9-28-2023

Remote Ischaemic Conditioning Combined With Bimanual Task Training to Enhance Bimanual Skill Learning and Corticospinal Excitability in Children With Unilateral Cerebral Palsy: A Study Protocol of a Single Centre, Phase Ii Randomised Controlled Trial

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BMJ Open

Remote ischaemic conditioning combined with bimanual task training to enhance bimanual skill learning and corticospinal excitability in children with unilateral cerebral palsy: a study protocol of a single centre, phase II randomised controlled trial

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To cite: Surkar SM, Willson JD, Cassidy JM, *et al*. Remote ischaemic conditioning combined with bimanual task training to enhance bimanual skill learning and corticospinal excitability in children with unilateral cerebral palsy: a study protocol of a single centre, phase II randomised controlled trial. *BMJ Open* 2023;**13**:e076881. doi:10.1136/bmjopen-2023-076881

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2023-076881).

Received 19 June 2023
Accepted 22 July 2023

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ABSTRACT

Introduction Children with unilateral cerebral palsy (UCP) have difficulty in bimanual coordination that restricts the child's independence in daily activities. Although several efficacious interventions to improve bimanual coordination exist, these interventions often require higher training doses and have modest effect sizes. Thus, there is a critical need to find an effective priming agent that, when paired with task-specific training, will facilitate neurobiological processes to enhance the magnitude of training effects and subsequently improve functional capabilities of children with UCP. The aim of this study is to determine the effects of a novel priming agent, remote ischaemic conditioning (RIC), combined with bimanual training on bimanual skill learning and corticospinal excitability in children with UCP.

Methods and analyses 46 children, aged 8–16 years, will be randomly assigned to receive RIC or sham conditioning combined with 5 days of bimanual skill (cup stacking) training (15 trials per session). RIC or sham conditioning will be performed with a standard conditioning protocol of five cycles of alternative inflation and deflation of a pressure cuff on the affected arm with the pressure of at least 20 mm Hg above systolic blood pressure for RIC and 25 mm Hg for sham conditioning. Primary outcomes will be movement time and corticospinal excitability measures determined with a single-pulse transcranial magnetic stimulation (TMS). Secondary outcomes include Assisting Hand Assessment, spatio-temporal kinematic variables and paired pulse TMS measures. All measures will be conducted before and immediately after the intervention. A mixed model analysis of variance will test the group×time interaction for all outcomes with group (RIC and sham) as between-subject and time (preintervention, postintervention) as within-subject factors.

Ethics and dissemination The study has been approved by the University Medical Centre Institutional Review Board (UMCIRB #21-001913). We will disseminate the study findings via peer-reviewed publications and presentations at professional conferences.

Trial registration number NCT05777070

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study is a unique clinical translation of a novel priming agent, remote ischaemic conditioning (RIC), for the first time in children with unilateral cerebral palsy to enhance a crucial tenet of rehabilitation, that is, motor learning.
⇒ The study capitalises on the neuroprotective mechanisms of RIC to enhance brain plasticity and will directly assess neuroplasticity related changes in response to RIC by examining corticospinal excitability using a reliable neurophysiological measure, transcranial magnetic stimulation.
⇒ The application of RIC is extremely easy and convenient due to small technological requirements (requires blood pressure cuff, which is easily available in any setting), low cost, minimal technical expertise and feasibility for application of intervention at research, clinic or home setting.
⇒ The study is not sufficiently powered to assess the responders and non-responders to RIC.

INTRODUCTION

Background

Unilateral cerebral palsy (UCP) is one of the leading causes of childhood disability with the prevalence of approximately 1 per 1000 live births in the USA.[1] An early brain injury damages the corticospinal tract (CST) and results in heterogenous sensorimotor deficits in the contralesional side of the body.[2] These deficits impair hand function, disturb bimanual coordination,[3–6] and result

in a profound burden of lifelong physical, social, and emotional disability in children with UCP.

Rehabilitation is paramount to improve functional use of the affected hand for performing bimanual coordination activities and for augmenting therapy (skill) dependent plasticity in children with UCP. One of the crucial tenets of rehabilitation is motor learning.[7] Specifically, structured skill practice is key for optimising motor learning and for driving cortical plasticity.[8] However, motor learning and skill dependent plasticity are both affected in children with UCP.[9–13] Specifically, children with UCP have a slower rate of learning, make more errors, require more practice to learn a motor sequence task, and the learning outcomes are dependent on CST reorganisation.[10 11] A plethora of brain mapping studies using transcranial magnetic stimulation (TMS) have shown that children with UCP have maladaptive cortical organisation,[4 14–18] which is associated with severe impairment in hand motor function and is a potential barrier in learning motor skills.[14–16 19]

In the past two decades, research has explored the efficacy of various task-specific intensive training approaches for improving the paretic hand function, bimanual coordination and cortical reorganisation in children with UCP.[20–24] The two most extensively investigated contemporary approaches include modified Constraint Induced Movement Therapy (mCIMT)[25 26] and Hand Arm Bimanual Intensive Therapy (HABIT).[27–29] Although both of these interventions have shown to be efficacious in improving unimanual and bimanual hand function, respectively, they require higher training doses (60–90 hours training within 2–3 weeks), have modest effect sizes, poor retention of training effects, and are highly variable in treatment efficacy across individuals.[26 30 31] Thus, clinical utility of these interventions is limited. More recently, non-invasive brain stimulations such as repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) combined with intensive behavioural interventions such as mCIMT and HABIT are emerging.[32–36] rTMS and tDCS are neuromodulatory interventions that are used to influence cortical excitability.[37] The results of these combination therapies reveal positive trends in improving hand function and cortical reorganisation[38 39] but overall effect sizes are modest.[21 22] Furthermore, clinical utility of these interventions is also significantly limited since they require technical expertise and expensive equipment. Taken together these studies suggest that the critical barriers in the rehabilitation of children with UCP are impaired motor skill learning and maladaptive plasticity that lead to higher training doses and poor retention of existing interventions. Therefore, there is an urgent need for a feasible, safe and cost-effective priming agent that when paired with training, will facilitate neurobiological processes to enhance motor performance (bimanual skill learning and coordination), neuroplasticity and functional outcomes in children with UCP.

Ischaemic conditioning is an endogenous phenomenon in which an organ exposed to a controlled, short-term, local, sublethal ischaemia is protected from subsequent ischaemic injury. Remote ischaemic conditioning (RIC) is a clinically feasible way of performing ischaemic conditioning where episodes of alternating, brief ischaemia and reperfusion are delivered with cyclic inflation and deflation of a blood pressure (BP) cuff on the arm or leg.[40] Ischaemic conditioning was first found to protect the heart from acute and lethal ischaemia, and these cardioprotective effects are now well established.[41–44] Of great importance to neurorehabilitation, preliminary studies have demonstrated that the effects of RIC extend into neuroprotection including reduced recurrence and severity of stroke.[45–50] Furthermore, our prior work suggests that when paired with task-specific training, RIC enhances motor learning in healthy young and older adults.[49 50] Although the exact mechanisms of RIC that promote motor learning are unknown, we speculate that the neuroprotective mechanisms of RIC overlaps with the mechanisms of neuroplasticity and motor learning. For example, substantial evidence from preclinical studies indicate that RIC promotes angiogenesis and neurogenesis[51]; modulates synthesis of glutamate,[52] N-methyl-D-aspartate,[53] gamma aminobutyric acid (GABA)[54]; and alters brain-derived neurotrophic factor expression.[55] While the neuroprotective role of RIC and mechanisms for neuroprotection are well appreciated, there is a substantial knowledge gap in understanding whether RIC can capitalise on these neuroprotective mechanisms to facilitate motor skill learning in children with UCP. Most importantly, RIC is safe in humans.[56 57]

Objectives

The overall objectives of this phase II randomised controlled trial (RCT) are to determine the effects of RIC plus bimanual task training on immediate bimanual skill learning, bimanual coordination and corticospinal excitability in children with UCP. Our central hypothesis is that the multifactorial mechanisms of RIC can be harnessed as a priming agent to enhance crucial tenets of rehabilitation, such as motor learning, and to augment neuroplasticity in children with UCP. We hypothesise that as compared with sham conditioning+bimanual training, RIC+-bimanual training will significantly enhance: (1) bimanual learning (decrease in movement time of bimanual speed stacking task), (2) bimanual coordination (improvement in spatio-temporal kinematic variables) of bimanual symmetric and asymmetric tasks, (3) bimanual function (increase in the Assisting Hand Assessment (AHA) scores), (4) increase cortical excitability from ipsilesional M1 (lower resting motor thresholds (rMTs) and larger amplitude of motor evoked potentials (MEPs)) and (5) reduce cortical inhibition in ipsilesional M1 (reduced short-interval intracortical inhibition (SICI) and increase in intracortical facilitation (ICF)).

