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Sensorimotor robotic changes of tDCS and HD-tDCS enhanced motor learning in children

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Sensorimotor robotic changes of tDCS and HD-tDCS enhanced motor learning in children

by

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Abstract

Non-invasive brain stimulation, such as transcranial direct-current stimulation (tDCS), can alter cortical excitability and human behavior but investigations to date have been limited in pediatrics. Emerging neurostimulation technologies such as high-definition tDCS (HD-tDCS) are unstudied in the developing brain. Application of tDCS can safely enhance motor skill acquisition in children but mechanisms are poorly understood. Robotics can objectively quantify sensorimotor function and may examine functional changes associated with motor learning and neurostimulation. We aimed to characterize the effects of tDCS and HD-tDCS on motor learning in healthy children. Our randomized, blinded, sham-controlled five day interventional trial demonstrated that both tDCS and HD-tDCS can enhance motor learning with medium to large effect sizes, lasting effects, and favorable safety and tolerability. To explore changes in sensorimotor function accompanying enhanced motor learning, a validated robotic protocol was performed before and after the trial. Motor training was associated with changes in sensory and motor function with less evident effects of stimulation. Both tDCS and HD-tDCS enhance motor learning in children while robotics can explore associated behavioural mechanisms, both of which promise to advance neurorehabilitation strategies in disabled children.

Preface

In fulfillment of a manuscript-based thesis, Chapter 2 has been submitted as, "Cole L*, Giuffre A*, Ciechanski P, Carlson HL, Zewdie E, Kuo H-C, & Kirton A. Effects of High-Definition and Conventional Transcranial Direct-Current Stimulation on Motor Learning in Children. *Cerebral Cortex.* 2018" and "Cole L, Dukelow SP, Giuffre A, Nettel-Aguirre A, Metzler MJ, & Kirton A. Sensorimotor robotic measures of tDCS and HD-tDCS enhanced motor learning in children. *Neural plasticity.* 2018".

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List of Symbols, Abbreviations and Nomenclature

Symbol	Definition
AMPED	Accelerated Motor Learning in Pediatrics
ANOVA	Analysis of variance
BDNF	Brain-derived neurotrophic factor
CIMT	Constraint-induced movement therapy
Cm	Centimeters
CP	Cerebral palsy
CSF	Cerebrospinal fluid
CST	Corticospinal tract
DCML	Dorsal column medial lemniscus
FDI	First dorsal interosseous
fMRI	Functional magnetic resonance imaging
GABA	γ -aminobutyric acid
HD-tDCS	High-definition Transcranial Direct-Current Stimulation
HICCUP	Healthy Infant and Children Clinical Research Program
IDE	Initial direction error
IHI	Interhemispheric inhibition
JTT	Jebsen-Taylor Test

KINARM	Kinesiological Instrument for Normal and Altered Reaching Movements
LTP	Long-term potentiation
M1	Primary Motor cortex
MEP	Motor evoke potential
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MT	Movement time
NMDA	N-methyl-D-aspartate
NSP	Number of speed peaks
PLR	Path length ratio
PPT_A	Purdue Pegboard Test assembly
PPT_L	Left hand Purdue Pegboard Test
PPT_{LR}	Left and right hand Purdue Pegboard Test
PPT_R	Right hand Purdue Pegboard Test
PPT_S	Purdue Pegboard Test sum of scores
PSR	Peak speed ratio
REDCap	Research Electronic Data Capture
RL	Response latency
RT	Retention time
rTMS	Repetitive transcranial magnetic stimulation
SEP	Somatosensory evoked potentials

$Shift_{xy}$	Systematic shift
SMA	Supplementary motor area
SRTT	Serial Reaction Time Test
SVIPT	Sequential visual isometric pinch-force task
TBI	Traumatic brain injury
tDCS	Transcranial direct-current stimulation
TMS	Transcranial magnetic stimulation
TMSMM	Transcranial magnetic stimulation motor mapping
Var_{xy}	Endpoint variability

Epigraph

A man who dares to waste one hour of time has not discovered the value of life.

- Charles Darwin

Chapter 1 - Introduction

1.1 Thesis Goals

Transcranial direct-current stimulation (tDCS) is a form of non-invasive brain stimulation with the potential to modulate cortical excitability and behaviour. There are promising advances in adult and pediatric literature suggesting the possible role of primary motor cortex (M1) targeting tDCS in the enhancement of motor learning. High-definition tDCS (HD-tDCS) has not been applied in a pediatric population and the safety, tolerability, and efficacy in motor learning requires exploration. The mechanisms underlying tDCS and HD-tDCS need to be defined in children to further optimize the effects of tDCS in clinical populations such as those with cerebral palsy (CP). The goals of this thesis are to determine: 1) the effects of tDCS and HD-tDCS on motor learning in healthy children, 2) the sensorimotor functional changes induced by tDCS and HD-tDCS enhanced motor learning in healthy children, and 3) assess the safety and tolerability of HD-tDCS in a pediatric population.

1.2 Background

1.2.1 Transcranial Direct-Current Stimulation

The first modern application of controlled polarized direct current across the scalp was performed by Priori and colleagues [1]. This group briefly applied current, which under certain paradigms, was capable of producing small changes in cortical excitability. This study motivated the seminal work of Nitsche and Paulus that established the modern approach to tDCS in 2000 [2]. Since this time, tDCS has been applied in a variety of clinical and behavioral contexts, such as stroke, CP, depression, Parkinson's disorder, and notably, motor skill enhancement. To date, over 33,000 sessions have been performed [3–7].

Early animal investigations suggested that the application of subthreshold current traveling from an anode to a cathode generated weak electric field in neurons in slice preparations [8–10]. These induced electric fields lead to a change in membrane potential and spontaneous neuronal firing patterns [2,11,12]. Different neurons were modulated to various extents. Importantly, layer V/VI pyramidal neurons may be depolarized or hyperpolarized [13].

Modern tDCS applied across the scalp also may shift cortical excitability resulting in a state of net excitation or inhibition [2,14,15]. The shift in cortical excitability is dependent on two important factors: the direction of current flow (polarity) and the amount of current (intensity). In the context of tDCS, polarity is typically classified by the terms anodal or cathodal stimulation. Anodal stimulation refers to placement of an anode over the targeted area on the scalp and a cathode over an inert location such as the supraorbital area or deltoid. The cathodal montage involves placing the cathode over the targeted brain region and the anode over an inert location. Anodal tDCS is thought to depolarize targeted neurons, whereas cathodal tDCS often hyperpolarizes the same neuronal populations. While this is a general rule of thumb, the precise relationship is complex and various exceptions to this rule have been recognized [14,16]. For example, under certain conditions these montages may have opposite effects on the excitability of the targeted region. Polarity-dependent effects are linked to neuronal-electric field interactions and may be explained by the direction of induced electric fields. When the electric fields are polarized in the direction of the dendrites towards the axons (as M1 neurons are in M1-targeting anodal tDCS), then the axon and soma will be depolarized and the dendritic tree will be hyperpolarized [13,17]. With cathodal stimulation, the opposite interaction is observed and the

electric field points from the axon to the dendrites, resulting in the axon and soma becoming hyperpolarized. The current intensity is also an important factor in determining tDCS effects, however a higher intensity of stimulation does not necessarily lead to larger changes in cortical excitability. Cathodal tDCS at 1mA has been found to decrease cortical excitability [14]. However, these effects are reversed at 2mA, where cathodal tDCS increases cortical excitability [14]. There are many other factors that complicate polarity-dependent effects of tDCS such as genetics, anatomy, sex, and hormones [18].

Various pharmacological investigations have probed the underlying mechanisms of tDCS. The modulatory effects of tDCS can be classified as immediate- or after-effects. Immediate effects refer to changes in neuronal excitability while the stimulation is being applied, whereas after-effects are still present after stimulation has been terminated. The immediate excitability modulating effects of anodal tDCS appear to be dependent on interactions with ion channels. The blockage of sodium channels with the application of carbamazepine, which reduces membrane permeability to sodium ions, completely abolishes changes in cortical excitability induced by anodal tDCS [19,20]. Likewise, the application of the calcium channel blocker flunarizine, reducing the permeability of calcium ions across them membrane, diminishes the cortical excitability increases seen with anodal tDCS [19,20]. In contrast, N-methyl-D-aspartate (NMDA) receptor antagonists [20] or γ-aminobutyric acid $(GABA)_A$ receptor agonists [21] do not influence immediate effects of anodal tDCS. Therefore, the interaction between electric fields and sodium and calcium channels appears to largely contribute to the immediate effects of anodal tDCS.

In addition to the immediate effects, there are lasting after-effects associated with tDCS [2,11]. These changes may be dependent on synaptic modulation of interneurons. The application of a partial NMDA agonist (D-cycloserine) extends the time of cortical excitability changes following the application of anodal tDCS [22]. Supporting the role of NMDA channels, the application of a NMDA receptor antagonist abolishes the after-effects observed of anodal stimulation [19]. GABA may also have a role in the lasting effects of tDCS. The addition of a $GABA_A$ receptor agonist initially delayed, but then prolonged and enhanced anodal tDCS generated changes in cortical excitability [23]. Pharmacological investigations of the acute and late effects of tDCS have not been studied in children, partially due to less experience in this population and challenges of drug trials in pediatrics.

The anodal and cathodal tDCS montages described earlier are referred to as 'conventional tDCS montages.' Conventional montages traditionally use large sponge electrodes soaked in saline (Figure 1A). These large electrodes lead to diffuse current distribution that effects the target area (Figure 1B), but in the process, also modulates 'off-target' cortical regions and deeper brain structures [24]. Recently, high-definition tDCS (HD-tDCS) montages have been developed and have been used to distribute current more focally to target cortical regions. The HD-tDCS montage involves a central anode surrounded by four cathodes (4x1 anodal HD-tDCS) (Figure 1C). The 4x1 anodal HD-tDCS montage can apply current in a relatively focal manner with generation of stronger electric fields in the target region compared to conventional tDCS [25] (Figure 1D). Modeling studies have suggested the 4x1 ring electrode configuration results in maximal electric field strength under the target electrode and current flow is constrained by the ring radius, resulting in more restricted electric fields and less off-target stimulation [26]. HD-

tDCS can also induce bi-directional changes in cortical excitability and the principle effects are similar to those of conventional tDCS [26]. The neurophysiological effects of HD-tDCS may be comparable but are not likely equivalent to conventional tDCS. For example, with conventional anodal tDCS, cortico-spinal excitability is increased to a larger extent immediately after tDCS, but then gradually returns to baseline levels. However in HD-tDCS, the induced plasticity reaches the peak change in excitability around 30 minutes after stimulation, and furthermore the after-effects lasted 30 minutes longer than conventional tDCS [26].

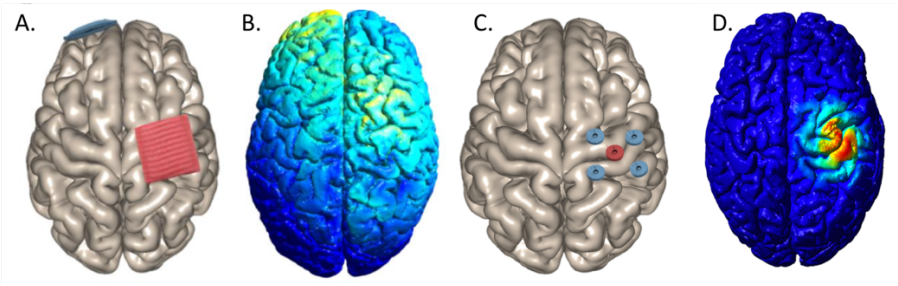

Figure 1.1 *tDCS montages. (A) Conventional 1x1 tDCS montage of the right primary motor cortex with (B) the accompanying computational model of induced electric fields with tDCS. (C) HD-tDCS montage targeting the right M1 with (D) the accompanying computational model of induced electric fields.*

Advanced computational models have been utilized to understand electric field strength and its distribution in the brain [27–30]. The effects of tDCS are dependent on the electric field direction and strength at the neuronal level. These factors are dependent on the bioavailable dose, which is influenced by neuroanatomical factors such as skull thickness, electrode position, and precise gyral neuroanatomy [24,31,32]. Combining MRI with finite-element modeling shows how individual elements such as soft tissue, bone, air, cerebrospinal fluid (CSF), white matter, and cortical and deep gray matter affect current flow. Current modeling studies confirm that

conventional tDCS has poorly focused current distribution with discrete clusters of electric field magnitude peaks [31]. The current maxima are often seen between electrodes, rather than under electrodes [33], and reflect idiosyncratic anatomical differences across individuals [34]. By using a restrictive electrode montage, such as that of HD-tDCS, the induced-electric fields are more limited to the target area under the active electrodes, and the peak electric fields fall within this region [27,34].

1.2.2 Neuroplasticity and motor learning

Neuroplasticity is the modulation and reorganization of neuronal connections that produce long-lasting changes in brain function. For an intervention to have persistent effects on behavior, it must demonstrate the ability to induce a lasting functional change in the brain [35]. Synaptic plasticity mechanisms induce persistent functional changes but are poorly understood, especially in the developing brain. The Hebbian theory proposes that mechanisms underlying neuroplasticity include neurons that fire together will establish connections and become more efficient over time [36]. This efficiency may manifest as strengthened connections or formation of new connections. This theory has been expanded and many mechanisms for synaptic strength modulation have been identified. Long-term potentiation (LTP) is a well-established mechanism by which synaptic strength is increased by repeated activation of neuronal connections [37,38]. LTP has been classically defined in memory formation where original studies observed the effects in dentate nuclei of the hippocampus. More recently, groups have demonstrated a form of LTP prominent in the motor cortex and motor learning [38,39]. This form of plasticity in the motor cortex is referred to as LTP-like plasticity.

The balance between neuronal excitation and inhibition is important in inducing LTP-like plasticity. Multiple neurotransmitter systems are implicated in these processes including glutamate and NMDA receptors. The induction of LTP-like plasticity is blocked when an NMDA receptor antagonist is applied, highlighting the role of glutamate in neuroplasticity [40]. Other neurotransmitters such as GABA are also important in the modulation of LTP-like plasticity in M1. Modulation of GABA may be necessary for LTP to occur [40], demonstrated through the induction of LTP-like plasticity when a $GABA_A$ receptor antagonist is applied [41].

Functional MRI (fMRI) studies have suggested that neuroplastic changes occur during motor skill training, and this may be linked to changes in cortical activation in M1 and other motor regions [42]. Animal studies have identified that learning and practicing a new skill is associated with plastic changes in the brain, particularly an increased efficacy of horizontal connections within M1 [43]. Horizontal connections found in layer II and III of M1 are strengthened following multi-day motor training [43]. These connections allow for an expansion of motor representations into surrounding areas [44]. These horizontal connections may play a role in explaining why an induction of LTP-like plasticity is observed with reduction of GABA activity. With reduced GABA activity, there is a lack of suppression on these connections allowing them to be modulated and strengthened. Further evidence suggests that following motor training, the balance of plasticity may be shifted to a state of reduced LTP and increased long-term depression (LTD) in M1 layer II and III horizontal connections [45]. This shift in plasticity demonstrates the possible role of LTP-like mechanisms underlying motor learning.

The neuroplastic changes following motor skill acquisition may also explain the phenomenon of skill ceilings, where additional training does not lead to further improvements in skill [45]. Because the available potential for LTP has been expanded by motor learning, the baseline level of M1 synaptic efficacy is already at the upper limit of the synaptic modification range. Therefore, further LTP cannot occur, occluding further skill gains.

Despite the recognition of these primary systems in motor learning and the ability to measure them in vivo with advanced imaging and neurophysiologic investigations, studies have been limited in pediatric populations.

The acquisition of new motor skills, and long-term retention of those skills, is crucial in our daily lives. Motor skill learning is defined as a process where repeated practice of movements leads to increased performance with greater speed and accuracy [46]. Motor learning is made up of skill acquisition and motor adaptation. Motor learning normally occurs in different phases. Often skills will develop fast initially and then progress at a more gradual improvement [47].

Skills may be acquired via two modes of learning, online and offline. Both online and offline learning processes can contribute to skill acquisition and long-term retention [48]. Online learning refers to skill learning within the task training period and is often associated with rapid, large improvements. The use of positron emission tomography and fMRI has explored rapid online learning in fast sequence learning task and identified an association between reaction time and activity in frontoparietal networks [49–51]. Online learning has been shown to change functional connectivity of the motor network and within session learning is mediated by

increased interconnectivity of the motor cortex, sensorimotor cortex, premotor cortex, and supplementary motor area (SMA) regions [51]. One study utilized motor sequence learning and MR spectroscopy (MRS) and identified a decrease in GABA in the sensorimotor cortex contralateral to the trained hand, which was associated with online learning of a motor sequence task [52]. Mouse models have suggested that a decrease in GABAergic inhibition has also been found to enhance LTP-like activity in the motor cortex [53,54]. Pharmacological studies have also suggested that plasticity in the sensorimotor cortex is modulated by local changes in GABA concentrations [55,56].

