anomaly/birth defect

- Is an important medical event

  Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event".

  For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

8.3 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event

Step 2: Grade the adverse event

Step 3: Determine whether the adverse event is related to the protocol therapy
Attribution categories are as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

Note: This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

Step 4: Determine the prior experience of the adverse event.
Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- The current known adverse events listed in the Agent Information Section of this protocol;
- The drug package insert;
- The current Investigator's Brochure

8.4 Reporting Requirements for Adverse Events/Unanticipated Problems Involving Risks to Subjects or Others

8.4.1 Expedited Reporting

- The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.

- The Cook Children's IRB must be notified of "any unanticipated problems involving risk to subjects or others" (UPIRTSO) as defined in Cook Children's Policy Number Cook Children's_HRPP_P08. UPIRTSOs, per HRPP policy P08 are considered to be those events that refer to any incident, experience, outcome or new information that: is unexpected, AND, Is related or possibly related to participation in the research, AND Indicates that subjects or others are at a greater risk of harm than was previously known or recognized, OR if a problem occurs with greater frequency, specificity, or severity than anticipated it should also be considered to be an unanticipated problem and should be reported to the IRB. UPIRTSOs are required to be reported to the IRB within 5 business days from the date that the PI determined the problem to be unanticipated using the Unanticipated Reportable Events Form in iMedRIS.

- All other adverse events - those that do not meet unanticipated problem criteria such as those that are expected, or are unlikely, or definitely not related, or those that are possibly related to the study participation - are not required to be reported to the IRB.

9. INTERVENTION DISCONTINUATION

9.1 Removal of Patients from Protocol Therapy

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The PI will be notified and the reason(s) for discontinuation, as well as the date patient was removed from study, will be documented and may include:

9.1.1 Patient voluntarily withdraws from treatment (follow-up permitted);

9.1.2 Patient withdraws consent (termination of treatment and follow-up);

9.1.3 Patient is unable to comply with protocol requirements;

9.1.4 Patient demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator);

9.1.5 Patient experiences toxicity that makes continuation in the protocol unsafe;

9.1.6 Treating physician judges continuation on the study would not be in the patient's best interest;

9.1.7 Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);

9.1.8 Lost to follow-up. Example language: If a research subject cannot be located to document survival after a period of 2 years, the subject may be considered "lost to follow-up." All attempts to contact the subject during the two years must be documented and approved by the Data Monitoring Committee.

9.2 Subject Withdrawals or Discontinuation of Study Intervention

Patients can withdraw from the study at any request or at the investigator's discretion for administrative, behavioral, or safety concerns. The patient will be taken out of the study on the day that the principal investigator is informed, and the reason(s) for stopping will be recorded, perhaps including:

a. The patient gives up willingly;
b. The patient is not able to follow the regimen;
c. The treating physician determines that continuing the research would not be in the patient's best interest.

9.3 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension

or termination, will be provided by the suspending or terminating party to the PI, DSMB, funding agency, the Investigational New Drug (IND) /Investigational Device Exemption (IDE) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.
Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination of futility.

10. STATISTICAL CONSIDERATIONS

Sample Size and Power Analysis: We will estimate the signal-to-noise ratio (SNR) at the sensor level, which will allow us to calculate the intra-subject variability. Given this SNR, we anticipate an effect size (Cohen's d) of greater than 0.5 in the primary motor cortex (M1). For an effect size of 0.5, 15 subjects per group are required to achieve 80% power at a significance level of 0.05. This estimation assumes a moderate effect of 6-Hz primed rTMS on motor function and meta-plasticity outcomes.

Handling Missing Data: Given the possibility of dropouts or incomplete data, particularly in a pediatric population, multiple imputation methods will be employed to handle missing data. We will also conduct sensitivity analyses to assess the impact of missing data on the results, ensuring robustness in our findings.

Multiple Comparisons and Adjustment: Considering the multiple outcome measures related to motor function, cortical excitability, and meta-plasticity, we will apply False Discovery Rate (FDR) adjustments to control for Type I error. This approach balances the need to maintain statistical rigor while not being overly conservative, which is crucial given the exploratory nature of some of our outcomes.