METHODS AND ANALYSIS

Patient and public involvement

Pilot data (NCT05355883) were collected on 12 children with UCP, RIC (n=7; age=12.9±2.2 years) or sham conditioning (n=5; age=10.2±3.5 years). Participating children and parents provided daily feedback on the feasibility, tolerability, acceptability and perceived safety of RIC. We consulted a family and their teenager with UCP who has been participating in different intervention programmes since the age of 2 years about the trial design and intervention protocol. The results of the study will be disseminated to study participants at the end of the study by sharing the summary of study findings in layman's language.

Trial design

Overall study design

The study design is a triple blind, parallel group, RCT with superiority framework, and with preintervention and postintervention assessments. The interventionist and assessor to participant ratio will be 1:1. Participants will be stratified based on Manual Ability Classification System levels and sex.

Participants

Study setting

All the training and testing will be performed in a research laboratory setting (Pediatric Assessment and Rehabilitation Lab (PeARL) and Human Movement Analysis Lab) at East Carolina University (ECU). The study started in September 2022 and anticipated to be completed in July 2024.

Sample size

Our sample size estimates are based on hypothesised effects (effect size=0.90) for the kinematic variable *goal synchronisation time* based on our preliminary data. This is the smallest effect size seen across all the primary outcome variables in our preliminary study. Sample size has been computed using G*Power and based on a two-sided t-test. Our preliminary study compared the effects of 75 speed stack trials (15 practice trials/session for 5 days) on bimanual performance (bimanual learning and coordination) between RIC and sham groups. Goal synchronisation time, which is defined as a lag between the initiation of the affected and less affected hand was the primary kinematic analysis outcome measure. Smaller time lag indicates greater bimanual coordination. Children with UCP in RIC group decreased their goal synchronisation time from pretraining to post-training by 256±25 ms and sham group decreased their goal synchronisation time by 282±33 ms. To detect a similar between group difference in the change score of goal synchronisation time, a total of 40 participants (20 in each group) will provide 80% power to detect an effect size of 0.90 (α=0.05). After accounting for a 15% drop out rate, we will need a total of 46 participants (23 in each group) for this investigation.

Participants

We intend to enrol an equal number of males and females, which will allow us to probe for sex differences in our final analyses and consider sex as a variable of interest in all analyses. We will enrol 46 children with UCP, including both sexes and from diverse ethnic and racial backgrounds, between ages 8 and 16 years based on the inclusion and exclusion criteria in table 1. Children will be recruited from a database of over 500 children with a diagnosis *exclusively* of UCP from the Physical Therapy clinic and mCIMT camp database of ECU, Brody School of Medicine, ECU Health Hospital, local therapy clinics, the University of North Carolina Hospital's Helping Kids with Hemiplegia programme and Pitt County Public School Districts.

Randomisation procedure

The study statistician will generate a randomisation schema in SAS V.9.4 using permuted blocks, which will be loaded into the REDCap (Vanderbilt University, Nashville, TN, USA)[58] project randomisation module to ensure allocation concealment. Participants will be randomly allocated to RIC or sham groups after baseline testing. Interventionist who will supervise bimanual training, assessors who will analyse the kinematic, TMS and behavioural assessments, and participants and their families will be blinded to the participant's group assignment. One study personnel (principal investigator (PI)) will not be blinded to group allocation since the PI will perform conditioning and will monitor safety, fidelity and overall adherence to the study protocol.

Interventions

Figure 1 shows general study protocol.

RIC versus sham conditioning

Before beginning RIC/sham conditioning, we will record resting heart rate, BP and oxygen saturation. Conditioning will be performed by cyclic inflation and deflation of a pressure cuff on the more affected arm using a Hokanson rapid cuff inflator (Hokanson). Conditioning for the RIC and sham conditioning groups will be delivered as five cycles of 5 min pressure cuff inflation followed by alternating 5 min of deflation resulting in a total conditioning time of 45 min.[47 49 50 59] Consistent with our previously published studies, the conditioning pressure in the RIC group will be >20 mm Hg above that visit's resting systolic BP since this pressure has shown as sufficient to induce ischaemia and as effective as the standard 200 mm Hg, with fewer side effects.[50 60 61] The conditioning pressure in the sham group will be 25 mm Hg since it gives the sensation of cuff inflation, but does not induce arterial occlusion.[62–64] All the participants will receive conditioning for the first seven visits. The first two visits will include conditioning only to prime the brain with RIC and to establish optimum environment to support motor learning. For the visits 3–7, conditioning will be paired with bimanual skill training.

Table 1 Enrolment criteria of the study participants

Enrolment criteria		
Inclusion criteria	**Rationale**	**Method of ascertainment**
Children diagnosed with unilateral cerebral palsy (UCP)	Target population for clinical translation of remote ischaemic conditioning (RIC) intervention in paediatric neurological population. Homogeneous population in terms of unilateral weakness for assessing bimanual skill training	Database of over 500 children with a diagnosis *exclusively* of UCP from the physical therapy clinic and mCIMT camp database of ECU, Brody School of Medicine, ECU Health Hospital, local therapy clinics, the University of North Carolina Hospital's Helping Kids with Hemiplegia programme and Pitt County Public School Districts
Age: 8–16 years	Maturation of hand function occurs until 7 years of age. Since study testing and training involves various hand functions such as grasping, releasing and manipulating of cups, inclusion of children above 7 years of age will ensure that impaired task performance is not due to the immature hand function. The development of the corticospinal tract continues until 16 years of age. Since the corticospinal tract is the primary pathway to control skilled voluntary hand movements, delivering the intervention in this developmental window may enhance the potential for improving hand function	Demographic details
Manual Ability Classification System (MACS) levels I–III	Children with MACS levels IV and V will not be able to perform the timed bimanual task independently	Enrolment of the study participants based on MACS assessment
Ability to complete a stack of three cups in 1 min	Bimanual training will not be too challenging for the child	Enrolment of the study participants based on cup stacking performance criteria
Mainstream in school	Sufficient cognition to follow the experiment instructions	Enrolment of the study participants based on Kaufman Brief Intelligence Test, score ±1 SD from normal
Exclusion criteria	**Rationale**	**Method of ascertainment**
Children with other developmental disabilities such as autism, attention deficit hyperactivity disorder, developmental coordination disorders	Other developmental disabilities may impact motor (bimanual) skill learning and affect the trial results	Medical records
Children with absent active motor threshold	Absence of active motor threshold indicates suppressed corticospinal excitability	EMG responses with TMS
Children with known cardiorespiratory, vascular and metabolic disorders	To ensure the safety of RIC application	Medical records and interview with participants and parents
Children with neoplasm and hydrocephalus	To ensure the safety of transcranial magnetic stimulation (TMS) application	Medical records and interview with participants and parents
Children who are currently receiving or received other adjunct therapies such as rTMS and tDCS in the past 6 months	Other adjunct therapies may interfere with the RIC effects	Medical records and interview with participants and parents
Children with active seizures or seizures within last 2 years and currently on antiseizure medications	Since seizures may be provoked by TMS application, active seizures or a history of seizures within last 2 years are a safety risk for TMS application. Antiseizure medications interfere with motor learning	Medical records and interview with participants and parents
Children with metal implants and incompatible medical devices	May interference with MRI scans posing risk of serious injury	Medical records and interview with participants and parents

ECU, East Carolina University; EMG, electromyography; mCIMT, modified Constraint Induced Movement Therapy; RIC, remote ischaemic conditioning; rTMS, repetitive transcranial magnetic stimulation; tDCS, transcranial direct current stimulation; UCP, unilateral cerebral palsy.