Motor sequence tasks are commonly used to examine online skill learning and further understand implicit and explicit learning paradigms. Explicit learning refers to conscious awareness of the task being learned while implicit learning occurs without recognition of the task. A neuroimaging study examined the diversity of brain regions associated with online explicit and implicit motor sequence learning [49]. They identified that explicit online learning was associated with increased activity in the posterior parietal cortex, premotor cortex, left SMA and left thalamus. Implicit online motor learning, however, was associated with increased activity in the sensorimotor cortex. The effects of implicit online learning were further explored and the same study identified increased activity in the premotor cortex and SMA [57]. This is a controversial topic, as a different study identified that with single session rapid learning there was a decrease, rather than an increase, in activity in the posterior parietal cortex, M1, and dorsolateral prefrontal cortex [50]. This diminished activity is thought to possibly reflect improved network efficiency, requiring recruitment of fewer neuronal resources.

Offline learning (consolidation) occurs after the training session has ended, is normally involved in skill stabilization or improvement, and is task dependent [58–60]. This stabilization involves neural network reorganization through changes in synaptic intracellular signal transduction cascades and neuronal protein synthesis [61]. M1 is thought to play an important role in adapting and modulating previously consolidated motor memories [62]. Evidence of this comes from an inability to modify previously consolidated motor memory after a temporary virtual lesion in M1 was created using transcranial magnetic stimulation (TMS) [62]. Sleep may be crucial in offline motor learning, as neuronal process that occur during sleep promote consolidation [63]. The effects of sleep on motor learning have been explored using sequential finger-tapping tasks, where a pattern is displayed and participants must tap the correct sequence on a keyboard as quickly as possible [60,64]. Explicit offline learning relied on sleep between training sessions, suggesting that higher order brain regions may be involved in consolidation [60,65,66]. However, sleep may not have a role in implicit offline motor learning [67]. When the serial reaction time task (SRTT), an implicit motor learning task, was administered in adults, offline learning depended on the passage of time between sessions [63]. Implicit learning reaction time was associated with increased activity in the primary sensorimotor cortex, compared to explicit motor learning that was correlated with activity of the frontoparietal network [49,68]. This difference between explicit and implicit offline learning may reflect differences in the modification of neural substrates [49,68].

Motor learning is not restricted to motor areas of the brain but may also involve changes to sensory areas. There are diverse connections between the M1 and somatosensory areas that may drive experience-dependent plasticity [69]. This study utilized a force field reaching task and

demonstrated changes in limb position sense [70]. There have been multiple studies examining the electrophysiological changes in sensory systems associated with motor learning. A study examining naïve monkeys trained in a novel tongue protrusion task found changes in orofacial somatosensory cortex activity following orofacial motor learning [71]. This study found rapid and long-lasting changes to both the M1 and the somatosensory cortex associated with behavioral improvements in task performance. Another study examined change in somatosensory-evoked potentials (SEP) following force-field learning in humans [70]. This group found that change in SEP magnitude was correlated with motor learning, where subjects who learned more showed greater changes in SEP magnitude. Others have examined changes in sensorimotor integration using SEP and concurrent performance changes following a repetitive task. They found that complex motor training led to an increase in sensorimotor integration processing [72]. Likewise, sensory training acts directly on the motor system to improve motor learning and increase the excitability of M1 [69].

The adaptation of internal models for new kinematic and dynamic experiences is also crucial in motor learning. Kinematic conditions refer to spatial information such as the angles of the arm or position of the hand. The dynamic experiences include forces acting on the joints during movement. With dynamic tasks, the brain adapts to forces by adjusting or reorganizing motor commands to generate new actions. There have been multiple behavioral, neurophysiological, and neuroimaging studies demonstrating that the acquisition of motor skills relies on sensory and motor plasticity [69].

There have also been novel neuroimaging studies exploring alterations in sensory areas of the

brain following motor learning. With force field adaptation training, there were resting-state network changes in the second somatosensory cortex, SMA, and ventral premotor cortex [73]. This finding possibly demonstrates a link between perceptual change and motor learning. Another study identified that the resting-state connectivity of the left supramarginal gyrus was strengthened after daily sequential finger movement training was performed for four weeks [74]. This study also identified an increase in resting-state functional connectivity in the right postcentral gyrus and right supramarginal gyrus in the first two weeks of training. These studies support that motor adaptation involves changes in both the motor and somatosensory systems. Other types of learning, such as perceptual learning, improve our ability to interpret sensory inputs [75]. Perceptual learning is driven by experience and is associated with plasticity of the sensory system. These plastic effects are dependent on afferent inputs from the periphery, as well as projections from motor areas [69]. Perceptual learning may produce changes in motor function and motor areas of the brain [69].

Measuring motor learning can be difficult as studies in the past have defined improvements as reduction in movement time, decrease in reaction time, or less variability. Robotics can be a useful tool in examining the effects of motor learning and this topic is discussed later.

1.2.3 Effects of tDCS and HD-tDCS on motor learning

When combined with behavioral tasks, tDCS may modulate task performance. There has been extensive literature describing the effects of tDCS on motor learning in the adult population. By altering cortical excitability concurrent to motor skill training, tDCS has been suggested to enhance motor learning in adults [2,76]. It has been shown that tDCS can be effective at

increasing the rate of skill acquisition in a variety of motor tasks [77]. The precise effect of tDCS on motor learning is dependent on montage, timing of application with motor task execution, and the type of training, among a host of factors [76]. There has been recent work examining the effects of tDCS in a pediatric population, which is discussed later, however most investigations are limited to adults.

Three different conventional tDCS montages are commonly used to modulate motor learning: anodal, cathodal, and bihemispheric tDCS. The montage used most frequently is anodal tDCS, where the anode is placed over the M1 contralateral to the trained hand and cathode over the supraorbital area. Anodal tDCS has been hypothesized to modulate LTP-like plasticity through NMDA receptors, resulting in an increase in postsynaptic calcium levels allowing for increased synaptic modification involved in motor learning [35]. The Hebbian plasticity models described earlier support that sustained improvements in a trained skill may be due to synaptic plasticity changes in M1 [36]. Therefore, by increasing the potential for synaptic modification, anodal tDCS may promote Hebbian plasticity. Enhancing motor learning requires certain crucial cortical or sub-cortical targets to be stimulated. For example, the application of anodal tDCS to the ipsilateral (dominant) M1 does not affect motor skill acquisition of the non-dominant hand, whereas application to the contralateral (non-dominant) M1 improves hand function [78]. This relationship may be dependent on the polarity of the stimulation, as application of cathodal tDCS over the ipsilateral M1 may also enhance skill acquisition [79]. Cathodal tDCS is thought to reduce M1 transcallosal inhibition, indirectly influencing the excitability of the contralateral (trained) M1 [76,79]. Both anodal and cathodal tDCS approaches have been combined into a

montage described as bihemispheric tDCS, which targets both M1. Bihemispheric tDCS has also been shown to improve motor skill learning [80].

The timing of when the task training occurs relative to tDCS application also influences the resulting effects. Motor training completed concurrent to tDCS or immediately after training has been shown to enhance motor performance [76]. Motor training paired before [81] and after [82] stimulation has been studied less frequently and results are mixed. Motor training is activity dependent and it is possible that homeostatic effects may occur in M1 when tDCS is applied directly before training [76].

Many studies have shown that tDCS enhances motor learning [83–85]. These studies have identified that targeting M1 with anodal tDCS, concurrent to hand training increases the rate of skill acquisition. Importantly, multiple days of a tDCS-paired training leads to larger effects than single day trials. The effects of tDCS on motor learning may be task-dependent. For example, fine motor skills may be more sensitive to improvement than gross motor skills [77]. The Purdue Pegboard Test (PPT) measures fine motor hand dexterity [86] and provides a unique tool to measure hand skill function. Kidgell et al.[87] paired the PPT with anodal tDCS and demonstrated hand skill improvements with anodal tDCS compared to sham. In their seminal study, Reis et al.[83] used a novel fine motor skill, the sequential visual isometric pinch-force task, to examine the effects of M1 anodal tDCS on skill acquisition. Unlike the single session studies, in this study participants trained for five days on this novel and challenging motor task. Compared to controls receiving sham tDCS, there were large improvements in skill in the anodal tDCS group, and these improvements persisted up to three months after the training. Importantly,

the effects of tDCS were observed predominantly in the form of consolidation (offline learning) rather than daily practice effects (online learning). Gross motor skills may show less improvement especially when performed with the dominant hand. Therefore, it is important to be cognizant of skill ceilings. Past studies have focused on using the non-dominant hand in motor learning studies to avoid this skill ceiling. The dominant hand skill may be closer to reaching this 'ceiling' at baseline, and therefore may have less potential to improve. These skill ceiling effects have been examined when anodal tDCS was applied during training of daily life tasks, assessed using the Jebsen-Taylor Hand Function Test (JTT). Improvements in score were only noted in the non-dominant hand and not the dominant hand [88]. Improved motor learning was also observed in the more difficult tasks that required speed and accuracy. This finding suggests that fine motor tasks may be more sensitive to change compared to gross motor tasks [88], especially over multiple days. Most multi-day motor learning studies have applied conventional tDCS, however bihemispheric M1 HD-tDCS paired with unimanual and bimanual motor training has also demonstrated bimanual hand dexterity improvements [89]. HD-tDCS has not been applied in a pediatric population in the context of motor learning.

The neurophysiological understanding of tDCS enhanced motor learning is growing but remains incompletely defined. TMS can be used to help elucidate changes in M1 cortical excitability induced by motor learning and tDCS. One TMS measure referred to as interhemispheric inhibition (IHI) is a measure of transcallosal inhibition from one M1 to the other. Changes in IHI may be linked to change in motor function. One study found that functional motor improvements of the non-dominant hand, measured using the JTT, were correlated with a decrease in IHI from the dominant to non-dominant hemisphere [90]. These findings suggested a greater activation of

the trained, non-dominant hemisphere compared to the untrained hemisphere. Advanced neuroimaging also provided insight on neuro-metabolic changes in M1 following tDCS. GABA-optimized MRS suggested the extent of GABA decrease in M1 by anodal tDCS was correlated with both the degree of motor learning and fMRI signal change within the left M1 [91]. Cellular investigations also help inform mechanisms of tDCS. Limited evidence suggests that brain-derived neurotrophic factor (BDNF) may play a role in the enhancement in motor learning seen with tDCS. Both animal and human subjects with a common BDNF polymorphism show impaired motor learning and response to tDCS [38], suggesting the importance of BDNF on neuroplasticity. While mechanisms are increasingly understood, there is an ongoing need for investigations of the underlying cellular and molecular mechanisms of tDCS.

1.2.4 tDCS in Pediatric Populations

Most tDCS investigations have been performed in adults and there is a lack of mechanistic investigations in the developing brain. Limited data indicates that tDCS effects are not identical between the two populations. Computational current modeling in the pediatric brain suggests increased strength of induced-electric fields compared to adults for the same tDCS dose [92]. Factors such as skull thickness, CSF volume, and head circumference may contribute to this difference. Current modeling in adults has suggested that the skull may impede current transmission by up to 50% [29]. Therefore, there is greater current transmission in the thinner skull of a developing child. CSF is highly conductive and allows for current to be shunted to different areas of the brain, as current often follows the path of least resistance. Children have reduced CSF volumes compared to adults and thus experience higher current densities due to reduced shunting. A pediatric tDCS modeling study looking at the peak current flow and

distribution across the brain of a single child with perinatal stroke found that tDCS at 0.7mA produced a peak brain current intensity comparable to an adult receiving 1.0mA with the same montage [93]. This finding suggests that tDCS current intensity may need to be reduced in a pediatric population. However, current intensity is a complex topic in children. The application of 0.7mA was applied in a bihemispheric montage in a pilot study in children with congenital hemiparesis, however there were no significant changes in TMS-assessed cortical excitability or behavioural data [94]. Electrode sizes comparable to adult studies are also used in pediatric studies, even though children have smaller head circumference, adding further complexity. Because the electrodes will occupy a larger relatively area of the head, this will lead to less focal and possibly more off-target stimulation [92]. The application of current using smaller electrodes in HD-tDCS may overcome this, allowing for a more targeted distribution of current.

Early neurophysiological investigations also suggest stronger electric fields in children. In a study of 19 healthy children and adolescents, both 1mA anodal and cathodal stimulation resulted in increased cortical excitability that was retained for one hour after stimulation ended [95]. A 1mA cathodal current will typically decrease cortical excitability in adults [11]. In contrast, 0.5mA cathodal tDCS decreased cortical excitability, suggesting that comparable stimulation intensities induce stronger electric fields in pediatrics. The precise relationship is complex however, as a 0.5mA anodal stimulation did not produce a change in cortical excitability [95]. Despite the findings that induced electric fields are stronger in children compared to adults, early studies suggest favorable safety in pediatric populations. This safety data will be discussed later.

The largest investigation of tDCS application in healthy children comes from our group. We conducted a double-blinded, randomized, sham-controlled trial to investigate the effect of tDCS on motor learning in children for the first time [96]. Participants were randomized into four intervention groups: 1mA anodal, 1mA cathodal, 2mA cathodal, or sham. Anodal stimulation was applied contralateral to the trained hand and cathodal stimulation was applied ipsilaterally. Over the course of three days, the groups receiving anodal or cathodal tDCS showed significant enhancement of motor learning compared to the sham group. These improvements persisted six weeks later. The anodal and cathodal tDCS groups appeared to have altered early and late elements of the learning curve, possibly facilitating enhanced skill acquisition. The sham group demonstrated clear learning but had a slower rate of early learning and reached a skill ceiling, where further improvements with training were limited. The dominant, untrained hand's skill also improved with tDCS stimulation with increased right hand PPT scores. In contrast, sham (training alone) did not significantly affect these scores. This study confirmed the safety and tolerability of tDCS in children. This early investigation suggested tDCS may be modulate both the motor networks.

There have also been multiple studies examining the effects of tDCS in pediatric clinical populations, despite the small number of tDCS studies in the healthy developing brain. The application of tDCS has been used in a diverse array of clinical populations such as perinatal stroke, CP, epilepsy, encephalitis, dystonia, attention-deficit hyperactivity disorder, childhood-onset schizophrenia, and autism [94,97–104]. The use of tDCS in a clinical population has been applied most often in CP. Gillick et al.[94] demonstrated preliminary findings that tDCS was safe, feasible, and tolerable in children with hemiparesis. A pilot, double-blind, randomized,

sham-controlled clinical trial in children with diparesis combined anodal tDCS with virtual reality gait training [105]. The tDCS group showed improvement in gross motor function, mobility, gait velocity, and cadence. Another trial found that anodal tDCS might reduce CP-related spasticity but did not improve range of motion [104]. Similar studies examined the effects of tDCS paired with motor therapy in children with unilateral CP [106]. Both sham and tDCS groups demonstrated improvements in hand function after intervention, however there were no significant differences between the intervention groups. Our group performed a randomized, controlled, double-blind clinical trial examining the effect of tDCS in children with hemiparetic CP [97]. Contralesional M1 1mA cathodal tDCS was paired with 10 days of goal-directed occupational therapy. We demonstrated that application of tDCS was feasible and safe in this population. There were subjective gains in function associated with tDCS, however no objective motor functions were demonstrated, possibly due to an under-dosing of therapy. Currently a phase III clinical trial is in progress examining the possible efficacy of tDCS in this population.

HD-tDCS has only been applied in a single pediatric study. This case study applied 10 days of HD-tDCS in a 30-month child with early-onset epileptic encephalopathy. The cathode was placed over the brain region exhibiting possible epileptogenic activity. There was no effect of HD-tDCS on the frequency of clinical seizures, however at the post-intervention period there was a significant change in electrical features of the epileptogenic activity. There is a clear lack of high-quality, rigorous trials applying HD-tDCS in children. To refine and advance application of tDCS in clinical populations, there is a need for investigation of the mechanisms and effects of tDCS and HD-tDCS on healthy developing children.

1.2.5 Safety of tDCS and HD-tDCS

Safety and tolerability of tDCS are well defined in the adult population. However, pediatric populations have been neglected in tDCS research where <5% of published studies have studied the effects in the developing brain. The use of conventional tDCS has been applied in over 33,000 sessions and 10,000 subjects with no serious adverse effects or irreversible injury [7]. An evidence based review conducted by an expert opinion panel examined the safety of tDCS in adults and children. This review found that tDCS is not associated with any tissue damage, irreversible behavioral changes, or serious adverse effects. When examining tDCS safety, it is important to consider dose metrics, intensity, duration, and charge density. According to Peterchev et al., the dose is defined as "the waveform of a single sustained direct current" applied to the scalp [107]. The tDCS intensity is defined as "the steady state intensity applied to the anode." The duration refers to the "length of time current is at the steady state level" and does not include the ramp up and ramp down timing. The charge density is defined as "charge divided by the electrode area is used as an average metric (A/m^2)".