Effect Size and Clinical Relevance: While statistical significance will be assessed using p-values, we will also report effect sizes (Cohen's d) to gauge the practical relevance of our findings. An effect size greater than 0.5, particularly in motor outcomes, will be considered clinically meaningful, contributing to the broader understanding of rTMS effects in pediatric CP.

Controlling for Confounding Variables: Behavioral scores, laterality, age, and gender will be included as covariates in our analysis to control for their potential confounding effects. We will employ Analysis of Covariance (ANCOVA) models to adjust for these variables, ensuring that the observed effects are attributable to the rTMS intervention rather than these extraneous factors.

Longitudinal Data Analysis: Given the repeated measures design, where participants will be assessed at multiple time points, we will use mixed-effects models to account for within-subject correlations. This approach will allow us to capture the temporal dynamics of rTMS effects on motor function and meta-plasticity accurately, providing a more comprehensive understanding of the intervention's impact over time.

10.1 Treatment Assignment Procedures

10.1.1 Behavioral Scores Statistical Analysis

Nonparametric Mann-Whitney U tests will examine relationships between continuous and categorical data. Spearman correlation will be used to evaluate the relationships between the behavioral ratings and the HD-EEG measures. Depending on the tests conducted, distinct univariate linear regressions will be carried out to assess the slope of the association between HD-EEG and behavioral scores. After that, multivariate general linear regression will be carried out for the candidate variables connected to the results of each behavioral test. Two-tailed $\alpha<0.05$ will be used in all tests.

10.1.2 TMS-EEG data analysis

TMS-EEG data analysis will be performed using Brainstorm[63] and EEGLAB[64] and TMS-EEG signal analyzer (TESA) toolbox[65,66] of the EEGLAB. Results are chosen for a good signal-to-noise ratio (SNR) and manually examined for MEPs quality to ensure each MEP accurately captures a motor response. For each examined muscle group, a voltage-graded color scale is created, with the maximum voltage exhibited in white and lower voltages displayed in red, to help visualize the motor mapping results. A distinct color scale for the minimum and maximum values is established to prevent meaningful comparisons between different muscle groups. Most intra-operative MRI guidance systems are compatible with the DICOM format in which mapping findings are exported. You can obtain this DICOM file upon request.

The data will be analyzed through the following steps:

a. **Preprocessing:** The initial step in analyzing HD-EEG data involves preprocessing to ensure data quality. This includes artifact correction to remove unwanted signals from sources such as eye movements, muscle activity, electrical noise, and TMS artifacts. After artifact correction, the data will be filtered using band-pass filters to isolate relevant frequency bands, such as theta, alpha, beta, and gamma, while removing irrelevant noise. The

continuous EEG data will then be segmented into epochs aligned with the timing of rTMS pulses and behavioral tasks.

b. TMS-Evoked Potentials: After segmenting the data around rTMS pulses and performing baseline correction, key components such as N15, P30, N45, P55, N100, P180, and N280 will be identified and measured for latency and amplitude.

c. Motor-Evoked Potentials: We will Identify MEPs by automated peak detection and quantify the MEPs by measuring their amplitude, latency, and duration.

d. Frequency Domain Analysis: In the frequency domain, power spectral density (PSD) will be computed to analyze how power is distributed across different frequency bands. Additionally, event-related spectral perturbation (ERSP) will be used to evaluate changes in power spectra time-locked to specific events, such as rTMS stimulation or task performance. These analyses help identify frequency-specific effects of the stimulation.

e. Source Localization: Source localization techniques, such as Minimum Norm Estimates (MNE), will be employed to estimate the neural sources of the observed EEG activity. This step also involves functional connectivity analysis to explore changes in connectivity between different brain regions, particularly those involved in motor functions and meta-plasticity.

f. Statistical Analysis: Statistical analysis compares EEG data between different stimulation conditions (low vs. high-frequency rTMS) and sham. Correlations between EEG findings and behavioral outcomes will be examined to understand the relationship between brain activity and motor function or meta-plasticity. Covariates such as age, gender, and behavioral scores will be included in statistical models to isolate the effects of rTMS.