The experimenter will continuously monitor the presence or absence of ischaemia in the RIC and sham conditioning groups, respectively, by monitoring a pulse oximeter placed on the index finger of the conditioning arm and by visually inspecting the colour of the conditioning arm. A reading of '0' for pulse and oxygen saturation on the pulse oximeter and the presence of pale dusky appearance of the conditioning arm will confirm the presence of ischaemia in the RIC group. Oxygen saturation and pulse equivalent to baseline or prior to

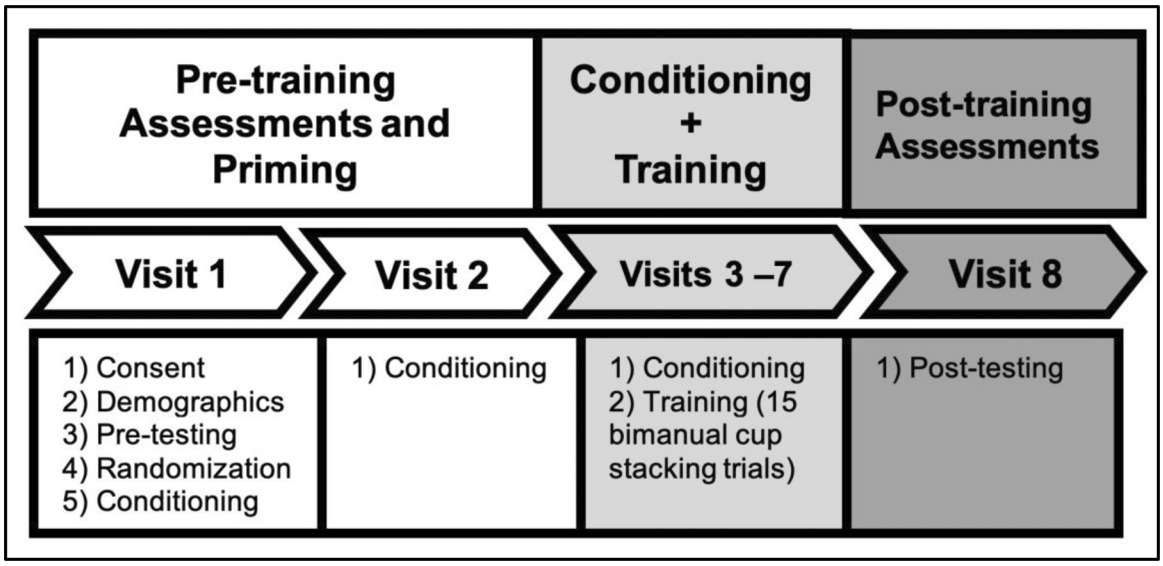

Figure 1 Schematics of the study protocol. Pretesting and post-testing involves movement time, spatio-temporal kinematics of bimanual task, assessment of hand function using the Assisting Hand Assessment, and measures of corticospinal excitability using transcranial magnetic stimulation.

initiation of conditioning and unchanged colour of the conditioning limb will confirm the absence of ischaemia in the sham conditioning group. In the RIC group, if the pulse or oxygen saturation reading appeared on the pulse oximeter anytime during the inflation cycle, the interventionist will increase the inflation pressure until confirmation of ischaemia on the conditioning arm and the total time will be adjusted to be consistent with 5 min of inflation cycle. Similarly, in the sham conditioning group, if oxygen saturation and pulse dropped below baseline measures, the conditioning pressure will be decreased until preconditioning pulse and oxygen saturation will be achieved, and the arm shows no visible evidence of ischaemia.

Conditioning safety will be monitored through measurement of BP, heart rate, oxygen saturation on the non-conditioning arm before, during and after each session. Pain due to conditioning will also be monitored. For both groups, conditioning will be terminated if: (1) systolic BP is <85 or >140 mm Hg; (2) diastolic BP is <50 or >90 mm Hg; (3) heart rate is <60 or >115; (4) oxygen saturation is <90%; and (5) pain is >6 on 10-point Likert pain scale.

At the end of the study, participants will be asked to indicate whether they believed they received the RIC or sham conditioning.

Training: bimanual task training

Rationale for bimanual task training using a speed stack

Bimanual training is selected based on evidence-based recommendations to improve bimanual function and successful demonstration of learning effects using the same task in prior studies.[9 13 65–68] A bimanual, high-speed cup stacking task based on the instructions developed by the World Sport Stacking Association will be used. This task has been previously used to assess motor learning and bimanual coordination in children with UCP, typically developing children, young and older adults, and in individuals with stroke.[9 65–67] The task demands grasping, moving and releasing objects that mimic upper limb skills that are trained in rehabilitation setting.

We will use 12 specialised cups specifically developed for speed stacking (Speed Stacks, Castle Rock, CO, USA). A non-slip mat (Speed Stacks) will be placed in front of the participant and a competition timer will be secured to latches on the front side of the mat. The timer features touch pads for beginning and stopping timing and a resolution of 0.001 s to ensure precise measurement. Before each trial, cups will be stacked together, upside down, inside each other on the mat with a three-cup stack on the right. The task consisted of three sequences of cup stacking and unstacking (figure 2). Once the last sequence will be stacked with no mistakes, the participant will unstack all the cups such that the cups will be once again in stacks of 3, 6 and 3.

Prior to beginning, participants will be instructed on how to perform the task with the instructions: (1) 'perform the task as fast as you can'; (2) 'always work from the left to the right, stacking first and then unstacking'; (3) 'use both hands'; (4) 'if error occurs, fix it and then continue until the cups are placed in the correct pattern'; (5) 'the cups must start and end in the same three stacks'. To ensure the affected hand's participation in cup stacking task, children will be instructed to use both hands so that hands touch the cups throughout the task. The experimenter will demonstrate the task and children will perform three practice trials before beginning the training. During practice trials, children will be encouraged to explore strategies to optimise performance and learning.

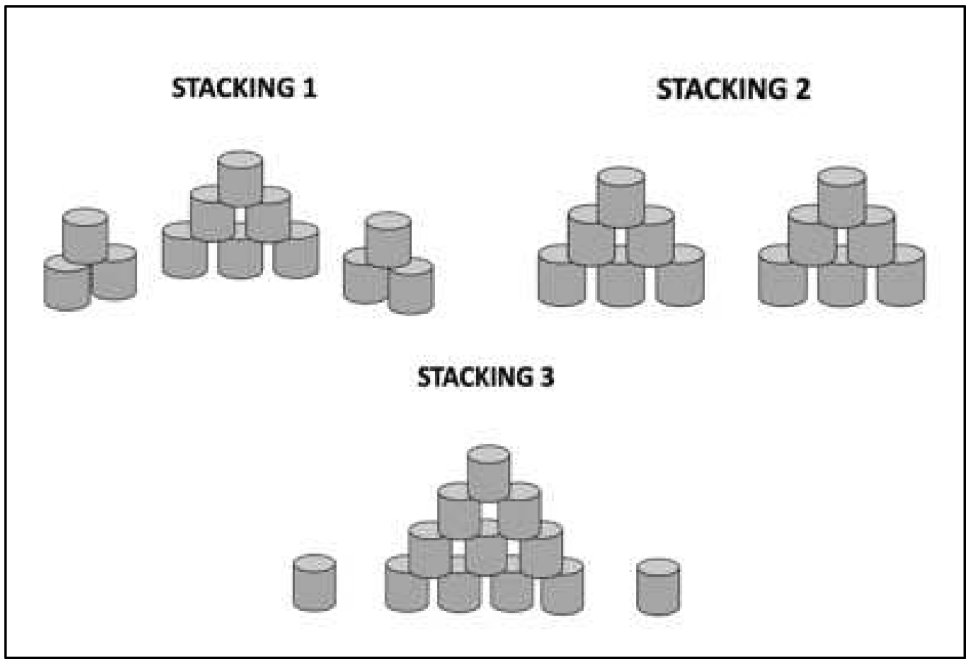

Figure 2 Stacking patterns of bimanual speed stack task. Children will practice 15 trials/day of these three stacking patterns for five consecutive days.