Animal lesion studies have been used to support existing safety data. There have been multiple studies assessing histological damage in epicranial direct current stimulation in rodents [108,109]. Bikson et al. has scaled these results to humans and hypothesized that the predicted minimum induced current density for detected damage ranged from 6.3-12A/m^2 [7]. This group further scaled these results and predicted human damage to occur around 67-120mA. These scaled factors largely exceeded maximum current levels applied during tDCS in humans. There are inherent limitations in using animal histology thresholds to base human safety standards.

These limitations include possible susceptibility differences in animal and human tissue to damage, experimental limits on detecting various models of damage, difference in proportions from rodent to human gross anatomy, and difference in method of stimulation such as transdermal in humans and epicranial in rodents. The highest tDCS intensity in human studies to date has not exceeded 4mA. A phase I study performed single session bihemispheric tDCS in adult stroke patients with current intensity reaching up to 4mA. This study reported transient skin redness in 50% of their patients, and likewise they did not find any rise in temperature or destruction of the skin barrier [110].

There have been multiple reviews to support the safety of tDCS applied in controlled human trials [111,112]. Mild skin redness is common during tDCS and resolves after stimulation has ended [113]. A MRI study found that tDCS was not associated with any edema or pathological alterations of the blood brain barrier or cerebral tissue damage [114]. There is also no evidence of neuronal damage examined by serum neuron-specific enolase after tDCS application [12,20]. There are no pathological electroencephalography (EEG) waveforms or worsening of neurophysiological measures observed after frontal lobe stimulation with duration of stimulation up to 20 minutes [115].

The tolerability of tDCS is also favorable and can be further improved by changing the electrolyte buffer between the electrode and the skin. Adjusting the concentration of the saline which the electrodes are soaked in may be a way to improve the tolerability and decrease sensation severity [116]. As well, increasing the separation distance between electrodes may improve tolerability of scalp current in HD-tDCS, however, this diminishes current focality [27].

In children, tDCS has been applied in at least 2800 sessions across nearly 500 subjects [7]. Among diverse clinical populations, there have been no serious adverse events. However, there is need for further studies in pediatric populations. A recent tDCS safety review in children and adolescents reported that the most common sensation was tingling, itching, and redness under the site of the anode [117]. This review concluded that tDCS is safe in children and adolescents with a variety of neurological conditions. The application of tDCS in children with CP is a rapidly developing field and there have been multiple studies examining its safety and tolerability [94,99,103,104,118–120]. The most commonly reported minor adverse event was tingling or discomfort under the electrodes [7]. Our lab has not reported any serious adverse events with tDCS application in children [96,97]. Additional safety outcomes included no decrease in motor task skill in the trained or untrained hand following intervention. Participants reported tinging or itching of the scalp in 55% of applications [96]. The safety literature presented is promising, however it is crucial to further understand the safety and tolerability of tDCS in the developing brain.

1.2.6 Motor and Sensory System Anatomy

There are two different types of organization of the neocortex defined by layers and columns. The neocortex has six cortical layers. From most superficial to deepest, these layers are referred to as the: molecular layer, external granular layer, external pyramidal layer, internal granular layer, internal pyramidal layer, and multiform layer [121]. The molecular layer is the first layer (layer I) and includes dendritic branches most commonly originating from pyramidal cells and axons from the thalamus or either hemisphere. Deeper layer Martinotti cells can also contribute

axons. Layer II is defined as the external granular layer and is made up of many small pyramidal cells and interneurons. The external pyramidal layer, layer III, consists of pyramidal cells that increase in size from the external to internal borders. The pyramidal cells in this layer project to the contralateral cortex and long horizontal projections connect to the M1 and sensory cortex. Layer IV is defined as the internal granular layer and is mostly made up of stellate cells. There are a small number of other interneurons and pyramidal cells. This layer receives sensory input from the thalamus and is crucial in cortical areas responsible for sensory function such as the primary somatosensory cortex. The M1 is referred to as agranular cortex, as it lacks layer IV. The internal pyramidal layer, layer V, is made up of larger pyramidal cells and interneurons. These neurons project to subcortical areas such as the striatum, brain stem, and spinal cord. Large pyramidal cells, called Betz cells, only occur in M1 and a small number are located in Layer V. Inputs to this region are from other cortical motor areas, somatosensory cortex, and posterior division of the ventral lateral thalamic nucleus. Layer VI is the deepest layer and is defined as the multiform layer. This layer is made up of fusiform cells, and various shapes of pyramidal cells and interneurons. These neurons project to the thalamus. The neocortex can also be organized in columns defined as small areas of vertically orientated neurons. Neurons within a column will generally have similar function and properties.

The motor cortex is made up by anatomically and functionally distinct areas that operate together to generate and organize a targeted movement [122]. A number of neurophysiological and imaging studies have identified the roles of M1, premotor cortex and supplementary motor area, corticospinal tract pathways, cerebellum, and basal ganglia in motor function [123].

The M1 is located in the precentral gyrus and is important in the execution of voluntary movements [124]. Wilder Penfield and colleagues used focal stimulation to motor map the M1, and found that disproportionally large areas were responsible for finely controlled body parts such as the fingers and face [125]. This finding suggested that a larger number of neurons may be needed for fine motor control [125]. Further mapping studies have found that neurons controlling hand, finger, and distal arm muscles are located in a horseshoe-shaped structure around the central core, commonly referred to the hand knob [126]. The M1 is confined to Brodmann area 4 which occupies the precentral gyrus and is rostrally orientated to the central sulcus [127,128]. Neurons in M1 are responsible for encoding specific kinematic parameters of voluntary movement. Individual neurons may be involved in the control of multiple muscles and studies have found that activating a single neuron in M1 can evoke activity in multiple arm muscles [129]. The M1 neuronal population that control various fingers overlap but appear to have some level of organization [130]. As discussed earlier, the unique organization of M1 allows for the redistribution and generation of new neuronal connections. Through these neuroplastic mechanisms, the motor network can be modulated.

The modulation of muscle activity in the contralateral arm is through neurons originating in M1 as well as subdivisions of the premotor cortex. These neurons project their axons into the cervical enlargement of the spinal cord and continue to their corresponding spinal level. The M1 is the only location that contains neurons with axons that directly terminate on the spinal motor neurons in the most ventral and lateral part of the spinal ventral horn [131]. The M1 is also involved in higher-order sensorimotor processing as it receives input from the parietal and

frontal areas [132]. The thalamus is also important to relay direct somatosensory information from the periphery to M1.

The premotor area is involved in motor function through its reciprocal connections with M1 and is a source of pyramidal and other descending motor pathways [133]. It is found in Brodmann area 6, anterior to the M1. The premotor areas receive connections from the ventro-anterior and anterior subdivision of the ventral lateral nucleus of the thalamus and pallidum of the corpus striatum. The premotor area is involved in the intention to perform a target movement. The premotor area is also connected with the posterior parietal cortex. This important relationship allows for integration of visual, proprioceptive, and other sensory information in the planning of movement [121].

The SMA is located in part of Brodmann area 6, on the medial surface of the hemisphere. It also receives input from the ventro-anterior and anterior subdivision of the ventral lateral nucleus of the thalamus. Both the premotor cortex and SMA are important in voluntary skilled movement. These regions are involved in modifying instructions for previously learned movements and generating new instructions for novel movements.

The corticospinal tract (CST) is a crucial white matter pathway involved in voluntary movement. The CST is made up of large pyramidal neurons which predominately originate from layer V of M1. It develops in the first trimester and continues to develop throughout adolescence [134–136]. The axons of the CST travel through the corona radiata, posterior limb of the internal capsule, and then the cerebral peduncles. Most CST axons cross the anatomical midline at the

junction between the brain stem and spinal cord, forming the pyramidal decussation, and enter the lateral aspect of the spinal cord to become the lateral CST (contralateral fibers). A small proportion of fibers remain uncrossed and descend anteriorly to the spinal cord (ipsilateral fibers). The contralateral fibers have been found to exert control over distal forearm and hand musculature [137]. The functional role of ipsilateral projections has not been well defined [138,139]. Abnormal persistence of these ipsilateral projections are a key element of CP models, discussed later. Several premotor and parietal cortical areas impact spinal motor function though their own corticospinal projections. The corticospinal fiber axons originating from M1, SMA, and cingulate motor areas terminate on interneuronal networks in the intermediate laminae of the spinal cord (Rexed laminae VI and VIII).

The sensory system begins with multiple peripheral receptors for different stimuli, including muscle spindles, joint receptors, cutaneous afferent fibers, and Golgi tendon organs. These peripheral receptors are crucial in proprioception. Neuronal cell bodies that mediate these afferent sensory inputs are found in the dorsal root ganglia, where they are classified as first-order neurons [140]. The dorsal root ganglion cells give rise to many peripheral axons, many of these being shorter axons that terminate in the dorsolateral spinal cord. These termination sites are oriented in a specific pattern to define unique sensory pathways within the central nervous system. One of these, the dorsal column medial lemniscus (DCML) pathway, carries proprioceptive information from mechanoreceptors in the periphery to the somatosensory cortex [140]. The second-order relay neuron in the dorsal column nuclei project their axons to the somatic sensory portion of the thalamus [140]. These neurons originate in the dorsal column nuclei and project into the dorsal side of the brain stem forming the internal arcuate tract. These

axons then cross the midline to form the medial lemniscus tract. When passing through the medulla, the ventral portion of the medial lemniscal axons carry information from the lower limbs, and the dorsal portion carries information from the upper limbs. The medial lemnsicus axons reach the ventral posterior lateral nucleus of the thalamus and from here are classified as the third order neurons of the DCML system. Another ascending tract involved in sensory perception is the spinocerebellar tract. The spinocerebellar tract carries proprioceptive information from the lower limb to the cerebellum.

The primary somatosensory cortex is located in the postcentral gyrus and is defined by Brodmann area 3a and b, 1, and 2 [121]. The ventral posterior nucleus is the main source of afferent fibers to the first somatosensory area. These thalamocortical projections travel through the internal capsule and cerebral white matter, carrying information for various sensations. The thalamocortical fibers for cutaneous sensation terminate preferentially in the first somatosensory area. Fibers for deep sensations, such as proprioception, end in more posterior aspects. The posterior parietal cortex, defined by Brodmann's area 7, also integrates this information [141]. The posterior parietal cortex is also important for visual-proprioceptive information integration. Lesion-to-function studies have identified that the parietal and temporal lobes are also important in the integration of proprioceptive information [142,143].

The primary somatosensory cortex and M1 have reciprocal connections that may be involved in coordinated movements [144]. U-shaped fibers running under the central sulcus connect the motor and somatosensory homunculi and follow topographic organization [145]. Previous investigations have identified a large number of connections between regions responsible for

finger movements compared to areas responsible for control of other body parts. These connections play an important role in motor control. The inactivation of the somatosensory cortex in monkeys resulted in deficits in fine motor control and an inaccurate control of grip forces [146,147]. When tactile sensation was removed through digital anesthesia in humans, there was inappropriate coordination of the thumb and finger movements demonstrating that connections between somatosensory cortex and M1 may play a crucial role in grasping movements [148]. Another study in humans demonstrated that the integrity of the somatosensory cortex and M1 connections correlated with motor skill performance on the PPT [149].

The cerebellum is involved in coordinating motor function and interpreting proprioceptive signals. Other structures such as the basal ganglia, which refers to a group of subcortical nuclei (corpus striatum, substantia nigra, and subthalamic nucleus), and cingulate motor areas are also involved in motor control [150].

1.2.7 Robotics

Understanding the behavioural effects of neuromodulation requires specific outcome measures. Clinical tools have been used in the past to quantify sensorimotor functions, however they suffer from many shortcomings including poor inter-rater reliability and a lack of sensitivity to small but important changes in function [151–153]. Modern robotic technology can now be used to more precisely quantify sensorimotor function in real time with a potentially profound impact on how we assess brain dysfunction and modulation [154–157].

The Kinesiological Instrument for Normal and Altered Reaching Movements (KINARM) can reliably monitor and manipulate upper limb movements [158]. This tool offers more accurate, objective, and reliable measures of arm sensorimotor function compared to clinical measures [151,154,155,157,159,160]. Additionally, this assessment tool provides continuous measures that appear to not have a ceiling or floor effects in contrast to current clinical measures. The KINARM has been described in a variety of adult clinical populations, most often stroke, where it has demonstrated utility in quantifying proprioceptive and other sensorimotor deficits [155,157].

The KINARM has a series of standardized assessments that can quantify proprioception. Proprioception can be quantified by the position matching and kinesthesia tasks. The position matching task can quantify position sense function through a variety of parameters such as variability, movement area, and spatial shift [155]. Participants passive arm (the arm being assessed) was moved to a variety of spatial locations and the active arm mirror matched the position [155]. This task can be performed with or without vision. Approximately half of stroke affected adults have position sense deficits, as assessed by the KINARM, demonstrating the need for rehabilitation [155]. The kinesthesia task quantifies a variety of kinesthetic functions such as spatial and temporal aspects such as response latency, peak speed ratio, initial direction error, and path length ratio [157]. The standard deviation across each parameter can be calculated to explore variability. In this task, the robot moves the passive arm to one of three spatial locations at a defined speed, direction, and magnitude, and the participants mirror match the speed and direction of this movement. In an adult stroke population, participants had difficulty matching the movement direction and magnitude [157]. Both tasks demonstrated good inter-rater

reliability [155,161]. A different robotic instruments using other tasks have also identified proprioceptive deficits in adult stroke participants compared to healthy controls [162]. Studies such as these demonstrate that the KINARM is a valuable tool that is capable of assessing and quantifying position sense.

The use of robotics to measure motor function in visually guided reaching has also been explored in the adult stroke population [154,163]. This task is highly correlated with activities of daily living scores such as the Functional Independence Measure [163,164], suggesting clinical relevance. The visually guided reaching task is capable of differentiating reaching movements into two components: initiating (feed-forward control) and corrective movements (feedback control). Visually guided reaching requires participants to stabilize their hand on a visual target and when prompted with a new visual target, move their hand into the new target location. Coderre et al.[154] examined the use of the KINARM visually guided task in subacute adult stroke participants and quantified differences in mild to moderate stroke severity compared to controls. This group found the task to have good inter-rater reliability. Past reports have suggested a link between position sense and motor recovery after stroke [165–167], however there has been conflicting results [168]. More recent work has identified that position sense deficits are independent from motor impairments [164].

A third task, the KINARM object hit task, also quantifies bilateral arm sensorimotor function. The task difficulty is increased over time to examine visuospatial attention and increased cognitive demand [169]. In the task, balls fall from the top of the screen and the participant hits

as many balls away from them as they can. Compared to healthy controls, the majority of adults suffering from stroke have impaired task performance [169].

There has been recent work examining the utility of the KINARM in quantifying the sensorimotor deficits of perinatal stroke affected children compared to typically developing controls [170–172]. There has been a large amount of research focused on motor deficits in this population, but accurately defining sensory dysfunction remains elusive. These studies identified that children with perinatal stroke have impaired position sense and kinesthesia when compared to healthy children [170,171]. Furthermore, children with arterial strokes showed more severe impairments than those with venous strokes. These impairments persisted when vision was restored. In perinatal stroke, the lesioned DCML tract integrity correlated with impairments in proprioception. This clear link between an anatomical and functional impairment suggests that the cortical sensory network may be a target for non-invasive brain stimulation [173]. Visually guided reaching is impaired in the affected limb of perinatal stroke children compared to controls [172]. The degree of impairment seen in both robotic and clinical measures in this population is correlated with diffusion properties in the lesioned CST. These findings highlight that the KINARM displays some degree of sensitivity in its ability to quantify and discriminate proprioceptive function, and therefore may be an appropriate tool in assessing sensorimotor function in children undergoing neuromodulation.

The utility of the KINARM has also been demonstrated in other clinical pediatric populations such as traumatic brain injury (TBI). Participants had impairments in object hit, suggesting possible impairment in their ability to shift attention to new visual stimuli [174]. Additionally,

participants with TBI tended to have problems in visually guided reaching such as difficulty in movement initiation. There were however no significant position sense impairments. A larger study utilizing the end-point KINARM robot explored sensorimotor and cognitive changes after concussion [156]. There was no overall difference of performance in children with concussion compared to controls. The re-test reliability was examined across the KINARM end-point robot in children [175]. This study identified intra-class coefficients ranging from moderate to high in most parameters. However, there was a significant learning effect observed in certain tasks such as the object hit task. These studies confirm that certain KINARM measures can be used to assess changes in proprioceptive function over time, without concern that changes in KINARM measures are attributed to learning of the KINARM tasks.

Robotic technology can also be used to apply novel mechanical loads to a limb in order to quantify how the limb learns to move with that load [176–179]. This force field adaptation is a form of motor learning. In these studies, the KINARM was used to examine the sensory and motor feedback from varying loads being applied to an elbow joint [179]. They identified an overlap between sensory input and motor output, which may be an important contributor to motor learning and adaption. Another study utilizing a KINARM force field reaching task examined the role of online visual feedback on acquiring and retaining motor skills [180]. This study found that online visual feedback may help increase the rate of learning and performance on reaching tasks. These studies demonstrate the utility of the KINARM in assessing physiological function in healthy subjects.