10.1.3 Visualization

Finally, data visualization is crucial for interpreting results. Topographic maps will be created to illustrate spatial distributions of EEG activity and changes in different frequency bands. Time-frequency representations will also be used to show how power in various frequency bands evolves over time relative to rTMS stimulation.

10 DATA MANAGEMENT AND MONITORING/AUDITING

10.2 Data Collection

The study group will gather PHI and clinical data. Once the data is entered into Epic, a REDCap database, or a secure database in the TMS room, only the research team will be able to access it.

10.3 Data Management

Cook Children's Health Care System Research Center (Cook Children's RAO) will handle data management, which involves creating forms, gathering, organizing, editing, storing, and reporting data; additionally, the research statistician will receive training and access the data. Cook Children's will update and maintain the eCRFs to reflect modifications to the protocol.

10.4 Data Storage

Data will be stored in an electronic database in REDCap created by Cook Children's RAO. User privileges will be granted only to the research team.

The database will be maintained on a database server with data entry via a web server both located securely behind the Cook Children's firewall. Only individuals listed as key study personnel approved by the IRB will be granted access to the study database.

Patient information will be de-identified prior to data analysis; the data will be transformed to make PHI undecipherable to others. When used for scientific abstract or publication, all PHI will be removed.

Any written study-related records will be maintained for at least 3 years following the completion of the study; then destroyed using a cross-cut shredder. Electronic study-related records and results will only be kept in aggregate form following completion of the study.

10.5 Procedures to Maintain Confidentiality

To minimize the chance of harm associated with PHI and identifiable study data collection, all information abstracted from the electronic medical record will be stored in a REDCap database maintained on Cook Children's servers by study staff. REDCap implements several measures of enforcing security including authentication to validate the identity of end-users that log in to the system, auto log-out, and various methods to protect against malicious users who may attempt to identify and exploit any security vulnerabilities in the system.

To maintain confidentiality, all patient identifiers will be removed prior to disclosure; any disclosed data will be for scientific abstract and publication to the medical community.

10.6 Quality Assurance

10.6.1 Training

To guarantee the precision and dependability of data notes and comments, all research team members who work with the data will receive training on appropriate safety protocols for all imaging modalities and study techniques. The team will meet regularly to ensure that standards are set and that data is handled and evaluated correctly and consistently. The team will ensure that these certificates are renewed annually and will comply with any Cook Children safety training requirements.

10.6.2 Quality in Data

- Edit checks and query resolution

  Data entered into the eCRF will subjected to auto-validation (ranges, data checks) and edits on a “real time” basis as well as batch edits.

  The batch edits will query inconsistent responses, missing forms, missing data, and data anomalies. The Data Manager will review each discrepancy report. Resolved queries will then be incorporated into the database.

- Audit trail

  An electronic audit trail of all changes is maintained by the system (i.e., by recording the change, name of user making the change, date/time).

11 STUDY MANAGEMENT

11.2 Conflict of Interest

It is mandatory for all Investigators and Key Study Personnel (KSP) to adhere to CCHCS RS policy 175 on Research Conflicts of Interest. A Declaration Regarding Financial Interests (DRFI) Form must be filled out and submitted by investigators and Key Study Personnel (KSP) for initial and ongoing assessment. Disclosure of any financial interest must be made on the DRFI form upon declaration. The Vice President of Internal Audit and Compliance, who also serves as the Conflict of Interest (COI) Official, reviews all DRFI forms and any disclosures. The COI Official has made decisions about conflicts of interest that, to the extent that they apply, comply with DHHS and FDA standards.

The COI Official reports all decisions made to the IRB. If financial conflicts of interest are found, the COI Official will advise on how to resolve or manage them and submit the results to the IRB. The PI and/or impacted individual will be informed by the IRB of these results and any measures for managing conflicts of interest. Until the conflict of interest has been resolved or the principal investigator has approved and carried out the management plan, the IRB will not grant final or continuing approval for any research study. For further details, see CCHCS RS policy 175 on Research Conflicts of Interest.