Dose of bimanual training

The dose of bimanual training in this study is determined based on the training dose of the earlier bimanual learning study and our previous RIC plus motor training studies.[9 49 50] Therefore, the bimanual training dose will be 15 trials/day (*Frequency*), 10–35 min/day (*Intensity*), for five consecutive days (*Time*). Based on motor learning literature, this dose is adequate to assess motor skill learning.[69–72] Furthermore, we will incorporate the principles of structured skill training by progressively increasing the spatial difficulty of the task, imposing skilled use, introducing variability of practice sequence to retain the child's engagement, and problem solving.[8 73] The experimenter will demonstrate the task and children will perform three practice trials before beginning the training.

On visit 1, for testing purposes, children will perform nine stacking sequences (trials) shown in figure 2 (not including the practice trial). The time to complete each trial, or movement time, will be recorded and nine trials will be averaged together for the baseline performance measurement. During five total training visits (visit 3–7), children will complete 15 trials/day. Therefore, there will be a total of 75 practice trials across five consecutive weekdays. Fifteen practice trials will be performed in three blocks of five trials each. Two to five minutes rest breaks will be provided after each block. In each block, the sequence of practice patterns will be in a random order and the experimenter will inform the child about performance or movement time after each trial. Children will be encouraged to be competitive and achieve better score in the next trial. No verbal information will be provided any time regarding specific strategy of stacking and unstacking cups outside of the demonstration and instructions. The experimenter will only provide cuing or correction in stacking patterns when the participant will be stuck or unaware of their mistake. A laminated card for each stacking sequence will be placed on the table for visual cuing each day. Children will be allowed to view the cards during stacking trials.

Children will first undergo RIC for 45 min followed by bimanual training during all five sessions. For the participants adherence to the intervention protocol, knowledge of their performance will be provided after each session. Participants will be monetarily compensated in the middle (visit 4) and at the end of the study (visit 8). Monetary compensation is for the participants time and will not be dependent on their performance for that visit.

Kinematics of bimanual coordination

Task and experimental setup

Separate speed stack tasks will be used to assess *symmetric performance* (figure 3A) *and bimanual coordination* (figure 3B) of the affected and less affected arm.[6 74–76] We will obtain 3D kinematic data using a 15-camera motion capture system (Qualisys AB, Gothenburg, Sweden) with reflective markers placed on the midpoint of participant's wrists. Data will be collected at 100 Hz and low pass filtered at 6 Hz. All sessions will also be video recorded.

Experimental procedure

Participants will be seated in an adjustable chair 15 cm in front of a table with elbows flexed to 90^0 and hands 30 cm apart at the edge of the table. During the test for *symmetric performance*, participants will be asked to use both hands concurrently to place two cups inside separate target areas 30 cm anterior to the starting position (figure 3A). During the test for *bimanual coordination*, participants will be asked to use both hands cooperatively to remove

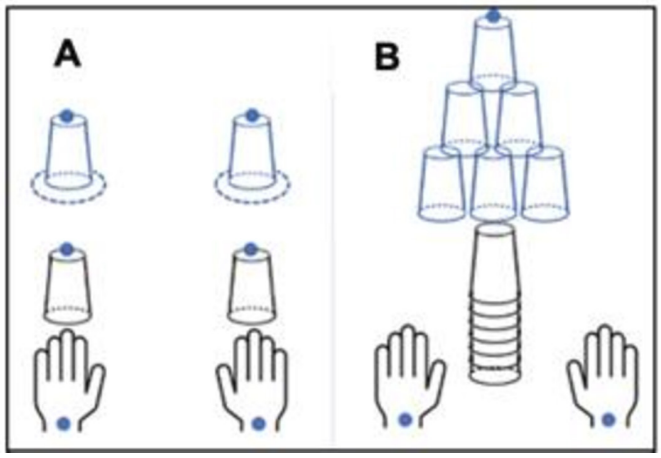

Figure 3 Kinematic task. (A) Symmetric performance task: participant will use both hands concurrently to move the cups at target position. (B) Bimanual coordination task: participants will use both hands in coordination with each other to remove the cups from the stack and create a pyramid of six cups.

cups from a stack and construct a pyramid of 6 cups (figure 3B). Participants will be encouraged to perform both tasks quickly and accurately using both hands on a 'GO' signal indicated by an LED light. One practice trial will be provided prior to recording five trials for each test.

Corticospinal excitability

Transcranial magnetic stimulation

Rationale for the use of TMS to assess ipsilesional motor cortex (M1) plasticity

Bimanual skill learning drives M1 plasticity.[8] TMS is a valid, non-invasive technique for assessing M1 plasticity.[77–80] The safety and tolerability of TMS in children is well-established.[81–83] Single-pulse measures, such as increase in MEP amplitude and area under the curve of resultant MEPs in a stimulus–response curve, are indicative of increase in M1 excitability.[84–87] Moreover, complex intracortical inhibitory and facilitatory networks underlie skill-dependent plasticity and motor function, which can be assessed with paired-pulse SICI and ICF paradigms.[88 89] RIC modulates GABA[54] and glutamate synthesis[52] and activates neural pathways,[90] which potentially enhances neuroplasticity. SICI reflects GABAergic and ICF reflects glutamate-mediated mechanisms of plasticity.[91 92] Hence, TMS outcomes will be surrogate measures to probe the underlying RIC driven neurophysiological mechanisms of plasticity. Reduction in SICI and an increase in ICF is associated with skill-dependent plasticity in children with CP.[93] Furthermore, a reduction in SICI is associated with enhanced motor learning.[94]

Transcranial magnetic stimulation

We will use two Magstim 200^2 magnetic stimulator with a 70 mm figure of eight coil and a peak magnetic field of 2.2 T (Magstim Company Limited, Dyfed, UK).

MRI data acquisition

Structural T1-weighted images will be acquired on a 1.5 T scanner (Philips Ingenia, USA).

Neuronavigation system

Prior to the TMS, we will identify the M1-Hand area using the child's structural brain images and a stereotactic neuronavigation system (Brainsight, Rogue Research, Montreal, QC, Canada). Using common anatomical landmarks, the child's head and the individual MR scan will be co-registered in a common reference frame. Following the co-registration, the M1-Hand will be localised by anatomical landmarks on the MR scan. After this, the TMS coil will be calibrated, which will allow a real-time display of the relative positions of the coil and the child's head and brain surface. The use of the neuronavigation system will ensure consistent coil placement over the course of TMS session.

Electromyography

For electromyography (EMG) recordings, we will use surface electrodes in a belly-tendon arrangement. We will record the surface EMG activity from bilateral first dorsal interossei (FDI) muscle with the electrode placed on the belly of muscle and the metacarpo-phalalngeal joint of the index finger. EMG signals will undergo bandpass (20 Hz to 2.0 kHz) and notch (60 Hz) filtering and digitisation at 5 kHz (Delsys Inc, USA). We will obtain and analyse TMS data from ipsilesional, as well as contralesional, M1. Since the role of ipsilesional M1 is more defined in training related neuroplasticity, we hypothesise that ipsilesional M1 will exhibit greater cortical excitability after training.[22 95–97]

Determination of hotspot and rMTs

Participants will sit comfortably in a reclining chair while our research team locates the motor hotspot—the location on the scalp that corresponds to motor cortex where TMS evokes an MEP of greatest amplitude with the lowest stimulation intensity. We will record the MEPs from the FDI. With the neuronavigation guide, we will position the TMS coil handle 45^0 posterolaterally on the motor hotspot region. We define rMT as the lowest stimulus intensity (percentage of machine output) required to produce a 50 or >50 µV MEP in at least 5 of 10 trials.[98] If rMT cannot be determined, we will acquire an active motor threshold (aMT). We will determine the participant's maximal voluntary isometric contraction (MVIC) from the FDI and then instruct them to contract at 30% of their max during single TMS pulse delivery. The participant will receive visual and auditory feedback from the oscilloscope while attempting 30% MVIC. We will define the aMT as the stimulation intensity necessary to produce an MEP amplitude of 200 µV MEP in 5/10 trials. We will repeat these procedures for the contralesional hemisphere.