In summary, the KINARM provides a unique opportunity to quantify complex neurophysiological processes and sensorimotor functions in the developing brain. The KINARM appears to be sensitive in that it can detect small changes in proprioceptive function that differ across various clinical conditions. Additionally, the high intra-class and inter-rater correlations seen in certain tasks suggests that it can be a valid tool in interventional studies, where it can quantify baseline proprioceptive function, and then reexamine changes following an intervention. Therefore, the KINARM is a valuable tool that may be used to examine changes in proprioceptive function in children undergoing motor learning enhanced through tDCS.

1.2.8 Translational application of tDCS-enhanced motor learning: Perinatal stroke

The perinatal period ranges from 20 weeks gestation to the first 28 days of life and is a high period of risk for a focal vascular injury. The first week of life carries the most focused lifetime risk for ischemic stroke [181]. Perinatal stroke is the leading cause of hemiparetic CP affecting 10,000 Canadian children [182]. Most perinatal stroke survivors will suffer from motor impairments but sensory dysfunction is also common [170,183]. The consequence is lifelong physical disabilities, which have adverse effects on quality of life for both the child and family.

Currently, there are limited therapies for sensorimotor disabilities. Previous literature in adult stroke and congenital hemiplegia trials have demonstrated significant effects of constraint-induced motor therapy (CIMT) [184]. This therapy involves constraining the less affected or non-paretic arm through intensive, structured manual therapy [185]. However, the efficacy of CIMT in hemiparetic CP has modest effect sizes and the mechanism is not fully understood [186,187].

Early focal brain injury models in animals and humans have identified cortical targets for neuromodulation including the non-lesioned M1 [4,182]. A general strategy towards improving post-stroke reorganization includes enhancing activity in the lesioned M1 or inhibiting the influence of the contralesional M1. Non-invasive brain stimulation has demonstrated utility in modulating motor function in adults with stroke-induced hemiparesis. The use of low-frequency repetitive TMS (rTMS) of the contralesional M1 may improve motor function [188,189]. Combining therapies such as CIMT and rTMS, may work synergistically. For example, a perinatal stroke trial demonstrated improved motor function using CIMT and contralesional rTMS [187]. However, rTMS is limited in its use because it cannot be simultaneously applied with therapy.

As previously discussed, the use of tDCS can alter cortical excitability, enhance motor learning, and be paired with motor therapy [83]. The use of contralesional cathodal tDCS may improve motor function in adult stroke trials [190,191], and the efficacy of tDCS in perinatal stroke has promise. The combination of non-invasive brain stimulation, such as tDCS, with CIMT has potential to improve motor function [97]. The lack of a mechanistic understanding of how tDCS enhances motor learning has limited the optimization of stimulation parameters to improve function most effectively.

1.3 Rationale and Aims

We have recently demonstrated that tDCS is safe and can produce lasting enhancement in motor learning in children [96]. While exciting, there is limited understanding of how such

improvements occur such as alterations in sensory motor function. The effects of HD-tDCS on motor learning have not been investigated in children, and the efficacy and safety require further exploration. Understanding the effects and mechanisms of tDCS and HD-tDCS on motor learning in children will advance clinical trials and new therapies for disabled children with CP.

Therefore, we propose the following aims:

Aim 1: Characterize the effects of tDCS and HD-tDCS on motor learning in healthy children.

Hypothesis 1: tDCS and HD-tDCS are associated with greater motor learning gains when compared to training alone (sham tDCS).

Aim 2: Characterize the changes in sensorimotor function induced by tDCS and HD-tDCS enhanced motor learning in children.

Hypothesis 2: tDCS is associated with improved position sense as measured by end point variability in the position matching task. HD-tDCS is associated with improved motor function as measured by visually guided reaching.

Aim 3: Assess the safety and tolerability of HD-tDCS in children.

Hypothesis 3: HD-tDCS is safe and well-tolerated in healthy children.

Chapter 2 – Effects of High-Definition and Conventional Transcranial Direct-Current Stimulation on Motor Learning in Children

The following work was submitted to Cerebral Cortex (Cole L*, Giuffre A*, Ciechanski P, Carlson HL, Zewdie E, Kuo H-C, Kirton A)

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2.1 Abstract

This study examined the effects of transcranial direct-current stimulation (tDCS) and high-definition tDCS (HD-tDCS) on motor learning in healthy children. Twenty-four children were recruited for a randomized, sham-controlled, double-blinded interventional trial to receive 1) right hemisphere (contralateral) primary motor cortex (M1) 1mA anodal conventional 1x1 tDCS (tDCS), 2) right M1 1mA anodal 4x1 HD-tDCS (HD-tDCS), or 3) sham. Over five consecutive days, participants trained their left hand using the Purdue Pegboard Test (PPT_L). The Jebsen-Taylor Test, Serial Reaction Time Task, and right hand and bimanual PPT were also tested at baseline, post-training, and 6-week retention time (RT). Both the tDCS and HD-tDCS groups demonstrated enhanced motor learning compared to sham (tDCS p=0.042, HD-tDCS p=0.049) with effects maintained at six weeks. Effect sizes were moderate-to-large for tDCS and HD-tDCS groups at the end of day 4 (Cohen's d tDCS=0.960, HD-tDCS=0.766) and day 5 (tDCS=0.655, HD-tDCS=0.851). Enhanced motor learning effects were also seen in the untrained hand. HD-tDCS was well tolerated and safe with no adverse effects. HD-tDCS and tDCS may advance motor rehabilitation therapies for children with motor disabilities such as cerebral palsy.

2.2 Introduction

Transcranial direct stimulation (tDCS) is a form of non-invasive brain stimulation with the potential to modulate cortical excitability, human brain function, and behaviour. Such promise has advanced studies across diverse brain disorders with over 33 000 sessions completed [7]. Safety and tolerability are well defined but mechanisms are poorly understood though both preclinical and human evidence suggests long-term potentiation (LTP)-like mechanisms are

involved [35]. As is often the case with emerging therapeutics, pediatric populations have been neglected in tDCS research where <2% of published studies have been dedicated to the developing brain.

The primary motor cortex (M1) is a key structure in motor skill learning and can be purposefully modulated with brain stimulation [192]. The application of anodal tDCS over M1 increases cortical excitability [2] and, when paired with training of the contralateral hand, has been demonstrated to improve motor performance within single [79,193,194] and multiple [83] sessions. Cathodal stimulation of the ipsilateral M1 can also improve motor skill acquisition, presumably via effects on transcallosal, interhemispheric motor networks [4]. Recently, we demonstrated that such M1 tDCS approaches can also enhance motor learning in healthy school-aged children over three days of training with retained effects and large effect sizes [195]. Stimulation was well-tolerated with no adverse events. Mechanistic investigations of tDCS in the developing brain have been lacking, however limited evidence suggests that the electric fields induced in the pediatric brain differ from those of adults [35,92,95,196].

Traditionally, tDCS has been applied using two large electrodes (1x1 tDCS). More recently, high-definition tDCS (HD-tDCS) montages have attempted to achieve more focal current delivery to better target functional cortical regions. By placing a central anode surrounded by four cathodes, such 4x1 HD-tDCS stimulation can be applied in a relatively focused manner with generation of stronger regional electric fields [25]. A recent adult study found that HD-tDCS M1 stimulation tended to increase motor adaptation within a single session [197]. A multi-day adult study applying bi-hemispheric M1 HD-tDCS paired with unimanual and bimanual motor training

also showed improvement in bimanual hand dexterity [89]. To date, HD-tDCS has not been examined in a pediatric population.

The ease of application and favorable tolerability of tDCS has promoted its early translation toward childhood disability and cerebral palsy (CP). Perinatal stroke is the leading cause of hemiparetic CP and affects more than 5 million people worldwide [198]. As a focal injury of defined timing in an otherwise healthy brain, perinatal stroke is an ideal human model of developmental plasticity where models have identified M1 as a potential target for therapeutic neuromodulation [199]. Most children with perinatal stroke suffer lifelong sensorimotor disabilities for which there are few effective treatments [183]. Although the underlying models are different, many trials in adult stroke hemiparesis suggest tDCS may facilitate motor rehabilitation [200,201]. Early trials of contralesional tDCS in children with perinatal stroke and hemiparesis have suggested favorable tolerability and safety with preliminary evidence of efficacy [97,106]. There is a pressing need to optimize tDCS-enhancement of motor learning in pediatrics to advance new therapies and better outcomes for disabled children.

We therefore conducted a sham-controlled, double-blind, randomized trial to compare the effects of M1 tDCS and HD-tDCS on motor learning in typically developing school-aged children.

2.3 Materials and Methods

2.3.1 Trial design and participants

Accelerated Motor Learning in Pediatrics (AMPED) was a randomized, double-blind, single-center, sham-controlled interventional trial. The study was registered at clinicaltrials.gov prior to

consent of the first participant (NCT03193580). The study was approved by the University of Calgary Research Ethics Board (REB16-2474).

Participants were recruited through community and school outreach programs and the Healthy Infants and Children Clinical Research Program (HICCUP), a population-based research cohort. The inclusion criteria were: 1) age 12-18 years, 2) right handed (self/parent report and Edinburgh Handedness Inventory laterality index greater than -28), 3) typical neurodevelopment, 4) no major medical conditions, and 5) informed consent/assent. Persons with neuropsychological or developmental diagnoses, implanted metal or medical devices, or who were taking neuropsychiatric mediations were excluded. At the time of enrollment, all participants or their guardians provided written informed consent/assent forms and were screened to meet safety criteria for non-invasive brain stimulation [202].

Each participant underwent the same testing, training, and stimulation procedures over five consecutive days. The complete study design and flow is shown in Figure 2.1.

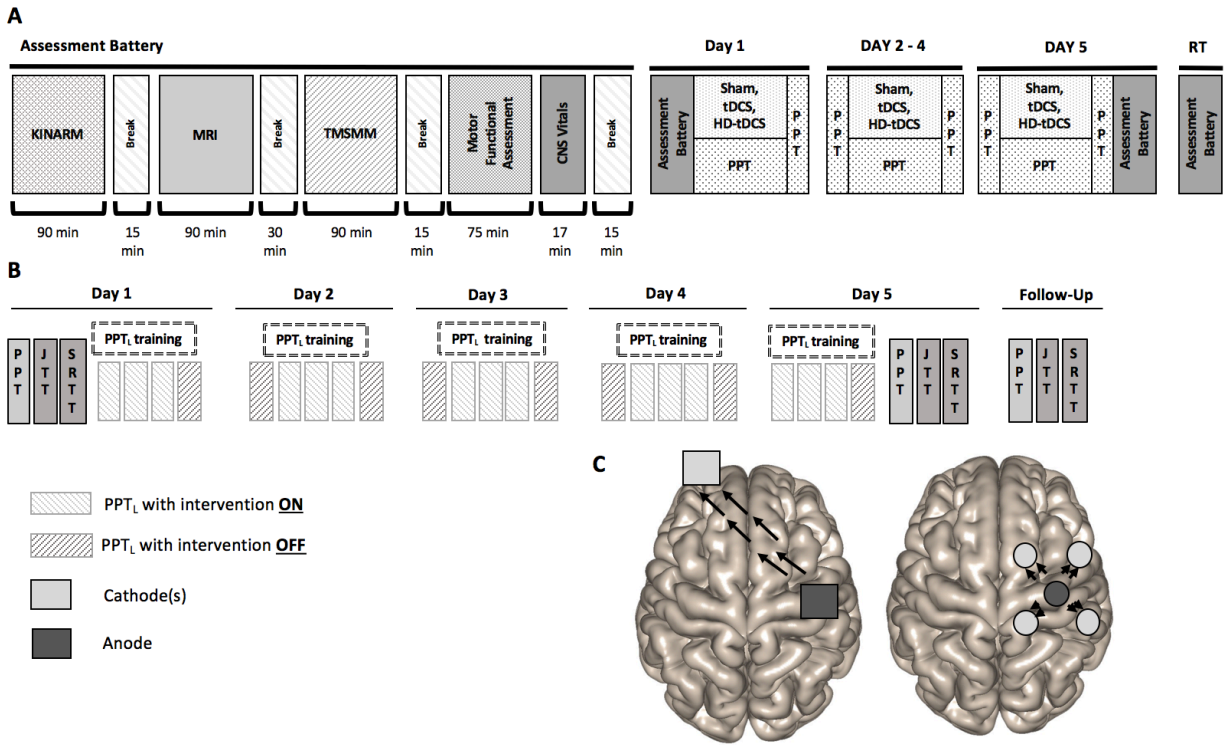

Figure 2.1 AMPED protocol. ***(A)*** *Participants received an MRI, complete tasks in a virtual reality KINARM robotic system, received TMS motor mapping (TMSMM), completed a series of motor assessments and then received training paired with non-invasive brain stimulation interventions. On days 2-4, subjects perform the PPT during intervention. Participants repeat the Day 1 tasks on Day 5 (with training) and at a six week retention testing follow up (RT).* ***(B)*** *PPT training is paired with stimulation by treatment groups with electrode montages* ***(C)*** *shown for tDCS (left) and HD-tDCS (right) where dark grey electrodes are anodes and light grey electrodes are cathodes. Black arrows represent the direction of current flow from anode to cathode(s).*

2.3.2 Randomization, blinding, and concealment

Following enrollment, participants underwent computer-generated randomization without stratification or minimization to one of three brain stimulation groups (1:1:1) using the Research

Electronic Data Capture randomization tool (REDCap). The groups were 1) right hemisphere (contralateral to the trained hand) M1 1mA anodal conventional 1x1 tDCS (tDCS), 2) right hemisphere M1 1mA anodal 4x1 HD-tDCS (HD-tDCS), and 3) sham. Participants and their parents were blinded to treatment assignment consistent with previous pediatric trials of tDCS [195]. A post-interventional questionnaire was administered requiring participants to guess which intervention they received and why. Only the investigator administering the stimulation was aware of the treatment group assigned. The remaining investigators were not present during stimulation and remained blinded during administration and scoring of all outcome measures. Treatment assignment remained blinded until study closure and final data analysis.

2.3.3 Motor learning measures

The primary motor learning measure was the Purdue Pegboard Task (PPT), a validated measure of hand dexterity commonly used in motor learning studies [86,203]. The PPT consists of four subtests, the first three requiring participants to place as many pegs as they can in a pegboard in 30 seconds with their left hand (PPT_L), right hand (PPT_R), and bimanually (PPT_{LR}). The total number of pegs was recorded. The final subtest was a bimanual assembly task where participants build as many structures as they can by placing a peg–washer–pin–washer complex in 60 seconds (PPT_A). The PPT_L was used to train and measure motor learning as it is a difficult enough task for children to learn without reaching a skill "ceiling" effect, producing optimal learning curves. The PPT_R was both a safety outcome (to ensure no decrease in function from receiving tDCS or HD-tDCS) and a measure of possible "spill-over" effects to the untrained hand.

Secondary motor outcomes assessed different components of motor function that were not trained. The Jebsen-Taylor Test of Hand Function (JTT) was used to assess six subtests of unimanual hand functions performed during daily activities: 1) card turning, 2) picking up small objects (pennies, paper clips, and bottle caps) and placing them in a container, 3) stacking checkers, 4) simulating feeding, 5) moving light objects, and 6) moving heavy objects. The fourth test is considered to be highly variable between individuals, and was therefore excluded from final analysis [204]. The six subtests were performed with the left hand first, followed by the right hand. All subtests are timed (seconds) and summed to generate a total score with lower scores corresponding to better motor function.

The Serial Reaction Time Task (SRTT) was used to measure reaction time and implicit motor learning [49,205]. The SRTT used was custom built using PsychoPy software [206]. Participants were seated in front of a computer and instructed to place the four fingers of their left hand on the keyboard buttons A, S, D, and F. A visual cue (green box) would appear at any one of the four positions on the computer screen corresponding to a keyboard button, which the participants were instructed to press as fast as possible. The test is divided into a total of eight trials, each containing 96 keystrokes. Trials 1 and 6 are a randomly generated string of keys, whereas trials 2-5, 7, and 8 have a 12 characters pattern that repeats itself eight times. A 250ms delay separated the keystrokes. Reaction times <200ms were excluded, as these may not indicate reaction based responses consistent with previous studies [193] Additionally, we also included all values in a separate analysis to explore potential differences that may have occurred in reaction times >200ms.

2.3.4 Intervention: tDCS and HD-tDCS

Participants received direct-current stimulation or sham during each training session using either a conventional 1x1 tDCS or a 4x1 HD-tDCS system (Soterix Medical Inc., New York, USA). Each participant's right M1 was localized and marked using neuronavigation (Brainsight2, Rouge Research Inc., Montreal, Canada). Robotic single-pulse transcranial magnetic stimulation (TMS) was used to localize the "hotspot" for the first dorsal interosseous (FDI) muscle of the left hand using established criteria [207]. The scalp location corresponding to the hotspot was marked and re-marked each day using a felt pen for consistent electrode placement.