11.3 Registration Procedures

All patients must be registered with the Cook Children's Research Administration Office before enrollment to study. Prior to registration, eligibility criteria must be confirmed with the PI.

11.4 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

11.5 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

However, for any such emergency modification implemented, an Unanticipated Reportable Event Form must be completed in iMedRIS IRB modification form must be completed within five (5) business days of making the changefrom the date that the event or intervention occurred. If the PI determines that the deviation or change that was implemented should be permanently included in the protocol, the following will be required for subsequent IRB submission: a Modification Form, revised protocol, revised consent form (if applicable), and revised assent form (if applicable).

11.6 Other Protocol Deviations/Exceptions/Violations

All other planned deviations (also called exceptions) from the protocol must have prior approval by the Principal Investigator, the sponsor and the IRB. According to the IRB, an exception is any circumstance in which the specific procedures called for in a protocol are not in the best interests of a specific patient/subject. Usually it is a violation that is anticipated and happens with prior agreement from the sponsor. Exceptions should be reported to the IRB using the Unanticipated Reportable Events Form within five (5) business days by the (PI), from the date of determining that the

exception should be made, post sponsor approval. Documentation of sponsor justification and approval should accompany this form.

According to the IRB, a protocol deviation is unanticipated and happens without any prior agreement. It is an accidental or unintentional change to the IRB approved protocol. Protocol deviations could also be any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

Per IRB policy, protocol deviations that result in harm to research subjects or resulted in an increase in risk or a decrease in benefit to individual subjects and/or the subject population should be reported to the IRB using the Unanticipated Reportable Events Form within 5 business days from the date that the PI became knowledgeable of the deviation.
An unplanned protocol variance is considered a violation if the variance:

- Has harmed or increased the risk of harm to one or more research subjects.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or Cook Children's policies.

If a deviation or violation occurs without prior approval from the Principal Investigator, follow the guidelines below:

Protocol Deviations: Personnel will report to any sponsor or data and safety monitoring committee in accordance with their policies. Any violation meeting IRB protocol deviation policy criteria should be reported to the IRB as described above. All other deviations should be summarized and reported to the IRB at the time of continuing review.

11.7 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

11.8 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until three years after the completion and final study report of this investigational study.

11.9 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

12 REFERENCES

1. Soleimani, F., Vameghi, R. & Biglarian, A. *213*. *Archives of Iranian Medicine* vol. 16 (2013).

2. Colver, A., Fairhurst, C. & Pharoah, P. O. D. Cerebral palsy. in *The Lancet* vol. 383 1240–1249 (Elsevier B.V., 2014).

3. Sun, Y. Y. *et al.* Effects of repetitive transcranial magnetic stimulation on motor function and language ability in cerebral palsy: A systematic review and meta-analysis. *Frontiers in Pediatrics* vol. 11 Preprint at https://doi.org/10.3389/fped.2023.835472 (2023).

4. Gupta, M., Rajak, B. L., Bhatia, D. & Mukherjee, A. Neuromodulatory effect of repetitive transcranial magnetic stimulation pulses on functional motor performances of spastic cerebral palsy children. *J Med Eng Technol* **42**, 352–358 (2018).

5. Reddihough, D. *Cerebral Palsy in Childhood*.

6. Colver, A., Fairhurst, C. & Pharoah, P. O. D. Cerebral palsy. in *The Lancet* vol. 383 1240–1249 (Elsevier B.V., 2014).

7. Hayes, C. Cerebral palsy: classification, diagnosis and challenges of care. *British journal of nursing* **19**, 368–373 (2010).

8. Chen, X. J. & Li, S. C. Definition, classification and diagnostic conditions of cerebral palsy in children. *Chinese Journal of Physical Medicine and Rehabilitation* **29**, 309 (2007).