Outcomes

All the outcomes will be measured at preintervention and immediately postintervention.

Measure of bimanual learning

Movement time

The time (seconds) to complete each trial of speed stack, which will be averaged across nine trials. A blinded assessor will perform the assessment and record the scores. The assessment will be video recorded. Increase in speed indicates motor learning; therefore, reduction in movement time will indicate bimanual skill learning.

Measure of hand function and bimanual coordination

Assisting Hand Assessment

AHA 5.0 is a reliable and valid functional outcome to assess the affected hand function and bimanual coordination, specifically in children with UCP.[99 100] It has 20 items in six different categories of the arm use and with a rating scale of 1–4 points: 1=does not use the arm, 4=effective use of the arm. A physical therapist will perform, and the certified AHA rater who will be blinded to the group and pretraining and post-training assessment time points will score the AHA. The raw scores will be converted into logit units (0–100) for the final analysis. A 5-point change in the AHA scale is considered clinically meaningful.[101]

Kinematic measures of bimanual coordination

The experimental tasks for kinematic analysis consist of the symmetric task and bimanual coordination task (Figure 3). Following spatio-temporal variables will be measured.

Symmetric performance and tangential velocities

Symmetric performance will be characterised as a time-lag between the affected and less affected arm during movement onset and task completion. Movement onset time is defined as a wrist marker reaching a tangential velocity greater than 2.0 cm/s for at least 100 ms. Amplitude of peak reaching tangential velocities for each arm will be calculated. Task completion is defined as the time when the tangential velocity of a reflective marker on each cup remains below 2.0 cm/s within the target area for at least 100 ms.

Hand trajectory

Hand trajectory is defined as the resultant 3D path length between the starting position and task completion.

Temporal coupling (normalised movement overlap time)

Normalised movement overlap time will be calculated as the percentage of total task completion time that both hands are participating in the stacking sequence during bimanual coordination task.[75]

Total participation time of each hand

Total participation time will be calculated as the total amount of time the affected and the less affected hand participate in bimanual coordination task. A hand will be considered as participating in the task any time the wrist marker tangential velocity remains over 2.0 cm/s for at least 100 ms.

Goal synchronisation

Goal synchronisation is defined as a time lag between the initiation of the affected compared with the unaffected arm.[75]

Total task duration

Total task duration will be defined as the duration from movement onset until the criteria for task completion is reached with both hands.

Kinematic analyses will be performed by the blinded assessor at preintervention and postintervention.

Expected outcomes

We predict that as compared with sham conditioning+training, RIC+training will significantly enhance: (1a) bimanual learning (decrease in movement time (seconds) to complete the bimanual speed stacking task), (1b) bimanual coordination (decrease in time-lag, increase in peak tangential velocity and decrease in hand path distance of the affected arm during the *symmetric task* and (1c) increased temporal coupling, total participation time of the affected arm and decrease in goal synchronisation time and total task duration during the *bimanual coordination task*) and (2) bimanual function (increase in the AHA scores).

TMS measures

The following TMS measures will be obtained from the ipsilesional as well as contralesional motor cortices. A blinded assessor will perform the TMS analyses.

Resting motor threshold

The rMT is the stimulator output required to produce an MEP of >50 µV in FDI muscle, which will be determined using maximum-likelihood parameter estimation by a sequential testing algorithm.[98] rMT is a measure of motor cortex excitability.

Active motor threshold

The aMT is the stimulator output required to produce an MEP of >200 µV in FDI muscle during 30% of MVIC of FDI muscle using a pinch grip.[102] aMT is a measure of motor cortex excitability.

Stimulus–response curve

To further assess M1 excitability, stimulus–response curves will be constructed for the ipsilesional as well as contralesional M1 at intensities of 90%, 100%, 110%, 120%, 130%, 140% and 150% of rMT (10 stimuli per intensity in random order). The peak-to-peak amplitude of MEPs and area under the curve of resultant MEPs to these intensities will be calculated.

MEP amplitude

The peak-to-peak amplitude of the EMG response from the affected as well as unaffected FDI muscle while stimulating the ipsilesional as well as contralesional motor cortex will be recorded at 100% rMT and averaged

across 10 single-pulse trials. MEP amplitude indicates the strength of motor response to TMS.

SICI and ICF

SICI and ICF will be obtained by applying a conditioning stimulus at 80% rMT intensity or AMT intensity followed by a test stimulus at 120% rMT intensity over the hot spot. The interstimulus interval between the conditioning and test stimulus will be 3 ms for obtaining measures of SICI and 15 ms for obtaining ICF.[103] We will collect 10 trials each of test stimulus alone, SICI and ICF in a random order[104] and ratios of conditioned (paired-pulse) to test (single-pulse) stimulus MEP amplitudes will be computed. A ratio <1 suggests intracortical inhibition and >1 suggests facilitation.

Expected outcomes

We predict that as compared with sham conditioning+training, RIC+training will significantly: (1) increase cortical excitability from ipsilesional M1 (larger amplitude of MEPs, greater area under the curve for stimulus–response curve and lower rMTs) and (2) reduce motor cortex inhibition (reduced SICI and increase in ICF in ipsilesional M1).

Data collection, management and analysis

Statistical analysis plans

For aim 1 (bimanual learning and bimanual coordination), we will use a linear mixed model to test the group×time interaction for all outcomes with group (RIC and sham) as between-subject and time (preintervention, postintervention) as within-subject factors accounting for correlation among the observations from the same subject using random subject effects. Additionally, arm (affected and less affected) will be the within-subject factor for symmetric movement performance of kinematic analyses. Contrasts will be used to test mean differences at times of interest. For aim 2 (corticospinal excitability), we will use a linear mixed model to test the group x time interaction for the primary and secondary outcomes with group (RIC and sham) as between-subject, and time (preintervention, postintervention) and hemisphere (ipsilesional and contralesional) as within-subject factors accounting for correlation among the observations from the same subject using random subject effects and accounting for random subject×hemisphere effect. Additionally, intensity will be a within-subject factor for stimulus–response curves.

For both aims, we will use Wald t-tests with Kenward Roger correction for small sample bias and Satterthwaite df. Contrasts will be used to test mean differences at times of interest. To address sex as a biological variable, we will conduct exploratory analyses stratified by biological sex.

Data management

The project PI will secure de-identified study data on a secured and password protected electronic REDCap database and this data will be backed up on a password protected lab drive managed by the ECU information technology and computing services. The pirate drive servers protect this information using advanced firewall technology. Only study investigators will know the password to files housing the participants' electronic data. All written documentation will be stored in a study binder housed in a locked cabinet within the PeARL, accessible only by the PI and coinvestigators. In this manner, only the primary investigator and coinvestigators will have access to individually identifiable private information about the participants, including signed consent forms. Data will be destroyed as appropriate 7 years after publication.

Data safety and monitoring

A Data Safety and Monitoring Committee (DSMC) has been established to address any issues that arise relating to personal health information and adverse events. The DSMC consists of two members who are independent of the research team and do not have any conflicts of interest. Both members are licensed physical therapists and investigators on the National Institutes of Health (NIH) funded grants. The DSMC will provide an independent voice for data management and safety issues. The DSMC will ensure integrity of systems for monitoring trial data on a regular basis and ensure participants' safety. DSMC members will meet once every 3 months and will systematically review the research protocol and plan for data safety and monitoring purposes. Additionally, they will review potential conflicts of interest, ethical issues, any scientific or therapeutic development that may impact the safety of the participants, and quality and reliability of the data. If an instance occurs requiring a report to officials, then this will be done immediately by DSMC members to the University and Medical Center Institutional Review Board (IRB) office. All serious adverse events will be reported to the IRB immediately using the following protocol set forth by the University and Medical Center IRB: (1) death—within 24 hours and (2) within 7 days for all other internal events. If any adverse events increasing the risk to participants occur, we will promptly stop the study and have an investigation conducted. Prior to resuming the study, a report regarding this incident will be produced. The NIH will be informed about the action taken by the University and Medical Center IRB for continuing review. The DSMC will maintain the confidentiality of trial data and results of monitoring. Interim analysis of the data will be performed every 6 months.