For tDCS and sham, two saline-soaked sponge electrodes (25 cm^2, SNAPpad, Soterix Medical Inc., New York, USA) were applied to the scalp with the anode centered over the localized right M1 while the reference electrode (cathode) was placed over the contralateral supraorbital area (Figure 1C). The electrodes were held in place with a light plastic "headband" (SNAPstrap, Soterix Medical Inc., New York, USA) sized for pediatric subjects. This was the same montage targeting training of the left hand described in both adult and pediatric tDCS motor learning studies [76,79,83,195]. The electrodes were then connected to a 1x1 DC SMARTscan Stimulator (Soterix Medical Inc., New York, USA).

For HD-tDCS, the montage targeted the same right M1 as described elsewhere [208–210]. The investigator applied a "cap" with pre-existing electrode holes on the participant's head. The active electrode (anode) was centered over the right M1 with the four return electrodes (cathode) spaced ~5 cm away to establish a ring-like orientation (Figure 1C) [26,27,211]. Such a 4x1 electrode ring has been predicted to target stimulation with maximal electric field strength under

the target electrode [209]. The electrodes were then connected to a 4x1 HD-tDCS Adaptor and a SMARTscan Stimulator (Soterix Medical Inc., New York, USA).

During active stimulation for both groups, current was initially ramped up to 1mA over 30 seconds. Stimulation was maintained for 20 minutes with automatic continuous current-control by the Soterix system (current control) and expert personnel. The current was then ramped down to 0mA over 30 seconds. Current density was 0.04mA/cm^2 (1.0mA of current intensity), and was current-controlled, based on sampling of resistance and changing the voltage to maintain a constant current. In the sham group, current was also initially ramped to 1mA over 30 seconds but then returned to 0mA where it remained for 20 minutes. During the final 30 seconds, the current ramped up to 1mA and back to 0mA. This sham procedure produces the same initial transient scalp sensations and has been validated in subjects naïve to tDCS in adults [212].

2.3.5 Training protocol

The sequence of motor measures, motor training, and stimulation are summarized in Figure 1B. Participants had the option to complete all tasks within a single session on Day 1 (~8 hours) or could split up Day 1 into two consecutive days (~4 hours each), always ending with stimulation. On Day 1, baseline skill was measured by completing all three motor tasks. Participants first performed all subtests of the PPT, followed by the JTT and the SRTT. Each participant then trained the PPT_L while receiving either tDCS, HD-tDCS, or sham at the end of Day 1. Training occurred over 20 minutes of stimulation, consisting of three trials per epoch performed at minute 5, 10, and 15. After stimulation, the electrodes were removed and participants completed a safety

and tolerability questionnaire (below). The PPT_L was then performed again (with no stimulation device worn).

On days 2, 3, and 4, participants performed the same PPT_L sequence, beginning with a baseline test followed by the same 20-minute training protocol during stimulation. On Day 5, participants repeated all assessments performed on Day 1, starting with the same training protocol. Participants returned 6+/-1 weeks later and performed the same order of assessments as Day 1.

Each assessment was video-taped and re-scored again by a blinded study team member for quality assurance and inter-rater reliability assessments. Videos only recorded the individual's hands, so the stimulation montage could not be identified by the rater. Learning curves generated for the PPT_L compared the score difference at each training point with the baseline score measured on Day 1. Skill decay was measured by comparing the 6-week follow-up average score with the Day 5 post-training score. Online effects (within-day training) were determined by comparing baseline and final scores of each day. Offline effects (consolidation) were measured by comparing baseline scores each day to the final score from the previous day. Average scores were also calculated as the average change in PPT_L score from Day 1 baseline for each day.

2.3.6 Safety and tolerability

Participants completed a modified pediatric non-invasive brain stimulation safety and tolerability questionnaire (Garvey et al. 2001) immediately following each training session (days 1-5). The duration and severity of any symptoms were reported including headache, neck pain, unpleasant tingling, itching, burning, fatigue, nausea, and lightheadedness. In addition, participants were

asked to rank the tolerability of their tDCS session compared to seven common childhood experiences. As the first study of HD-tDCS in children, a neuropsychological battery was also completed at baseline and following the final stimulation session. A validated, computerized neurocognitive assessment software (CNS Vital Signs) evaluated the neurocognitive of status of the participants throughout the study [215]. An interim safety analysis was conducted by two blinded researchers after the first eight subjects to exclude any drop-in motor function (reduction of PPT score) of either hand or serious adverse events possibly associated with intervention.

2.3.7 Sample size

Previously published evidence of tDCS-enhanced motor learning in children [195] suggested moderate-to-large effect sizes of tDCS while defining typical means and variances for learning curves. In this study, participants in the sham condition improved their PPT_L by 1.83 pegs and participants in the anodal tDCS condition improved by 1.5 to 2-fold compared to sham. The mean standard deviation for this sample was 0.71. Combining these with α=0.05 and power of 85% estimated a total requirement of 24 participants (8 per stimulation group).

2.3.8 Statistical analysis

For the primary hypothesis that change in PPT score would be greater in tDCS compared to HD-tDCS and sham, a linear mixed model analysis was employed (SPSS, IBM, Armonk, NY, USA). A linear mixed effects model for PPT_L score was performed with fixed effects for Group, Day, and the interaction of Group and Day and covariate for Subjects to account for repeated measures. Analysis for secondary outcomes (SigmaPlot 12.5, Systat Software Inc; San Jose, USA) for continuous variables included one-way analyses of variance (ANOVA) and Chi-

square/Fischer exact test (for dichotomous variables) to compare group demographics, baseline motor scores, and tolerability ratings. Paired t-tests analyzed differences in left and right-hand baseline motor scores, skill decay between final training block versus six week follow-up, and online versus offline effects. Effect sizes were reported as Cohen's *d*. Repeated measures ANOVA was used to analyze changes in JTT and SRTT scores. Tolerability scores across groups were compared using a one-way ANOVA. Holm-Sidak post-hoc corrections were performed to correct for multiple comparisons. To examine possible effects of baseline function on intervention susceptibility, participants were divided into high and low performers based on baseline PPT_L score falling above or below the median.

2.3.9 Replication

To evaluate replicability of previous studies while increasing the power of the current study, we also combined our data with that of a previous, similar trial [195]. Both studies had the same inclusion criteria and applied right M1 tDCS (1mA) or sham during 20 minutes of PPT_L training over three consecutive days. Accordingly, learning curves over three days of training from sham controls and anodal tDCS groups were combined (n=14 for both groups). The linear mixed modeling analysis described above was repeated with the combined data.

2.4 Results

2.4.1 Population characteristics

Twenty-four participants were recruited (median age 15.5 years, range 12-18, 52% female). All participants completed all stages and outcome assessments with no drop-outs. Demographics and baseline motor function across intervention groups are shown in Table 1. Age, sex, handedness,

and baseline PPT, JTT, and SRTT scores did not differ between intervention groups (all p>0.3). All groups demonstrated higher PPT_R compared to PPT_L scores (p<0.001).

Table 2.1 Participant demographics and baseline motor function by intervention group

				Baseline PPT Scores			Baseline JTT Scores				
Stimulation Group	**Age (Years)**	**Laterality Index**	**Sex F:M**	**Left Hand**	**Right Hand**	**Left vs Right *p* value**	**Left Hand**	**Right Hand**	**Left vs Right *p* value**	**Baseline Reaction Time Excluded (ms)**	**Baseline Reaction Time Included (ms)**
HD-tDCS	14.77 (+/-2.0)	81.25 (+/-14.7)	4:4	13.91 (+/-1.9)	15.79 (+/-1.55)	*p*<0.001	21.91 (+/-2.1)	20.23 (+/-2.2)	*p*=0.053	540 (+/-82.6)	540 (+/-82.6)
tDCS	15.94 (+/-1.5)	82.5 (+/-13.1)	3:5	13.50 (+/-1.3)	15.21 (+/-1.9)	*p*=0.011	22.92 (+/-3.1)	20.63 (+/-2.9)	*p*<0.001	525 (+/-99.0)	525 (+/-98.7)
Sham	15.81 (+/-1.3)	81.9 (+/-22.8)	6:2	13.83 (+/-1.3)	15.16 (+/-1.9)	*P*=0.013	21.09 (+/-2.9)	18.92 (+/-1.9)	*p*=0.003	538 (+/-78.5)	550 (+/-99.2)
Mean	15.51 (+/-1.7)	81.88 (+/-16.6)	13:11	13.75 (+/-1.5)	15.4 (+/-1.7)	*p*<0.001	21.97 (+/-2.7)	19.92 (+/-2.4)	*p*<0.001	540 (+/-84.7)	544 (+/-91.8)
Between group	*p*=0.324	p=0.879	*p*=0.309	*p*=0.846	*p*=0.741	---	*p*=0.424	*p*=0.342	---	*p*=0.924	*p*=0.859

Purdue Pegboard Test (PPT), Jebsen-Taylor Test of Hand Function (JTT), Serial Reaction Time Test (SRTT). Values are group mean +/- standard deviation

2.4.2 Motor learning

Learning curves of similar overall morphology were generated across subjects and groups. Daily motor learning and daily average learning curves by intervention group are shown in Figure 2. Regardless of intervention, participants demonstrated motor learning over the five training days with the rate of learning dissipating over time (p<0.001). Linear mixed effects modeling demonstrated a significant interaction effect of day and intervention on the rate of learning. The model suggested that both active stimulation groups had enhanced learning (increase in pegs per day) compared to sham over the training period (tDCS p=0.042, HD-tDCS p=0.049). The sham group improved their PPT_L score by 0.508+/-0.190 pegs per day as compared to 0.703+/-0.269

for the tDCS group and 0.697+/-0.269 for the HD-tDCS group. At both day 4 and 5, the tDCS and HD-tDCS groups had larger improvements in the daily average PPT_L score compared to sham (both $p<0.05$). Moderate to large effect sizes were observed in the tDCS and HD-tDCS groups at the end of day 4 (Cohen's *d* tDCS=0.960, HD-tDCS=0.766) and day 5 (Cohen's *d* tDCS=0.655, HD-tDCS=0.851).

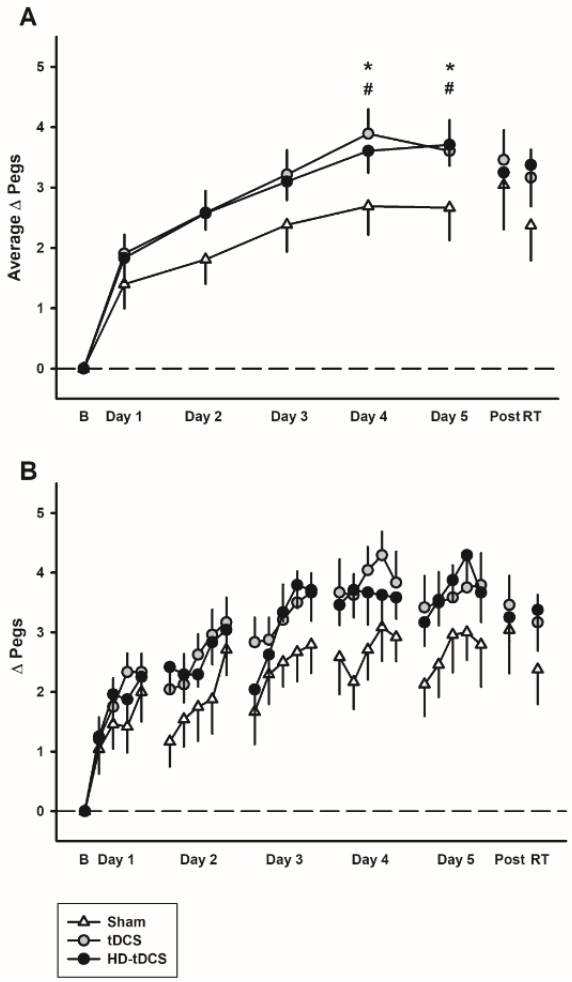

Figure 2.2 Motor learning by treatment group. (A) *The mean daily change from baseline (B) in left Purdue Pegboard (PPT_L) learning curves for sham (white triangles) were lower than both tDCS (grey circles) and HD-tDCS (black circles). Effects decayed by six week retention time (RT) for sham but not tDCS groups.* ***(B)*** *Daily mean scores per repetition of the PPT_L are shown*

*for the same 3 groups. Error bars represent standard error. *p<0.05 for tDCS vs. sham, # p<0.05 for HD-tDCS vs. sham.*

2.4.3 Retention

Learning effects were retained in the tDCS and HD-tDCS groups with no significant decrease in skill performance between end of training and the retention assessment. In contrast, significant skill decay in PPT_L scores was observed in the sham group (p=0.034). All groups scored higher at retention testing on the PPT_L compared with baseline (all p<0.003). At retention testing, there was no significant difference in PPT_L scores between intervention groups.

2.4.4 Online/Offline learning

Participants showed improvements in performance primarily through online learning (Figure 3). There were no differences seen between tDCS or HD-tDCS and sham in the amount of online learning. There was significantly more online learning compared to offline learning in all three groups (all p<0.003). Offline effects contributed to a significant loss of skill in the sham and HD-tDCS group (sham p=0.004, HD-tDCS p=0.046) but not the tDCS group (p=0.140).

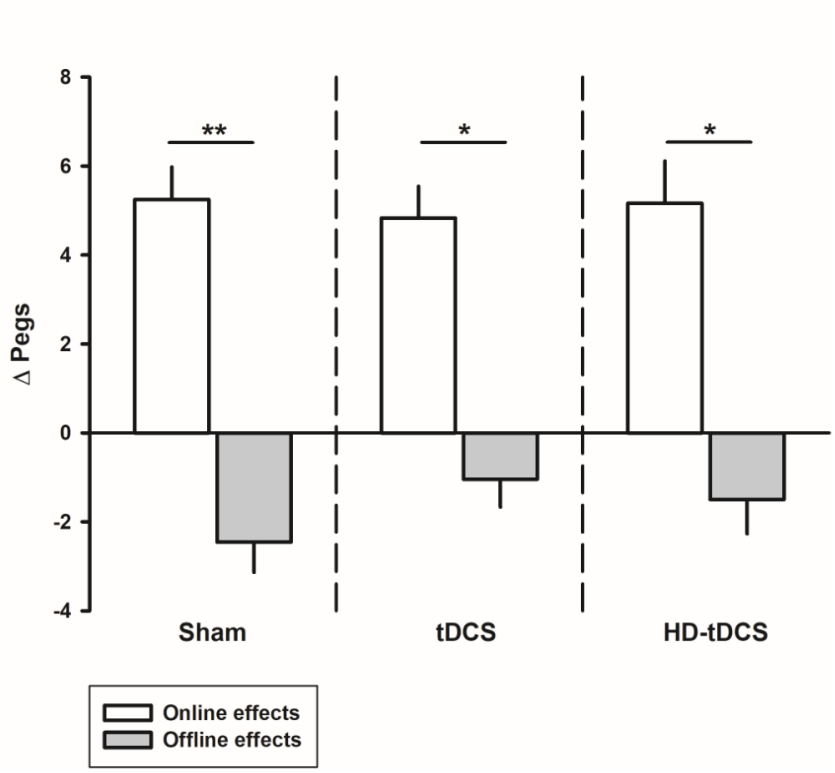

Figure 2.3 *Online and offline learning effects on left hand Purdue Pegboard (PPT_L) for the three intervention groups. The online effects represent the difference in PPT_L score from the first and last training point of the day. The offline learning represents the difference between the last training time point of the day to the first training point of the following day. Error bars represent standard error. *p<0.05, **p<0.01*

2.4.5 Low versus high performers

Participants were divided into high and low performers to explore if baseline motor skill was associated with response to intervention. The median baseline PPT_L score for all participants was 13.33 pegs. Eleven participants were above this score and classified as the high performer group and the remaining participants were classified as the low performer group (Figure 4). In the low performance group, participants in the tDCS and HD-tDCS groups demonstrated greater

improvements compared to sham. There was a significant difference between sham and HD-tDCS for all days of training (all $p<0.05$). The tDCS group had significantly more improvement compared to sham at days 2, 3, 4 and 5 (all $p<0.044$). There was a significant time effect over the five days of training in all intervention groups ($p<0.001$). The high performer group did not show any difference in learning across groups.