9. Reddihough, D. *Cerebral Palsy in Childhood*.

10. Green, L. B. & Hurvitz, E. A. Cerebral Palsy. *Phys Med Rehabil Clin N Am* **18**, 859–882 (2007).

11. Bar-On, L. *et al.* Spasticity and its contribution to hypertonia in cerebral palsy. *BioMed Research International* vol. 2015 Preprint at https://doi.org/10.1155/2015/317047 (2015).

12. Diego, A. & Leung, A. Transcranial direct current stimulation for improving gross motor function in children with cerebral palsy: A systematic review. *British Journal of Occupational Therapy* **83**, 030802261989788 (2020).

13. Stanley, F. J., Blair, E., Alberman, E. & Alberman, E. D. *Cerebral Palsies: Epidemiology and Causal Pathways*. (Cambridge University Press, 2000).

14. Yeargin-Allsopp, M. *et al.* Prevalence of cerebral palsy in 8-year-old children in three areas of the united states in 2002: A multisite collaboration. *Pediatrics* **121**, 547–554 (2008).

15. Kuo, H., Ferre, C. L., Chin, K. Y., Friel, K. M. & Gordon, A. M. Mirror movements and brain pathology in children with unilateral cerebral palsy. *Dev Med Child Neurol* **65**, 264–273 (2023).

16. Fling, B. W. & Seidler, R. D. Task-dependent effects of interhemispheric inhibition on motor control. *Behavioural brain research* **226**, 211–217 (2012).

17. Cox, B. C., Cincotta, M. & Espay, A. J. Mirror movements in movement disorders: a review. *Tremor and other hyperkinetic movements* **2**, (2012).

18. Takeuchi, N., Oouchida, Y. & Izumi, S.-I. Motor control and neural plasticity through interhemispheric interactions. *Neural Plast* **2012**, 823285 (2012).

19. Azizi, S. *et al. The Impact of an Anti-Gravity Treadmill (AlterG) Training on Walking Capacity and Corticospinal Tract Structure in Children with Cerebral Palsy*. *Conference proceedings: ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference* vol. 2017 (2017).

20. Hameed, M. *et al.* Transcranial Magnetic and Direct Current Stimulation in Children. *Curr Neurol Neurosci Rep* **17**, (2017).

21. Kirton, A. Advancing non-invasive neuromodulation clinical trials in children: Lessons from perinatal stroke. *European Journal of Paediatric Neurology* **21**, 75–103 (2017).

22. Chung, M. G. & Lo, W. D. Noninvasive brain stimulation: The potential for use in the rehabilitation of pediatric acquired brain injury. *Archives of Physical Medicine and Rehabilitation* vol. 96 S129–S137 Preprint at https://doi.org/10.1016/j.apmr.2014.10.013 (2015).

23. Chung, M. G. & Lo, W. D. Noninvasive brain stimulation: the potential for use in the rehabilitation of pediatric acquired brain injury. *Arch Phys Med Rehabil* **96**, S129–S137 (2015).

24. Pascual-Leone, A., Amedi, A., Fregni, F. & Merabet, L. B. The plastic human brain cortex. *Annu. Rev. Neurosci.* **28**, 377–401 (2005).

25. Casanova, M. F. *et al.* Effects of transcranial magnetic stimulation therapy on evoked and induced gamma oscillations in children with autism spectrum disorder. *Brain Sci* **10**, 423 (2020).

26. Elbanna, S. T., Elshennawy, S. & Ayad, M. N. Noninvasive brain stimulation for rehabilitation of pediatric motor disorders following brain injury: systematic review of randomized controlled trials. *Arch Phys Med Rehabil* **100**, 1945–1963 (2019).

27. Gillick, B. T. *et al.* Primed low-frequency repetitive transcranial magnetic stimulation and constraint-induced movement therapy in pediatric hemiparesis: A randomized controlled trial. *Dev Med Child Neurol* **56**, 44–52 (2014).