Resource sharing plan

We will follow the standard guidelines in documenting and depositing data sets that will be generated from the behavioural, kinematic, neurophysiological and neuroimaging data generated during the course of this investigation. All the data will be shared on the NIH Figshare and/or centralised resource such as NICHD's Data and Specimen Hub. All the data will be made available for secondary analyses. We will make de-identified raw, as well as processed, data resulting from the TMS assessments available for use to any external group. Similarly,

the anonymised MRI data will be made available to any external group on request. Furthermore, the PI will disseminate the findings of this research through national and international conferences, institution meetings, lab meetings and publications in relevant scientific journals.

The results of the study will be posted on the clinicaltrials.gov website in a timely manner. The PI, coinvestigators and their universities will adhere to the NIH Grants Policy on Sharing of Unique Research Resources, including the Sharing of Biomedical Research Resources: Guidelines for Recipients of NIH Grants and Contracts on Obtaining and Disseminating Biomedical Research Resources.

Ethics and dissemination

Research ethics and approval

The study has been approved by ECU's University and Medical Center IRB (#21-001913) and is registered with clinicaltrials.gov (NCT05777070). Any protocol modifications including changes to the eligibility criteria, outcomes or analyses will be reported to IRB, DSMC and clinical trials.

Informed consent

PI will contact and screen the potential candidates prior to study enrolment and obtain parental consent and child assent as set forth in HHS regulations at 45 CFR 46.408. The PI will thoroughly explain the study procedures in appropriate language and any questions from the parent and child will be answered. The consent process will include discussion of potential risks and benefits associated with participation, safeguards, and how the decision to participate or withdraw from the study at any time is entirely up to the participants.

Confidentiality

All the data will be de-identified. Only research team members will have access to the participants' demographic details. Consent forms will be kept in a locked cabinet in the PI's office. All the de-identified data will be saved on a secured, password-protected lab drive and only research team members will have access to it.

Declaration of interest

None of the investigators has any financial or other competing interests for overall trial.

Access to data

The research team members, IRB and DSMC personnel will have access to the participants data.

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Acknowledgements We thank the parents and a child adviser for their contributions to the trial design and study protocol, as well the children who participated in the pilot study.

Contributors SS is a principal investigator. She has substantially contributed to the conception and design, writing the protocol, critically revising, registering the trial, obtaining the institutional review board permission and final approval. JDW is a coinvestigator. He has substantially contributed to the design of the study specifically the kinematics of bimanual coordination section, drafting the protocol, critically revising and final approval. JMC is a coinvestigator. She has substantially contributed to the design of the study specifically the transcranial magnetic stimulation (TMS) section, drafting the protocol, critically revising and final approval. SK is a consultant. He has substantially contributed to the design of the study, drafting the protocol, critically revising and final approval. CP is a coinvestigator. She has substantially contributed to the design of the study specifically the statistical analysis of the study, drafting the protocol, critically revising and final approval. All authors are accountable for the integrity and accuracy of the work.

Funding Research reported in this publication was supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under Award Number R03HD107644 and APTA's Pediatric Physical Therapy (grant no. 21-0810).

Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained from parent(s)/guardian(s)

Provenance and peer review Not commissioned; peer reviewed for ethical and funding approval prior to submission.

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REFERENCES

1 Yeargin-Allsopp M, Van Naarden Braun K, Doernberg NS, *et al*. Prevalence of cerebral palsy in 8-year-old children in three areas of the United States in 2002: a multisite collaboration. *Pediatrics* 2008;121:547–54.
2 Lehman LL, Rivkin MJ. Perinatal arterial ischemic stroke: presentation, risk factors, evaluation, and outcome. *Pediatr Neurol* 2014;51:760–8.
3 Jaspers E, Byblow WD, Feys H, *et al*. The Corticospinal tract: a biomarker to categorize upper limb functional potential in unilateral cerebral palsy. *Front Pediatr* 2015;3:112.
4 Holmström L, Vollmer B, Tedroff K, *et al*. Hand function in relation to brain lesions and corticomotor-projection pattern in children with unilateral cerebral palsy. *Dev Med Child Neurol* 2010;52:145–52.
5 Steenbergen B, Hulstijn W, de Vries A, *et al*. Bimanual movement coordination in spastic hemiparesis. *Exp Brain Res* 1996;110:91–8.
6 Hung YC, Charles J, Gordon AM. Bimanual coordination during a goal-directed task in children with hemiplegic cerebral palsy. *Dev Med Child Neurol* 2004;46:746–53.
7 Krakauer JW, Mazzoni P. Human sensorimotor learning: adaptation, skill, and beyond. *Curr Opin Neurobiol* 2011;21:636–44.
8 Friel KM, Kuo H-C, Fuller J, *et al*. Skilled bimanual training drives motor cortex plasticity in children with unilateral cerebral palsy. *Neurorehabil Neural Repair* 2016;30:834–44.
9 Hung YC, Gordon AM. Motor learning of a bimanual task in children with unilateral cerebral palsy. *Research in Developmental Disabilities* 2013;34:1891–6.
10 Gagliardi C, Tavano A, Turconi AC, *et al*. Sequence learning in cerebral palsy. *Pediatr Neurol* 2011;44:207–13.
11 Carneiro MIS, Russo C, Masson R, *et al*. Motor learning in unilateral cerebral palsy and the influence of corticospinal tract reorganization. *Eur J Paediatr Neurol* 2020;27:49–59.