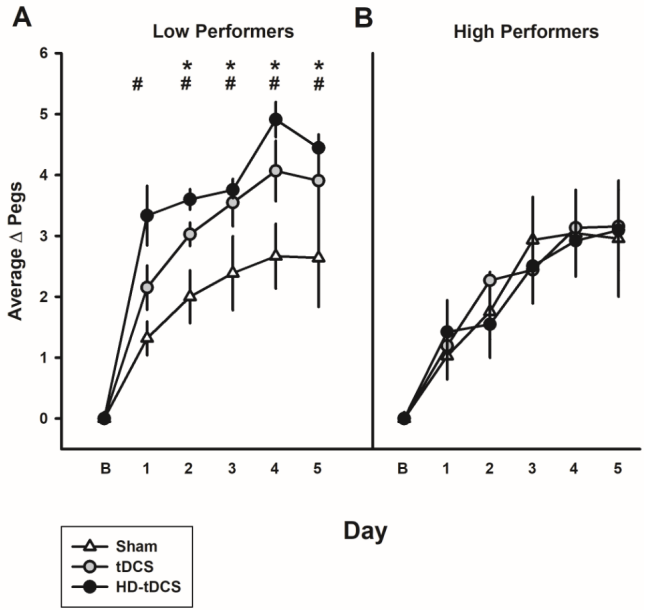

Figure 2.4 Effect of performance status on motor learning enhancement. (A) *Low performers (baseline PPT_L below the median score) demonstrated marked separation of PPT_L learning curves with tDCS (grey circles) and HD-tDCS (black circles) outperforming sham (white triangles).* ***(B)*** *Treatment group effects were not observed for high performers. B refers to baseline. Error bars represent standard error. *p<0.05 for sham vs. tDCS, #p<0.05 for sham vs. HD-tDCS.*

2.4.6 Secondary, untrained motor outcomes

The effects of intervention group on the secondary measures of hand function are shown in Figure 5. At post-training and RT, there was no difference in raw PPT_L versus PPT_R score for all groups despite the laterality seen at baseline. PPT_R scores increased following training in the tDCS and HD-tDCS groups (both $p<0.042$) but did not change in the sham group (Figure 5A, $p=0.076$). PPT_R scores were not correlated with PPT_L improvements ($r=0.266$, $p=0.208$). There was no decay in PPT_R scores at retention testing in all groups.

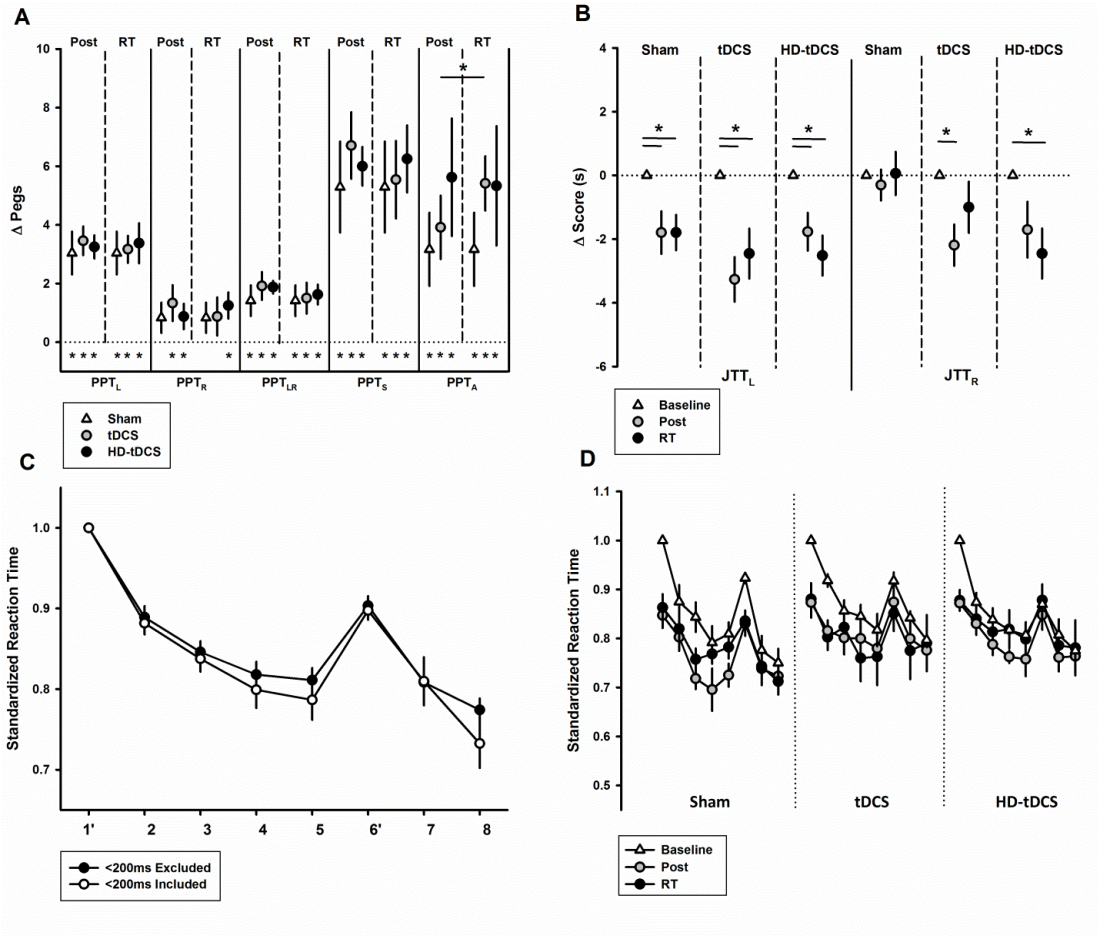

Figure 2.5 Secondary motor outcomes. (A) *Change in Purdue Pegboard Test (PPT) scores at post-training and retention time (RT) demonstrated treatment group effects for PPT_A. PPT subtests are left (PPT_L), right (PPT_R), bimanual (PPT_{LR}), sum of scores (PPT_S), and assembly (PPT_A). *$p<0.05$.* ***(B)*** *Jebsen-Taylor Test of Hand Function left and right (JTT_L, JTT_R) demonstrated treatment group effects bilaterally at post-training and RT.* ***(C)*** *Serial Reaction*

Time Task (SRTT) curves with and without <200ms responses are shown. Blocks 1 and 6 are random while all others follow a 12-character sequence. ***(D)*** *SRTT by intervention group with <200ms responses excluded. Error bars represent standard error.*

PPT_{LR} scores improved in all groups: sham ($p=0.016$), tDCS ($p=0.003$), and HD-tDCS groups ($p<0.001$) with no skill decay at six weeks. There was no significant difference between the three groups at post-training ($p=0.664$). PPT_{LR} scores at post-training were correlated with change in PPT_L ($r=0.564$, $p=0.004$). Regardless of group, participants showed improvements in PPT_S scores (all $p<0.006$), again without decay. There was also a significant improvement in PPT_A for all groups (all $p<0.03$) and no skill decay at retention testing. There was no significant difference between the intervention groups for change in PPT_A score ($p=0.506$). There was a significant improvement in PPT_A from post-training to retention in the tDCS group ($p=0.05$). There was no correlation between PPT_A and PPT_L improvements ($r=-0.032$, $p=0.881$).

Training effects on JTT performance are summarized in Figure 5B. There was a significant learning effect in the JTT_L across all treatment groups (all $p<0.008$). All three treatment groups improved their JTT_L scores from baseline to post-training ($p<0.003$) and baseline to retention testing ($p<0.019$).

In the untrained hand, JTT_R scores improved over time from baseline to post-training ($p=0.005$) and from baseline to retention testing ($p=0.019$). JTT_R scores significantly improved in the tDCS group from baseline to post-training ($p=0.016$) and in the HD-tDCS group from baseline to retention testing ($p=0.026$). However, in the sham group, there were no significant improvements in JTT_R scores ($p=0.857$).

The baseline SRTT curves with and without <200ms measures are summarized in Figure 5C, where a shift downward in the SRTT curves indicates improved reaction time. A negative correlation was observed between baseline reaction time and age ($r=-0.488$, $p=0.016$). To correct for age effects, the reaction time of each block was standardized to baseline Block 1 reaction time. When reaction times <200ms were excluded from the analysis, there was a significant learning effect from baseline to post training (both $p<0.051$) and baseline to retention (both $p<0.036$) in the sham and tDCS groups. When reaction times <200ms were included in the analysis, there was a visible downward shift in SRTT curves of all groups. There was a significant learning effect from baseline to post-training (both $p<0.010$) and baseline to retention (both $p<0.009$) in the sham and tDCS groups. Regardless of whether reaction times <200ms were excluded or included, there was no significant learning effect in the HD-tDCS group. There was no decay in reaction time seen at any time-point or in any stimulation group.

2.4.7 Replication

The results of the combined PPT_L dataset for three days of training for sham (n=14), tDCS (n=14) and HD-tDCS (n=8) are shown in Figure 6. There was no significant difference in learning between either the sham groups ($p=0.402$) or 1x1 tDCS ($p=0.980$) groups from both studies. For the combined data, linear mixed modeling showed a significant interaction effect of day and intervention group (rate of learning). Effect sizes were larger than the primary study alone with sham participants placing 0.666+/-0.226 more pegs each day compared to 1.04+/-0.375 more pegs for tDCS and 1.00+/-0.320 more pegs for HD-tDCS. The tDCS and HD-tDCS group outperformed the sham group in terms of the rate of pegs placed over the course of three

days (tDCS p=0.001, HD-tDCS p=0.012). Effect sizes for the combined group at the end of Day 3 were large for tDCS (Cohen's *d*=1.265) and HD-tDCS group (Cohen's *d*=0.995).

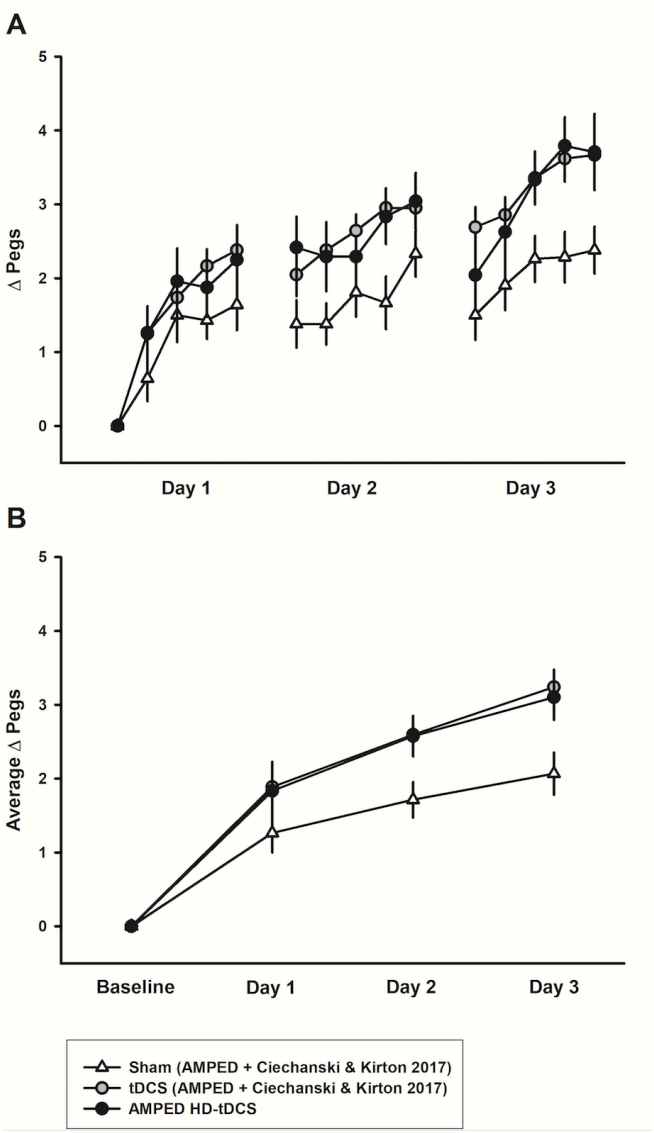

Figure 2.6 *Combined PPT_L training data for sham and tDCS groups over 3 days.* ***(A)*** *Sham (white triangles, n=14) learning curves were inferior to both tDCS (grey circles, n=14) and HD-tDCS (black circles, n=8) groups.* ***(B)*** *Mean daily learning for the same three groups form the combined studies. Both the tDCS and HD-tDCS groups placed more pegs each day as compared to sham. Error bars represent standard error.*

2.4.8 Inter-rater assessment correlation

A high degree of reliability was observed between raters in PPT and JTT scoring. The average intraclass correlation was 0.996 [95% CI: 0.994-0.988 ($F(29,58)=276.265$, $p<0.001$)]. A similar degree of reliability was observed between raters of the JTT where average intraclass correlation was 0.974 [95% CI: 0.960-0.983 ($F(59,118)=38.136$, $p<0.001$)].

2.4.9 Safety and tolerability

A total of 120 tDCS sessions were performed without any complications or serious adverse events. The most common reported sensation was itching (56%) ranging from mild (75%) to moderate (25%) in severity. Itching was more common with tDCS (78%) compared to sham (48%, $p=0.006$) and HD-tDCS (43%, $p=0.001$). Additional sensations included: unpleasant tingling 24% (mild in 90%) and burning 37% (mild in 80%), neither of which differed by treatment group. In the sham group, most sensations (90%) lasted less than 2 minutes. Sensations reported in tDCS and HD-tDCS persisted for the duration of the stimulation in 23% of tDCS and 3% of HD-tDCS sessions. One HD-tDCS participant reported a headache lasting less than 2 minutes. One tDCS participant felt mildly nauseated in a single session. The tolerability ranking of sham was 3.8+/-1.1, tDCS was 4.1+/-1.0 and HD-tDCS was 3.9+/-1.2 on an 8-point scale, comparable to watching TV (2.4+/-1.0) or a long car ride (4.9+/-1.2). There was no significant difference in tolerability scores between the three intervention groups ($p=0.51$). Participants were unable to correctly predict their treatment group (56% accuracy, where 50% indicates a chance guess). All participants completed all cognitive assessments (Table 2). Baseline cognitive performance in all three domains was comparable across groups (all $p>0.70$). No changes in

cognitive function were found over the three time points for any group (all $p>0.07$) with the single largest drop being in visual memory for the sham group from post-training to retention testing.

Table 2.2 Group mean performance on the CNS Vital Signs neurocognitive battery

Neurocognitive Domain	**Participant group**		
Time point	Sham	tDCS	HD-tDCS
Visual memory			
Baseline	65.4 (20.0) [32-92]	67.4 (26.6) [18-92]	67.9 (25.2) [25-96]
Post	69.1 (19.9) [37-92]	75.0 (20.7) [45-96]	59.6 (24.7) [25-95]
Follow-up	43.5 (27.0) [7-92]	61.4 (26.3) [10-97]	65.3 (28.0) [14-88]
Reaction time			
Baseline	61.6 (35.0) [2-95]	53.9 (36.5) [3-95]	72.0 (17.4) [37-87]
Post	78.4 (12.3) [53-96]	61.3 (26.6) [30-96]	80.4 (18.9) [45-98]
Follow-up	68.1 (26.6) [10-96]	59.4 (34.1) [12-90]	66.4 (25.0) [14-94]
Simple attention			
Baseline	62.4 (19.0) [23-79]	60.4 (27.6) [13-79]	69.4 (11.1) [50-79]
Post	60.8 (19.5) [23-79]	57.5 (32.6) [2-79]	60.9 (24.2) [13-79]
Follow-up	56.4 (26.1) [1-79]	49.8 (32.0) [1-79]	73.4 (11.0) [50-79]

Values are mean percentiles with (SD) and [range], higher values indicate better performance. tDCS: conventional anodal tDCS, HD-tDCS: high-definition tDCS.

2.5 Discussion

We evaluated the ability of anodal conventional and HD-tDCS to enhance motor learning in healthy school aged children. Our findings suggest that application of both conventional and HD-tDCS is feasible, safe, and tolerable in children. We demonstrated that pairing non-dominant hand training with either tDCS or HD-tDCS of the contralateral M1 can enhance skill learning compared to training alone. Children with lower performance at baseline appeared to be more responsive to the effects of tDCS. Skill enhancement was not restricted to the trained hand or task, with spillover to untrained tasks and the untrained hand.

Improvement in motor learning with tDCS has been well established in adults. Multiple studies support that both single and multi-day application of tDCS concurrent to skill training produce extensive effects on motor learning [83–85]. Despite these promising findings, studies of pediatric applications of tDCS are limited. We identified a faster rate of learning in the tDCS and HD-tDCS group compared to sham. Our findings suggest that anodal conventional and HD-tDCS have a moderate effect on potentiating non-dominant hand skill learning. Training alone, as characterized by our sham tDCS group, demonstrated clear learning, however a skill ceiling was reach by the 4^{th} day of training. Furthermore, skill decay was observed six weeks following training in this group. These findings have not been reported previously [195] and suggest that PPT performance improvements may be susceptible to decay when trained without active tDCS. The concurrent application of anodal conventional or HD-tDCS appeared to prevent this decay of skill, as participants showed retention at the six-week follow-up.