28. Gupta, M., Rajak, B. L., Bhatia, D. & Mukherjee, A. Effect of repetitive transcranial magnetic stimulation on motor function and spasticity in spastic cerebral palsy. *Int J Biomed Eng Technol* **31**, 365–374 (2019).

29. Naeser, M. A. *et al.* Improved naming after TMS treatments in a chronic, global aphasia patient–case report. *Neurocase* **11**, 182–193 (2005).

30. Kirton, A. Modeling developmental plasticity after perinatal stroke: defining central therapeutic targets in cerebral palsy. *Pediatr Neurol* **48**, 81–94 (2013).

31. Abraham, W. C. & Bear, M. F. Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci* **19**, 126–130 (1996).

32. Daskalakis, Z. J., Farzan, F., Radhu, N. & Fitzgerald, P. B. Combined transcranial magnetic stimulation and electroencephalography: its past, present and future. *Brain Res* **1463**, 93–107 (2012).

33. Thut, G. & Pascual-Leone, A. A review of combined TMS-EEG studies to characterize lasting effects of repetitive TMS and assess their usefulness in cognitive and clinical neuroscience. *Brain Topogr* **22**, 219–232 (2010).

34. Ilmoniemi, R. J. & Kičić, D. Methodology for combined TMS and EEG. *Brain Topogr* **22**, 233–248 (2010).

35. Massimini, M., Tononi, G. & Huber, R. Slow waves, synaptic plasticity and information processing: insights from transcranial magnetic stimulation and high-density EEG experiments. *European Journal of Neuroscience* **29**, 1761–1770 (2009).

36. Pellicciari, M. C., Veniero, D. & Miniussi, C. Characterizing the cortical oscillatory response to TMS pulse. *Front Cell Neurosci* **11**, 38 (2017).

37. Barr, M. S., Farzan, F., Davis, K. D., Fitzgerald, P. B. & Daskalakis, Z. J. Measuring GABAergic inhibitory activity with TMS-EEG and its potential clinical application for chronic pain. *Journal of Neuroimmune Pharmacology* **8**, 535–546 (2013).

38. Gupta, J. *et al.* Brain Stimulation and Constraint Induced Movement Therapy in Children With Unilateral Cerebral Palsy: A Randomized Controlled Trial. *Neurorehabil Neural Repair* **37**, 266–276 (2023).

39. Lee, J. C. *et al.* Strategies for augmentation of high-frequency left-sided repetitive transcranial magnetic stimulation treatment of major depressive disorder. *J Affect Disord* **277**, 964–969 (2020).

40. Rajak, B. L., Gupta, M., Bhatia, D. & Mukherjee, A. Increasing Number of Therapy Sessions of Repetitive Transcranial Magnetic Stimulation Improves Motor Development by Reducing Muscle Spasticity in Cerebral Palsy Children. *Ann Indian Acad Neurol* **22**, (2019).

41. Cassidy, J. M. *et al.* A comparison of primed low-frequency repetitive transcranial magnetic stimulation treatments in chronic stroke. *Brain Stimul* **8**, 1074–1084 (2015).

42. Gillick, B. T. *et al.* Primed low-frequency repetitive transcranial magnetic stimulation and constraint-induced movement therapy in pediatric hemiparesis: A randomized controlled trial. *Dev Med Child Neurol* **56**, 44–52 (2014).

43. Tallabs, F. A. & Hammond-Tooke, G. D. Theta priming of 1-Hz rTMS in healthy volunteers: Effects on motor inhibition. *Journal of Clinical Neurophysiology* **30**, 79–85 (2013).

44. Kakuda, W. *et al.* Application of combined 6-Hz primed low-frequency rTMS and intensive occupational therapy for upper limb hemiparesis after stroke. *NeuroRehabilitation* **29**, 365–371 (2011).

45. Carey, J. R., Anderson, D. C., Gillick, B. T., Whitford, M. & Pascual-Leone, A. 6-Hz primed low-frequency rTMS to contralesional M1 in two cases with middle cerebral artery stroke. *Neurosci Lett* **469**, 338–342 (2010).