12 van der Kamp J, Steenbergen B, Masters RSW. Explicit and implicit motor learning in children with unilateral cerebral palsy. *Disability and Rehabilitation* 2018;40:2790–7.
13 Nemanich ST, Rich TL, Gordon AM, *et al*. Bimanual skill learning after transcranial direct current stimulation in children with unilateral cerebral palsy: a brief report. *Dev Neurorehabil* 2019;22:504–8.
14 Eyre JA, Smith M, Dabydeen L, *et al*. Is Hemiplegic cerebral palsy equivalent to amblyopia of the corticospinal system *Ann Neurol* 2007;62:493–503.
15 Nezu A, Kimura S, Takeshita S, *et al*. Functional recovery in hemiplegic cerebral palsy: ipsilateral electromyographic responses to focal transcranial magnetic stimulation. *Brain Dev* 1999;21:162–5.
16 Guzzetta A, Bonanni P, Biagi L, *et al*. Reorganisation of the somatosensory system after early brain damage. *Clin Neurophysiol* 2007;118:1110–21.
17 van der Aa NE, Verhage CH, Groenendaal F, *et al*. Neonatal neuroimaging predicts recruitment of contralesional corticospinal tracts following perinatal brain injury. *Dev Med Child Neurol* 2013;55:n
18 Zewdie E, Damji O, Ciechanski P, *et al*. Contralesional corticomotor neurophysiology in hemiparetic children with perinatal stroke. *Neurorehabil Neural Repair* 2017;31:261–71.
19 Staudt M, Grodd W, Gerloff C, *et al*. Two types of ipsilateral reorganization in congenital hemiparesis: a TMS and fMRI study. *Brain* 2002;125(Pt 10):2222–37.
20 Sakzewski L, Ziviani J, Boyd RN. Efficacy of upper limb therapies for unilateral cerebral palsy: a meta-analysis. *Pediatrics* 2014;133:e175–204.
21 Reid LB, Rose SE, Boyd RN. Rehabilitation and neuroplasticity in children with unilateral cerebral palsy. *Nat Rev Neurol* 2015;11:390–400.
22 Inguaggiato E, Sgandurra G, Perazza S, *et al*. Brain reorganization following intervention in children with congenital hemiplegia: a systematic review. *Neural Plast* 2013;2013:356275.
23 Dong VA-Q, Tung IH-H, Siu HW-Y, *et al*. Studies comparing the efficacy of constraint-induced movement therapy and bimanual training in children with unilateral cerebral palsy: a systematic review. *Dev Neurorehabil* 2013;16:133–43.
24 James S, Ziviani J, Ware RS, *et al*. Randomized controlled trial of web-based multimodal therapy for unilateral cerebral palsy to improve occupational performance. *Dev Med Child Neurol* 2015;57:530–8.
25 Charles JR, Wolf SL, Schneider JA, *et al*. Efficacy of a child-friendly form of constraint-induced movement therapy in hemiplegic cerebral palsy: a randomized control trial. *Dev Med Child Neurol* 2006;48:635.
26 Hoare BJ, Wallen MA, Thorley MN, *et al*. Constraint-induced movement therapy in children with unilateral cerebral palsy. *Cochrane Database Syst Rev* 2019;4:CD004149.
27 Alahmari K, Tedla JS, Sangadala DR, *et al*. Effectiveness of hand-arm bimanual intensive therapy on hand function among children with unilateral spastic cerebral palsy: a meta-analysis. *Eur Neurol* 2020;83:131–7.
28 Charles J, Gordon AM. Development of hand-arm bimanual intensive training (HABIT) for improving bimanual coordination in children with hemiplegic cerebral palsy. *Dev Med Child Neurol* 2006;48:931–6.
29 Surkar SM, Hoffman RM, Willett S, *et al*. Hand-arm bimanual intensive therapy improves Prefrontal cortex activation in children with Hemiplegic cerebral palsy. *Pediatr Phys Ther* 2018;30:93–100.
30 Eliasson AC, Krumlinde-Sundholm L, Gordon AM, *et al*. Guidelines for future research in constraint-induced movement therapy for children with unilateral cerebral palsy: an expert consensus. *Dev Med Child Neurol* 2014;56:125–37.
31 Sakzewski L, Ziviani J, Boyd RN. Best responders after intensive upper-limb training for children with unilateral cerebral palsy. *Archives of Physical Medicine and Rehabilitation* 2011;92:578–84.
32 Nemanich ST, Rich TL, Chen C-Y, *et al*. Influence of combined transcranial direct current stimulation and motor training on corticospinal excitability in children with unilateral cerebral palsy. *Front Hum Neurosci* 2019;13:137.
33 Gillick BT, Krach LE, Feyma 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* 2014;56:44–52.
34 Elbanna ST, Elshennawy S, Ayad MN. Noninvasive brain stimulation for rehabilitation of pediatric motor disorders following brain injury: systematic review of randomized controlled trials. *Arch Phys Med Rehabil* 2019;100:1945–63.
35 Kirton A, Chen R, Friefeld S, *et al*. Contralesional repetitive transcranial magnetic stimulation for chronic hemiparesis in subcortical paediatric stroke: a randomised trial. *The Lancet Neurology* 2008;7:507–13.
36 Ciechanski P, Kirton A. Transcranial direct-current stimulation can enhance motor learning in children. *Cereb Cortex* 2017;27:2758–67.
37 Gillick BT, Gordon AM, Feyma T, *et al*. Non-invasive brain stimulation in children with unilateral cerebral palsy: a protocol and risk mitigation guide. *Front Pediatr* 2018;6:56.
38 Hilderley AJ, Metzler MJ, Kirton A. Noninvasive neuromodulation to promote motor skill gains after perinatal stroke. *Stroke* 2019;50:233–9.
39 Kuo HC, Zewdie E, Ciechanski P, *et al*. Intervention-induced motor cortex plasticity in hemiparetic children with perinatal stroke. *Neurorehabil Neural Repair* 2018;32:941–52.
40 Kharbanda RK, Nielsen TT, Redington AN. Translation of remote ischaemic preconditioning into clinical practice. *Lancet* 2009;374:1557–65.
41 Bøtker HE, Kharbanda R, Schmidt MR, *et al*. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *The Lancet* 2010;375:727–34.
42 Brevoord D, Kranke P, Kuijpers M, *et al*. Remote ischemic conditioning to protect against ischemia-reperfusion injury: a systematic review and meta-analysis. *PLoS One* 2012;7:e42179.
43 Le Page S, Bejan-Angoulvant T, Angoulvant D, *et al*. Remote ischemic conditioning and cardioprotection: a systematic review and meta-analysis of randomized clinical trials. *Basic Res Cardiol* 2015;110:11.
44 Cheung MMH, Kharbanda RK, Konstantinov IE, *et al*. Randomized controlled trial of the effects of remote ischemic preconditioning on children undergoing cardiac surgery: first clinical application in humans. *J Am Coll Cardiol* 2006;47:2277–82.
45 England TJ, Hedstrom A, O'Sullivan SE, *et al*. Remote ischemic conditioning after stroke trial 2: a phase IIb randomized controlled trial in hyperacute stroke. *J Am Heart Assoc* 2019;8:e013572.
46 England TJ, Hedstrom A, O'Sullivan S, *et al*. RECAST (remote ischemic conditioning after stroke trial): a pilot randomized placebo controlled phase II trial in acute ischemic stroke. *Stroke* 2017;48:1412–5.
47 Meng R, Asmaro K, Meng L, *et al*. Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. *Neurology* 2012;79:1853–61.
48 Meng R, Ding Y, Asmaro K, *et al*. Ischemic conditioning is safe and effective for Octo- and nonagenarians in stroke prevention and treatment. *Neurotherapeutics* 2015;12:667–77.
49 Sutter EN, Mattlage AE, Bland MD, *et al*. Remote limb ischemic conditioning and motor learning: evaluation of factors influencing response in older adults. *Transl Stroke Res* 2019;10:362–71.
50 Cherry-Allen KM, Gidday JM, Lee JM, *et al*. Remote limb ischemic conditioning enhances motor learning in healthy humans. *J Neurophysiol* 2015;113:3708–19.
51 Esposito E, Hayakawa K, Maki T, *et al*. Effects of postconditioning on neurogenesis and angiogenesis during the recovery phase after focal cerebral ischemia. *Stroke* 2015;46:2691–4.
52 Zhang W, Miao Y, Zhou S, *et al*. Neuroprotective effects of ischemic postconditioning on global brain ischemia in rats through upregulation of hippocampal glutamine synthetase. *J Clin Neurosci* 2011;18:685–9.
53 Brassai A, Suvanjeiev R-G, Bán E-G, *et al*. Role of synaptic and nonsynaptic glutamate receptors in ischaemia induced neurotoxicity. *Brain Res Bull* 2015;112:1–6.
54 Jia J, Hu Y-S, Wu Y, *et al*. Pre-ischemic treadmill training affects glutamate and gamma aminobutyric acid levels in the striatal dialysate of a rat model of cerebral ischemia. *Life Sci* 2009;84:505–11.
55 Wu H, Yang SF, Dai J, *et al*. Combination of early and delayed ischemic Postconditioning enhances brain-derived neurotrophic factor production by upregulating the ERK-CREB pathway in rats with focal ischemia. *Mol Med Rep* 2015;12:6427–34.
56 Gonzalez NR, Connolly M, Dusick JR, *et al*. Phase I clinical trial for the feasibility and safety of remote ischemic conditioning for aneurysmal subarachnoid hemorrhage. *Neurosurgery* 2014;75:590–8.
57 Gonzalez NR, Hamilton R, Bilgin-Freiert A, *et al*. Cerebral hemodynamic and metabolic effects of remote ischemic preconditioning in patients with subarachnoid hemorrhage. *Acta Neurochir Suppl* 2013;115:193–8.
58 Harris PA, Taylor R, Thielke R, *et al*. Research electronic data capture (Redcap)--a metadata-driven methodology and workflow process for providing translational research Informatics support. *J Biomed Inform* 2009;42:377–81.