Our findings suggest that conventional and HD-tDCS may enhance motor skill learning with retained effects. Motor learning is a process that involves both online and offline skill gains, where online effects are those that occur within the period of active training and/or stimulation while offline effects occur between training sessions (consolidation). Previous studies in adults suggested that motor learning occurs mainly through online effects but tDCS enhancement acted more selectively on offline consolidation [83]. Limited work in pediatric populations suggests that tDCS may enhance motor learning more by modulating online motor learning systems [195]. Our findings here may occupy a middle ground between these bodies of evidence whereby some degree of offline effect was suggested for tDCS and possibly HD-tDCS where between session decay appeared to be less pronounced as compared to sham. Extension of previous adult studies demonstrating effects of tDCS administered *after* training [82] have not been replicated in children and represent a potential avenue to further elucidate potential mechanisms.

Mechanisms of tDCS are difficult to study in humans with current knowledge being even more limited in the developing brain. Anodal tDCS may modulate neuronal excitability leading to increased spontaneous neuronal firing rates [8,12]. Such LTP-like enhancement and strengthening of neuronal activity between stimulated and distal locations, may be similar to natural motor learning processes [9]. Human studies suggest that tDCS paired with motor training may improve the efficacy of synaptic connections with lasting effects on cortical networks [38]. GABA systems are likely crucial in motor learning [35] and anodal tDCS may modulate M1 GABA in adults [91]. The use of advanced imaging before and after such trials may shed further light on the mechanisms of tDCS enhanced motor learning in children.

Motor learning and stimulation effects were not limited to the trained hand. Increases in motor cortical excitability [2], facilitation of motor performance [88,193,194,216] potentiation of the formation of motor memories [217], and improvement in motor learning of the contralateral hand have all been reported after application of tDCS over M1. Functional motor improvements of the untrained hand could be secondary to various mechanisms. The "callosal access" and "bilateral access" hypothesis proposes that practice-induced motor engrams developed in the dominant hemisphere that underlie performance of the trained hand are located in homologous regions that the opposite motor cortex can access via the corpus callosum, facilitating performance in the untrained limb (Anguera et al. 2007; Lee et al. 2010). Others have suggested that the improvement in the untrained hand reflects an increase of excitatory (or decrease of inhibitory) drive towards M1 [221]. Supporting evidence comes from studies using paired-pulse TMS to demonstrate suppression of intracortical inhibitory system after tDCS [221]. MR spectroscopy imaging studies have also shown a decrease in GABA at rest after tDCS applied over M1 [222]. Increases in brain-derived neurotrophic factor (BDNF) induced by tDCS have also been hypothesized to modulate neuroplastic potential with possible effects on the untrained hand [221].

Both pediatric and adult studies have also shown tDCS-related improvements in the untrained hand in hemiparetic and healthy individuals using the JTT [195,223,224]. Schambra et al, conducted a study where subjects received tDCS over the left or right M1 and trained the sequential visual isometric pinch task (SVIPT) and the SRTT [84]. Left M1 tDCS had moderate effects in the right and left hand while right M1 tDCS showed a moderate effect in the left hand and a small negative effect in the right hand. Pediatric data is limited but we have previously

shown that M1 tDCS is associated with improvements in both the trained task in the untrained hand as well as spillover to other untrained motor tasks [195]. Our findings in the current study further suggest that stimulation-enhanced training effects may be transferred to the untrained hand and bimanual skills. This transfer of skill was evident only in the anodal conventional and HD-tDCS groups, suggesting that cortical modulation may be necessary for the process to occur. Even though right hand assessments were performed hours after stimulation, changes in neuronal excitability may be sustained and play a role in affecting the untrained task [12,95]. Another possible theory for a transfer of skill included mirror visual feedback that affects M1 plasticity and enhances motor function [225,226]. These phenomena have been described in conventional tDCS but require further study, particularly in HD-tDCS and other emerging neurostimulation approaches.

The development of computational current models assists in understanding brain electric field strengths as well as important potential differences of tDCS in children (Datta et al. 2009; Kessler et al. 2013). Differences may be attributable to tissue structure of the skull and age-dependent differences in grey and white matter content and CSF volume that change dynamically through development [227]. Pediatric current modeling suggests that electrodes placed on M1 produce diffuse cortical effects including contralateral M1 and bilateral premotor and supplementary motor areas [92](Ciechanski et al. *under review*). In contrast, HD-tDCS has been found to produce more focal stimulation with peak electric fields approximating functional cortical targets directly under the active electrode [27]. Despite this potentially increased specificity, there have been few studies of HD-tDCS in motor learning and none in children that we are aware of. Improvement in motor behaviours in single [197] and multiday [89] HD-tDCS

training studies appear consistent with our findings here. Our direct comparison of conventional to HD-tDCS provides further insight, though implications for mechanism remain speculative. One simple interpretation would be that stimulation of M1 is most important for enhancing motor learning effects as both montages accomplished this, likely to a comparable degree. A different hypothesis was that the stimulation of larger areas of the motor network (e.g. premotor and supplementary motor areas) and other frontal regions (prefrontal cortex) might be mediating the previously described effect of 1X1 tDCS. If this was the case, we would have expected the tDCS group to outperform the HD-tDCS group. That this did not occur provides indirect but informative evidence suggesting that M1 remains a major target for motor system neuromodulation approaches. We cannot rule out however that these other motor regions, or other areas such as primary sensory cortex, were not involved in the tDCS effects observed here.

Our study adds new safety data for both HD-tDCS and children. tDCS has been applied to thousands of subjects and a diverse array of brain disorders without evidence of harm [7]. Given that the distribution of conventional tDCS-induced electric fields is thought to be quite wide-spread across the brain and there were no previous HD-tDCS studies in children, we conducted screening tests of cognitive function before and after intervention. We observed no changes in neurocognition over the course of the study and this supports safety of both applications in children as well as the standing premise of functional targeting; neurostimulation likely modulates neurophysiological processes that are themselves undergoing plastic change. In our population, tDCS was well-tolerated with itching and tingling being common as reported previously [195]. HD-tDCS had comparable sensations and tolerability to tDCS. Adjusting saline concentration may be a method to improve tolerability and decrease sensation severity in

children for conventional tDCS [116]. Increasing the separation distance between stimulation electrode may improve the tolerability of increased scalp current with HD-tDCS but diminishes focality [27]. While most sensations reported in the sham group lasted less than two minutes, this did not appear to influence the ability to correctly distinguish their treatment group.

The AMPED trial served as a replication and validation of previous work [195]. In both studies, participants received 20 minutes of 1mA tDCS or sham; HD-tDCS was specific to the AMPED trial. The learning curves in each study were similar though our sham group may have outperformed that of the previous study, perhaps contributing to smaller effect sizes. Both alone and in combination, these studies support the principle that M1 stimulation can enhance motor learning in children. This important result should promote future research using non-invasive stimulation to modulate neuronal networks involved in motor learning in children.

The translational significance of our findings is evident for the 17 million people in the world living with CP, the leading cause of lifelong disability [198]. With M1 as the primary target, four early phase clinical trials of non-invasive stimulation paired with intensive motor therapy have shown evidence of safety and possibly efficacy in children with hemiparetic CP [97,106,187,228]. That lower baseline skill level was also predictive of responding to stimulation-enhanced motor learning in our study is consistent with previous results in young adults [229] and also potentially therapeutically relevant if in fact those with poor motor skills may be more responsive. Clinical trials of neurostimulation in disabled pediatric populations require special considerations [199] but should be driven by the first principles established in healthy populations as presented here.

Several important limitations are noted. Our study was limited by our modest sample size which was evidence based but we encountered higher variability in outcomes than anticipated, limiting our power to somewhat less than intended. This in turn may have decreased our ability to fully define the efficacy of, and any potential differences between, tDCS and HD-tDCS. There are many known factors that may dictate differences in both motor learning and response to neurostimulation including genetics (e.g. BDNF genotype), gender, age, past experience, and other environmental influences [38]. Fatigue is one such factor and our study protocol was very intense for young participants. We took extensive measures to ensure consistency in the protocol with multiple set breaks to try to diminish possible fatigue effects but these cannot be excluded.

In conclusion, tDCS and HD-tDCS of the primary motor cortex can enhance motor learning in children over days with lasting effects. Trials of existing and emerging neurostimulation approaches can be safe, are imminently feasible, and are required to define and expand therapeutic potential for children suffering from cerebral palsy and other motor disabilities.

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2.7 Acknowledgements

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2.8 Bridge

We demonstrated lasting improvements in motor learning with both tDCS and HD-tDCS. However, the possible changes in sensorimotor function underlying this improved motor skill is unknown and warrants further study. To investigate this, we used the KINARM robot to quantify sensorimotor changes associated with tDCS and HD-tDCS enhanced motor learning.

Chapter 3 – Sensorimotor robotic measures of tDCS and HD-tDCS enhanced motor learning in children

The following work was submitted to Neural Plasticity (Cole L, Dukelow SP, Giuffre A, Nettel-Aguirre A, Metzler M, and Kirton A)

Author LC's contribution to this study include: study design, participant recruitment, data collection, data analysis, and drafting and revising the manuscript. Author SPD's contribution to this study include: study design, data analysis, and revising the manuscript. Author AG's contribution to this study include: participant recruitment, data collection, data analysis, and revising the manuscript. Author ANA's contribution to the study include: data analysis and revising the manuscript. Author MM's contribution included technical support and revising the manuscript. Author AK's contribution to the study include: obtaining funding, study design, and revising the manuscript.

3.1 Abstract

Transcranial direct-current stimulation (tDCS) enhances motor learning in adults. We have demonstrated that anodal tDCS and high-definition (HD)-tDCS of the motor cortex can enhance motor skill acquisition in children but behavioral mechanisms remain unknown. Robotics can objectively quantify complex sensorimotor functions to better understand mechanisms of motor learning. We aimed to characterize changes in sensorimotor function induced by tDCS and HD-tDCS paired motor learning in children within an interventional trial. Healthy, right-handed children (12-18y) were randomized to anodal tDCS, HD-tDCS, or sham targeting the right primary motor cortex during left hand Purdue Pegboard Test (PPT) training over five consecutive days. A KINARM robotic protocol quantifying proprioception, kinesthesia, visually guided reaching, and an object hit task was completed at baseline, post-training, and six weeks later. Effects of treatment group and training on changes in sensorimotor parameters were explored. Twenty-four children (median 15.5 years, 52% female) completed all measures. Compared to sham, both tDCS and HD-tDCS demonstrated enhanced motor learning with medium effect sizes. At baseline, multiple KINARM measures correlated with PPT performance. Following training, visually-guided reaching in all groups was faster and required less corrective movements in the trained arm ($p=0.010$). Aspects of kinesthesia including initial direction error improved across groups with sustained effects at follow-up ($p=0.011$). No changes with training or stimulation were observed for position sense. For the object hit task, the HD-tDCS group moved more quickly with the right hand compared to sham at post-training ($p=0.044$). Robotics can quantify complex sensorimotor function within neuromodulator motor learning trials in children. Correlations with PPT performance suggest KINARM metrics can assess motor learning effects. Understanding how tDCS and HD-tDCS enhance motor learning may be

improved with robotic outcomes though specific mechanisms remain to be defined. Exploring the mechanisms of neuromodulation may advance therapeutic approaches in children with cerebral palsy and other disabilities.

3.2 Introduction

Transcranial direct-current stimulation (tDCS) is a form of non-invasive brain stimulation that can modulate cortical excitability with associated behavioral changes [2]. Conventional tDCS has traditionally been applied using two large sponge electrodes (1x1 tDCS), inducing broad electric fields between the anode and cathode. More recently, modified montages have created options for high-definition tDCS (HD-tDCS) with more focal application of current to targeted cortical areas. Such montages may involve a central anode surrounded by 4 cathodes (4x1 HD-tDCS). There are many reasons to suspect that tDCS effects differ in the developing brain including current modeling investigations that suggest more intense and diffuse electric fields are induced by tDCS in children [92]. Therefore, there is a need to investigate tDCS applications and mechanisms in the developing brain.

One of the most well studied effects of tDCS is its ability to enhance motor learning [76]. When paired with training of the contralateral hand, anodal tDCS centered on the primary motor cortex may improve motor acquisition and retention of skill. We have demonstrated in healthy school aged children that M1-targetted tDCS over three consecutive days of training enhances motor learning as assessed by improvements on the Purdue pegboard task (PPT) [195]. These improvements were retained six weeks later. HD-tDCS has not been commonly applied in the context of motor learning but one adult study suggests that M1 HD-tDCS may increase visuo-

motor adaptation assessed through tracing time and accuracy in several mirror drawing tasks within a single session [230]. Bi-hemispheric M1 HD-tDCS paired with unimanual and bimanual motor training for three consecutive days also showed improvements in bimanual hand dexterity [89]. Recently, we described similar effects of both anodal 1x1 tDCS and high definition tDCS (HD-tDCS) motor cortex stimulation over five days of training in healthy school-aged children [231].

Importantly, understanding the mechanisms that underlie tDCS modulation are limited [35] and virtually unstudied in the developing brains of children. While progress continues at the preclinical, cellular, imaging, and other systems level approaches, few studies have examined the behavioral mechanisms by which tDCS might enhance motor learning [83,193,196,232]. Reaching is a crucial function for everyday life that requires intricate integration between motor and sensory systems. Strong connections are evident between M1 and the somatosensory cortex, and the stimulation of M1 may impact somatosensory processing [144]. The integrity of M1 and somatosensory cortex connections have also been previously correlated with PPT scores [149]. Studies of the effects of motor learning on sensory function suggest direct interactions between M1 and the primary sensory cortex [69]. Training on motor tasks not only improves motor performance but may also enhance sensory function [70]. Position sense is independent of reaching task performance, however its integration is functionally relevant for therapeutic applications [164]. Proprioception is a composite of position sense, which refers to the static sense of limb position, and kinesthesia, the dynamic sense of limb motion [233–235]. Functional proprioception is vital in providing feedback required for motor control, coordination, and

learning. How all of these elements change during motor learning and neuromodulator are unknown in children.

The question of how sensorimotor performance changes with learning and interventions like brain stimulation can be more accurately investigated using robotics. Robotic tools have been used extensively to examine motor learning for the last few decades [236,237]. Robotic tools improve on many of the observer based clinical tools that are historically utilized to quantify sensory and motor function. Most of these clinical tools have short-comings, including a lack of sensitivity to small changes in function and poor inter-rater reliability [151–153]. Members of our team have helped develop more accurate, objective, and reliable measures of upper limb sensory and motor function using robotic technology [151,154,155,157,159,160]. We have used this technology to study healthy adults, clinical-populations, and more recently, to quantify sensorimotor function in children [170–172]. KINARM measures have been correlated with evidence-based functional outcomes and imaging biomarkers, confirming clinical relevance [172]. The KINARM provides a unique opportunity to quantify complex changes in sensorimotor function during motor learning and its neuromodulation. Such studies serve to investigate the mechanisms of motor learning, as well as increase our understanding of how tDCS influences motor and sensory function in the developing brain.

An improved understanding of motor learning neuromodulation mechanisms has immediate translational relevance for clinical populations. For example, perinatal stroke is the leading cause of hemiparetic cerebral palsy [238] where sensorimotor dysfunction results in lifelong disability [170,183]. Robotic measures of visually guided reaching, kinesthesia, and position sense in

affected children have defined mechanisms of disability, imaging biomarkers, and novel therapeutic targets [170–173]. The improved models of sensorimotor development that result from these studies inform novel targets for neuromodulation including translation into multiple recent controlled trials [94,239]. The ability to measure detailed sensorimotor functions with robotics before and after such interventions has the potential to further inform mechanisms of interventional plasticity.

Here, we aimed to characterize sensorimotor changes within a blinded, controlled interventional trial of tDCS and HD-tDCS enhanced motor training in healthy children. We hypothesized that both motor and sensory measures would change with motor learning, with tDCS specifically conferring an improvement in position sense.

3.3 Materials and Methods

Participants

Participants were recruited from our population-based Healthy Infant and Children Clinical Research Program (HICCUP) and from the community. Inclusion criteria were: 1) typical neurodevelopment, 2) right handed (Modified Edinburgh Handedness Inventory was applied at enrollment to confirm a laterality index of ≥-28), 3) healthy (no major medical condition). Exclusion criteria were: 1) neuroactive medications, or 2) non-invasive brain stimulation [202] or MRI contraindications. The Research Ethics Board at the University of Calgary approved all experimental procedures. All participants provided written consent and assent if applicable.

Study design

Accelerated Motor Learning in Pediatrics (AMPED) was a double blind, randomized, sham controlled interventional trial to determine the effects of tDCS and HD-tDCS on motor learning in children (ClinicalTrials.gov: NCT03193580). Participants were computer randomized into one of three intervention groups: 1) sham, 2) right (contralateral to the trained hand) hemisphere 1mA anodal tDCS (tDCS), or 3) right (contralateral) hemisphere 1mA anodal HD-tDCS (HD-tDCS). Participants completed a series of assessments, including KINARM measures (see below) at baseline (pre-training), post-training, and at a six week follow up (follow-up). Training consisted of five consecutive days of left hand training paired with stimulation. Details of the protocol are described elsewhere [231] and summarized in the Supplementary Figure.