46. Todd, G., Flavel, S. C. & Ridding, M. C. Priming theta-burst repetitive transcranial magnetic stimulation with low-and high-frequency stimulation. *Exp Brain Res* **195**, 307–315 (2009).

47. Gillick, B. T. *et al.* Primed low-frequency repetitive transcranial magnetic stimulation and constraint-induced movement therapy in pediatric hemiparesis: A randomized controlled trial. *Dev Med Child Neurol* **56**, 44–52 (2014).

48. Jung, S. H., Shin, J. E., Jeong, Y. S. & Shin, H. I. Changes in motor cortical excitability induced by high-frequency repetitive transcranial magnetic stimulation of different stimulation durations. *Clinical Neurophysiology* **119**, 71–79 (2008).

49. Palisano, R. *et al.* Gross motor function classification system for cerebral palsy. *Dev Med Child Neurol* **39**, 214–223 (1997).

50. Russell, D. J., Rosenbaum, P., Wright, M. & Avery, L. M. *Gross Motor Function Measure (GMFM-66 & GMFM-88) Users Manual*. (Mac keith press, 2002).

51. Harb, A. & Kishner, S. Modified ashworth scale. in *StatPearls [Internet]* (StatPearls Publishing, 2023).

52. Krumlinde-Sundholm, L. & Eliasson, A.-C. Development of the Assisting Hand Assessment: a Rasch-built measure intended for children with unilateral upper limb impairments. *Scand J Occup Ther* **10**, 16–26 (2003).

53. Manual Ability Classification System (MACS) http://www.macs.nu/ .

54. Assessment of Motor and Process Skills (AMPS): Access can be found at http://www.innovativeotsolutions.com/content/amps/ .

55. Melbourne Assessment of Unilateral Upper Limb Function (MUUL): Access can be found at http://www.rch.org.au/melbourneassessment/ .

56. Jebsen-Taylor Hand Function Test (JHFT): http://www.strokengine.ca/assess/jhft/ .

57. Canadian Occupational Performance Measure (COPM) http://www.thecopm.ca/ .

58. Zewdie, E. *et al.* Safety and tolerability of transcranial magnetic and direct current stimulation in children: prospective single center evidence from 3.5 million stimulations. *Brain Stimul* **13**, 565–575 (2020).

59. Lerner, A. J., Wassermann, E. M. & Tamir, D. I. Seizures from transcranial magnetic stimulation 2012–2016: results of a survey of active laboratories and clinics. *Clinical Neurophysiology* **130**, 1409–1416 (2019).

60. Zewdie, E. *et al.* Safety and tolerability of transcranial magnetic and direct current stimulation in children: prospective single center evidence from 3.5 million stimulations. *Brain Stimul* **13**, 565–575 (2020).

61. Gilbert, D. L. *et al.* Should transcranial magnetic stimulation research in children be considered minimal risk? *Clinical neurophysiology* **115**, 1730–1739 (2004).

62. Croarkin, P. E., Wall, C. A. & Lee, J. Applications of transcranial magnetic stimulation (TMS) in child and adolescent psychiatry. *International Review of Psychiatry* **23**, 445–453 (2011).

63. Tadel, F., Baillet, S., Mosher, J. C., Pantazis, D. & Leahy, R. M. Brainstorm: A user-friendly application for MEG/EEG analysis. *Comput Intell Neurosci* **2011**, 879716 (2011).

64. Iversen, J. R. & Makeig, S. MEG/EEG data analysis using EEGLAB. *Magnetoencephalography: From signals to dynamic cortical networks* 391–406 (2019).

65. Rogasch, N. C. *et al.* Analysing concurrent transcranial magnetic stimulation and electroencephalographic data: A review and introduction to the open-source TESA software. *Neuroimage* **147**, 934–951 (2017).

66. Mutanen, T. P., Biabani, M., Sarvas, J., Ilmoniemi, R. J. & Rogasch, N. C. Source-based artifact-rejection techniques available in TESA, an open-source TMS–EEG toolbox. *Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation* **13**, 1349–1351 (2020).