59 Hyngstrom AS, Murphy SA, Nguyen J, *et al*. Ischemic conditioning increases strength and volitional activation of paretic muscle in chronic stroke: a pilot study. *Journal of Applied Physiology* 2018;124:1140–7.
60 Cherry-Allen KM, Gidday JM, Lee JM, *et al*. Remote limb ischemic conditioning at two cuff inflation pressures yields learning enhancements in healthy adults. *J Mot Behav* 2017;49:337–48.
61 Surkar SM, Bland MD, Mattlage AE, *et al*. Effects of remote limb ischemic conditioning on muscle strength in healthy young adults: a randomized controlled trial. *PLoS One* 2020;15:e0227263.
62 Groothuis JT, van Vliet L, Kooijman M, *et al*. Venous cuff pressures from 30 mmHg to diastolic pressure are recommended to measure arterial inflow by plethysmography. *J Appl Physiol (1985)* 2003;95:342–7.
63 Lorentsen E, Hansteen V, Sivertssen E. The influence of venous collecting pressure on measurements of calf blood flow by venous occlusion plethysmography. *Angiology* 1970;21:661–77.
64 Pallarés LC, Deane CR, Baudouin SV, *et al*. Strain gauge plethysmography and doppler ultrasound in the measurement of limb blood flow. *Eur J Clin Invest* 1994;24:279–86.
65 Lessa HT, Chiviacowsky S. Self-controlled practice benefits motor learning in older adults. *Hum Mov Sci* 2015;40:372–80.
66 Tretriluxana J, Taptong J, Chaiyawat P. Dyad training protocol on learning of bimanual cup stacking in individuals with stroke: effects of observation duration. *J Med Assoc Thai* 2015;98 Suppl 5:S106–12.
67 Udermann BE, Mayer JM, Murray SR, *et al*. Influence of cup stacking on hand-eye coordination and reaction time of second-grade students. *Percept Mot Skills* 2004;98:409–14.
68 Shierk A, Lake A, Haas T. Review of therapeutic interventions for the upper limb classified by manual ability in children with cerebral palsy. *Semin Plast Surg* 2016;30:14–23.
69 Pixa NH, Pollok B. Effects of tDCS on bimanual motor skills: a brief review. *Front Behav Neurosci* 2018;12:63.
70 Valvano J, Newell KM. Practice of a precision Isometric grip-force task by children with spastic cerebral palsy. *Dev Med Child Neurol* 1998;40:464–73.
71 Gordon AM, Charles J, Duff SV. Fingertip forces during object manipulation in children with hemiplegic cerebral palsy. II: bilateral coordination. *Dev Med Child Neurol* 1999;41:176–85.
72 Sullivan KJ, Kantak SS, Burtner PA. Motor learning in children: feedback effects on skill acquisition. *Phys Ther* 2008;88:720–32.
73 Brandão MB, Ferre C, Kuo H-C, *et al*. Comparison of structured skill and unstructured practice during intensive bimanual training in children with unilateral spastic cerebral palsy. *Neurorehabil Neural Repair* 2014;28:452–61.
74 Rudisch J, Butler J, Izadi H, *et al*. Kinematic parameters of hand movement during a disparate bimanual movement task in children with unilateral cerebral palsy. *Hum Mov Sci* 2016;46:239–50.
75 Hung YC, Casertano L, Hillman A, *et al*. The effect of intensive bimanual training on coordination of the hands in children with congenital hemiplegia. *Research in Developmental Disabilities* 2011;32:2724–31.
76 Surkar SM, Hoffman RM, Davies B, *et al*. Deficits in planning sequential goal-directed action impact motor execution in children with hemiplegic cerebral palsy: a kinematic analysis. *J Mot Learn Dev* 2019;7:122–40.
77 Chen CY, Rich TL, Cassidy JM, *et al*. Corticospinal excitability in children with congenital hemiparesis. *Brain Sci* 2016;6:49.
78 Bleyenheuft Y, Dricot L, Gilis N, *et al*. Capturing Neuroplastic changes after Bimanual intensive rehabilitation in children with unilateral spastic cerebral palsy: a combined DTI, TMS and fMRI pilot study. *Res Dev Disabil* 2015;43–44:136–49.
79 Mackey A, Stinear C, Stott S, *et al*. Upper limb function and cortical organization in youth with unilateral cerebral palsy. *Front Neurol* 2014;5:117.
80 Wittenberg GF. Motor mapping in cerebral palsy. *Dev Med Child Neurol* 2009;51 Suppl 4:134–9.
81 Garvey MA, Mall V. Transcranial magnetic stimulation in children. *Clin Neurophysiol* 2008;119:973–84.
82 Quintana H. Transcranial magnetic stimulation in persons younger than the age of 18. *J ECT* 2005;21:88–95.
83 Allen CH, Kluger BM, Buard I. Safety of transcranial magnetic stimulation in children: a systematic review of the literature. *Pediatr Neurol* 2017;68:3–17.
84 Devanne H, Lavoie BA, Capaday C. Input-output properties and gain changes in the human corticospinal pathway. *Exp Brain Res* 1997;114:329–38.
85 Ridding MC, Rothwell JC. Stimulus/response curves as a method of measuring motor cortical excitability in man. *Electroencephalography and Clinical Neurophysiology/ Electromyography and Motor Control* 1997;105:340–4.
86 Maegaki Y, Maeoka Y, Ishii S, *et al*. Mechanisms of central motor reorganization in pediatric hemiplegic patients. *Neuropediatrics* 1997;28:168–74.
87 Hallett M. Transcranial magnetic stimulation: a primer. *Neuron* 2007;55:187–99.
88 Ziemann U, Rothwell JC, Ridding MC. Interaction between Intracortical inhibition and facilitation in human motor cortex. *J Physiol* 1996;496 (Pt 3)(Pt 3):873–81.
89 Auriat AM, Neva JL, Peters S, *et al*. A review of transcranial magnetic stimulation and multimodal neuroimaging to characterize post-stroke Neuroplasticity. *Front Neurol* 2015;6:226.
90 Malhotra S, Naggar I, Stewart M, *et al*. Neurogenic pathway mediated remote preconditioning protects the brain from transient focal ischemic injury. *Brain Res* 2011;1386:184–90.
91 McDonnell MN, Orekhov Y, Ziemann U. The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp Brain Res* 2006;173:86–93.
92 Werhahn KJ, Kunesch E, Noachtar S, *et al*. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. *J Physiol* 1999;517 (Pt 2)(Pt 2):591–7.
93 Mall V, Berweck S, Fietzek UM, *et al*. Low level of Intracortical inhibition in children shown by transcranial magnetic stimulation. *Neuropediatrics* 2004;35:120–5.
94 Ziemann U, Muellbacher W, Hallett M, *et al*. Modulation of practice-dependent plasticity in human motor cortex. *Brain* 2001;124(Pt 6):1171–81.
95 Friel K, Chakrabarty S, Kuo HC, *et al*. Using motor behavior during an early critical period to restore skilled limb movement after damage to the corticospinal system during development. *Journal of Neuroscience* 2012;32:9265–76.
96 Juenger H, Kuhnke N, Braun C, *et al*. Two types of exercise-induced neuroplasticity in congenital hemiparesis: a transcranial magnetic stimulation, functional MRI, and magnetoencephalography study. *Dev Med Child Neurol* 2013;55:941–51.
97 Walther M, Juenger H, Kuhnke N, *et al*. Motor cortex plasticity in ischemic perinatal stroke: a transcranial magnetic stimulation and functional MRI study. *Pediatr Neurol* 2009;41:171–8.
98 Rossini PM, Barker AT, Berardelli A, *et al*. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. *Electroencephalogr Clin Neurophysiol* 1994;91:79–92.
99 Krumlinde-Sundholm L, Holmefur M, Kottorp A, *et al*. The assisting hand assessment: current evidence of validity, reliability, and responsiveness to change. *Dev Med Child Neurol* 2007;49:259–64.
100 Holmefur M, Krumlinde-Sundholm L, Eliasson AC. Interrater and Intrarater reliability of the assisting hand assessment. *Am J Occup Ther* 2007;61:79–84.
101 Krumlinde-sundholm L. Reporting outcomes of the assisting hand assessment: what scale should be used *Dev Med Child Neurol* 2012;54:807–8.
102 Zewdie E, Ciechanski P, Kuo HC, *et al*. Safety and tolerability of transcranial magnetic and direct current stimulation in children: prospective single center evidence from 3.5 million stimulations. *Brain Stimulation* 2020;13:565–75.
103 Kujirai T, Caramia MD, Rothwell JC, *et al*. Corticocortical inhibition in human motor cortex. *The Journal of Physiology* 1993;471:501–19.
104 Ziemann U, Rothwell JC, Ridding MC. Interaction between Intracortical inhibition and facilitation in human motor cortex. *J Physiol (Lond)* 1996;496:873–81.