Motor training and transcranial direct-current stimulation

Participants trained their left, non-dominant hand on the Purdue Pegboard Test (PPT_L) for 20 minutes for five consecutive days. The PPT is a validated measure of hand dexterity [86]. Participants have 30 seconds to place as many pegs as they can with their left hand (PPT_L). The average total number of pegs placed over three trials was scored. The non-dominant hand was used to assess motor learning to achieve a steeper learning curve and avoid a possible "skill ceiling." The PPT_L was performed prior to stimulation each day. After stimulation began, the PPT_L was completed 5, 10, and 15 minutes into the stimulation period, and again after stimulation ended (three repetitions per time point). The same training was repeated on days 2 to 5. After the final training block on Day 5, participants underwent the same series of assessments as Day 1. Participants returned six weeks later and repeated all assessments to examine retention of acquired motor skills.

Non-invasive brain stimulation was administered by experienced personnel according to established tDCS methods in adult and pediatric populations [83,97,195]. Participants' 'hotspot' (region of the M1 that evoked maximum response of the first dorsal interosseous muscle) was identified using transcranial magnetic stimulation (Magstim, Cardiff UK and Axilum Robotics, Strasbourg, France). This location was co-registered to each participants' neuroanatomical T1 MRI and marked on the scalp. Participants randomized to tDCS or sham had two saline-soaked sponge electrodes (25 cm^2 EASYpads, Soterix Medical Inc., NY, USA) placed on the scalp and held in place by a commercially available light plastic headband sized for children (SNAPstrap, Soterix Medical Inc., NY, USA). The anode was centered over the 'hotspot' with the cathode over the contralateral supraorbital area. Electric current was applied using a Soterix 1x1 Stimulator (Soterix Medical Inc., NY, USA). For participants randomized to the HD-tDCS group, an EEG cap with a ring of four cathodes surrounding a single anode (SMARTscan Stimulator, HD-tDCS Adaptor, Electrodes, electrode holder, and gel; Soterix Medical Inc., NY, USA) was placed over the right M1. Electrodes were connected to a 1x1 stimulator attached to a 4x1 HD-tDCS Adapter (Soterix Medical Inc.). In all conditions, the strength of current applied was 1mA. For participants receiving tDCS or HD-tDCS, the current was ramped up from 0 to 1mA over 30 seconds, held at 1mA for 20 minutes, and then ramped down to 0mA over 30 seconds. For participants randomized to sham, current was also ramped up from 0 to 1mA over 30 seconds, and then immediately ramped down to 0mA. This sham protocol produces similar sensations to active forms of tDCS, however it does not induce any lasting changes in cortical excitability[240].

Robotic assessment of sensorimotor function

The primary outcomes of the current study were derived from a standardized assessment of sensorimotor function using the KINARM robot. Robotic assessments were performed at Alberta Children's Hospital using the KINARM robotic exoskeleton (BKIN Technologies Ltd., Kingston, Ontario, Canada) to measure a variety of sensorimotor tasks as previously described in healthy and stroke affected adults [154,155,157] and children [170,171]. Participants sat in a modified wheelchair base with each arm supported in the horizontal plane by the exoskeleton [158]. To achieve comparable arm positioning for smaller children, modifications were made by adding up to 5 cm of padding under the seat cushion. After the participant was set-up in the KINARM, they were wheeled into an augmented reality work station where virtual targets were projected through a semi-transparent screen (see Figure 1). Four tasks were completed in a standardized sequence.

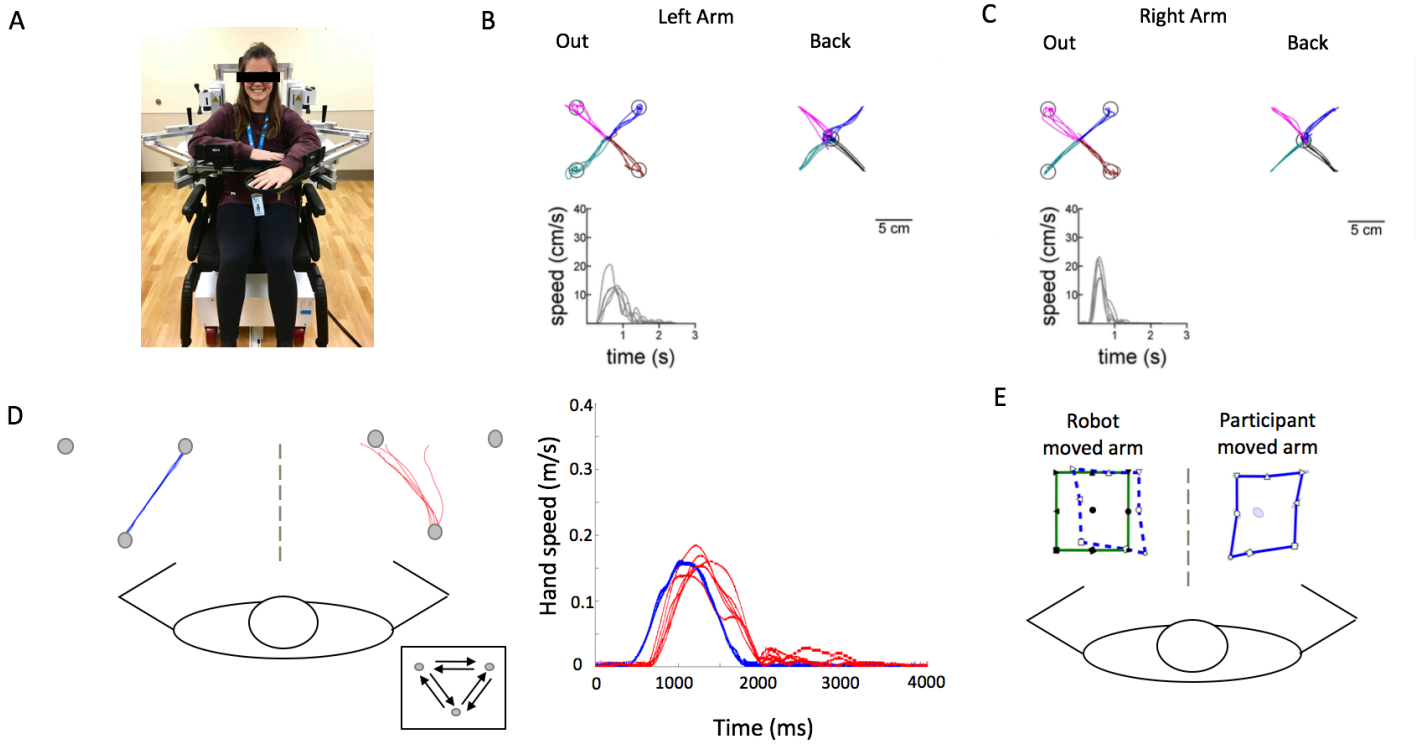

Figure 3.1 *Pediatric KINARM robot tasks for visually guided reaching, kinesthesia, and position matching tasks for an exemplar 18 year old female. (A) Frontal view of the KINARM robotic apparatus. (B) Visually guided reaching performance for the left hand and speed profile for the movement out from the center to the bottom left target. (C) Visually guided reaching performance for the right hand and speed profile for the movement from the center out to the bottom left target. The participant reached out from a central target to one of the four peripheral targets and reached back to the center target. (D) Kinesthesia single direction hand paths. Blue lines represent the robot movement of the passive left arm, red lines represent the active arm path. Grey circles represent the location of robotic movement targets. Hand speed profile show the speed of the passive (blue) and active (red) arm. (E) End positions for the position matching task. Closed symbols represent the positions where the robot moved the participants' passive left arm. The solid green lines represent the border of the outer 8 targets. Open symbols on the right represent where the participant mirror matched with their active right arm. The ellipses represent variability of matching (1SD). Open symbols on the left are the mirrored representation of the subject's attempts to match so the readers can easily compare the two arms.*

A. Visually guided reaching

In a unimanual visually guided reaching task [154,164], participants were instructed to reach as quickly and accurately from a fixed central target (2 cm diameter) to one of four peripheral circular targets (2 cm diameter) located 6 cm away. The robot did not assist or provide resistance. Participants first completed the task with their dominant arm, followed by the non-dominant arm. We analyzed performance in the non-dominant arm. A total of 20 reaches were completed with each arm (five reaches per target). Targets were presented in a pseudo-randomized order. The task was identical to previous work and used the same metrics described elsewhere [172]. Performance was quantified by six parameters:

1. Postural hand speed: A measure of upper limb postural control while trying to hold at the center target, measured by mean hand speed for 500ms before the peripheral target appears (cm/s).
2. Reaction time: The time between appearance of peripheral target and onset of movement (seconds).
3. Initial direction error (IDE): The angular deviation between a straight line from the central target to peripheral target and the actual path taken in the initial phase of movement (degrees).
4. Number of speed peaks per movement (NSP): The number of hand speed maxima between movement onset and movement offset.
5. Total movement time (MT): The total movement time measured in seconds.
6. Maximum hand speed: the maximum speed reached in the task (cm/s).

B. Kinesthesia task

The kinesthesia task assessed participants' sense of upper limb motion. With vision of the arms occluded, the participants' arms were brought into a mirrored starting position at one of three possible positions. To do this, the robot moved the non-dominant arm passively to a position, the subject then placed the index finger of their opposite active arm, represented by a white circle in the virtual environment, into a red target presented in a mirrored position in the workspace. When the subject placed the white circle in the red target, a trial was initiated [157]. The target then disappeared from the workspace and the robot initiated the movement of the non-dominant (passive) arm at 0.18m/s for 12 cm to one of the other 2 targets in the workspace. As soon as participants felt the initiation of movement, they were asked to mirror match the speed,

amplitude, and direction of movement with their dominant (active) arm. The order of targets was pseudo-randomized, and participants completed six blocks of six trials. The task was completed with vision of the arms occluded. The task was identical to previous work [171], and the parameters have been thoroughly described elsewhere [157,171,241]. Task performance was measured by eight parameters:

1. Response latency (RL): The time to initiate a matching movement in response to the robotic movement (milliseconds).
2. Initial direction error (IDE): The angular deviation from the direction of the robotic movement and active arm length (degrees).
3. Peak speed ratio (PSR): The ratio of how well the participant matched the peak speed of the active arm to that of the robotic arm. Ratios <1 indicate movement slower than the robot, >1 indicates faster than the robot.
4. Path length ratio (PLR): The ratio of the length of active arm movement relative to the length of the robot moved passive arm.

The standard deviations across all movements were also classified as the variability of each parameter: RLv, IDEv, PSRv, and PLRv.

C. Position matching task

The arm-position matching task measured position sense ability [155]. With bell-shaped velocity, the robot moved the participants' non-dominant (passive) arm to one of nine spatial targets, each separated by 6 cm. When the movement was complete, participants were instructed to match their dominant (active) arm to the mirror-image location of the passive arm. A total of 54 movements were performed involving six blocks of trials where the targets were pseudo-

randomized. This task was identical to previous work [170] and the parameters are described elsewhere [155,170]. The task was completed with vision occluded. The performance was quantified by three parameters:

1. Variability (Var_{xy}): Endpoint variability (mean standard deviation) of the active arm position in the matched location, measured in centimeters.
2. Contraction/expansion: The ratio of area moved over by active hand relative to the area moved over by the passive hand (values <1 demonstrate contraction).
3. Systematic shift ($Shift_{xy}$): The spatial translation of the workspace between the passive and active hands, measured in centimeters.

D. Object hit task

The KINARM object hit task assessed rapid visuomotor function, decision-making skills, and bilateral motor control [169]. Virtual balls fell from the top of the workspace towards the participant who used 5 cm virtual paddles located at their hands to hit the balls away. A total of 300 balls fell from 10 bins separated equally across the top of the workspace [156]. As the task continued, the difficulty increased, where balls fell at greater speeds and appeared more often. Twelve parameters were collected as previously described [169]. A learning effect has been observed in children and this effect was diminished after performing the task twice [175]. Therefore, a practice effect trial was completed at the beginning of each assessment. Performance was quantified with seven variables:

1. Total balls hit: total number of balls successfully hit throughout task.
2. Total balls with left or right hand: total balls hit with either left or right hand.

3. Median error: the percentage of the task that is complete at the time subjects make 50% of their errors (percentage).
4. Mean hand speed for the right and left hands: the average hand speed for each hand (cm/s).
5. Hand bias hits: quantifies hand dominance in balls hit. Calculated as:

$$\frac{Total\_RightHand\_Hits - Total\_LeftHand\_Hits}{Total\_RightHand\_Hits + Total\_LeftHand\_Hits}$$

6. Hand movement bias area: quantifies differences in size of workspace of each hand.
7. Hand bias speed: quantifies the difference between mean hand speeds of the left and right hand.

Statistical analysis

Non-parametric statistics were used to examine differences in KINARM task scores due to relatively small sample size and the lack of knowledge on whether the true distribution of the measurements were normal. Shapiro-Wilk tests determined the normality of the sampled data distributions. Spearman correlation was used to identify associations between baseline robotic scores, PPT performance, and age. Kruskal-Wallis One-way Analysis of Variance on Ranks was used to examine possible differences in baseline scores across intervention groups, and examine intervention effects at each time point. Post-hoc analysis employed Dunn's test. One-way analyses of variance (ANOVA) and Chi-square/Fischer Exact tests compared group demographics and baseline PPT_L score. Paired t-tests examined the differences in the left and right-hand baseline PPT scores. Significance values were adjusted for multiple comparisons

using Bonferroni corrections. The Friedman test was used to explore training effects within and across intervention groups with post-hoc Wilcoxon-Signed Rank test Analysis was performed using SigmaPlot 12.5 (Systat Software Inc.; San Jose USA) and SPSS (IBM, Armonk, NY, USA).

3.4 Results

Population characteristics

Twenty-four children were recruited and completed all training and robotic measures (median age 15.5 years, range 12-18 years, 52% female). Age, sex distribution, self-reported handedness, and baseline clinical function measures did not differ between intervention groups ($p>0.323$). All groups demonstrated a higher PPT_R compared to PPT_L scores ($p<0.001$). Baseline KINARM robotic scores did not differ between groups ($p>0.05$). Population characteristics by intervention group are summarized in Table 1.

Table 3.1 Baseline demographics and motor function

Stimulation group	Age (SD)	Laterality Index (SD)	Sex F:M	Baseline PPT_L (SD)	Baseline PPT_R (SD)	Baseline PPT_L vs. PPT_R
Sham	15.8 (1.3)	81.9 (22.8)	3:5	13.8 (1.3)	15.2 (1.9)	p=0.013
tDCS	15.9 (1.5)	82.5 (13.1)	6:2	13.5 (1.3)	15.2 (1.9)	p=0.011
HD-tDCS	14.8 (2.0)	81.3 (14.6)	4:4	13.9 (1.9)	15.8 (1.6)	p<0.001
Mean	15.5 (1.7)	81.9 (16.6)	13:11	13.8 (1.5)	15.4 (1.7)	p<0.001
Between groups	p=0.324	p=0.879	p=0.309	p=0.846	p=0.741	---

Age = age in years at enrollment, laterality index measured through the Modified Edinburgh Handedness Inventory, Baseline PPT_L = left hand Purdue pegboard score measured at baseline, Baseline PPT_R = right hand Purdue pegboard score measured at baseline.

Motor learning

The effects of intervention on motor learning were described in detail elsewhere [231]. In summary, all participants demonstrated an increased number of pegs placed over the five days of training on the primary training task (PPT_L), regardless of intervention group ($p<0.001$). Participants receiving tDCS or HD-tDCS had significantly enhanced rates of learning compared to sham (tDCS p=0.042, HD-tDCS p=0.049) with moderate to large effect sizes (Cohen's d tDCS=0.655, HD-tDCS=0.851) and sustained effects at 6 weeks.

Visually guided reaching

Outcomes for left hand visually guided reaching are summarized in Figure 2. At baseline, no associations were observed with age in any of the six robotic parameters (all $r<0.209$, all $p>0.072$). Baseline left hand visually guided reaching reaction time was negatively correlated with baseline PPT_L score ($r=-0.582$, $p=0.003$) (Figure 2A), where quicker reaction times correlated with higher PPT_L score. Reaction time in the left hand was not significantly altered by training or intervention (both factors $p>0.214$) (Figure 2B). Movement time with the left hand

across all groups decreased over the training period ($p=0.010$) (Figure 2C), where post-hoc comparisons identified a significant reduction from pre-training to follow-up ($p=0.042$). There was also an overall significant effect of training in the number of speed peaks ($p=0.019$) (Figure 2D), meaning subjects tended to make less sub-movements during left hand reaching. Post-hoc comparisons identified a marginal reduction from pre-training to follow-up ($p=0.063$). The left hand visually guided reaching initial direction error, postural speed, and left hand maximum speed were not significantly affected by training or intervention group (all $p>0.05$).

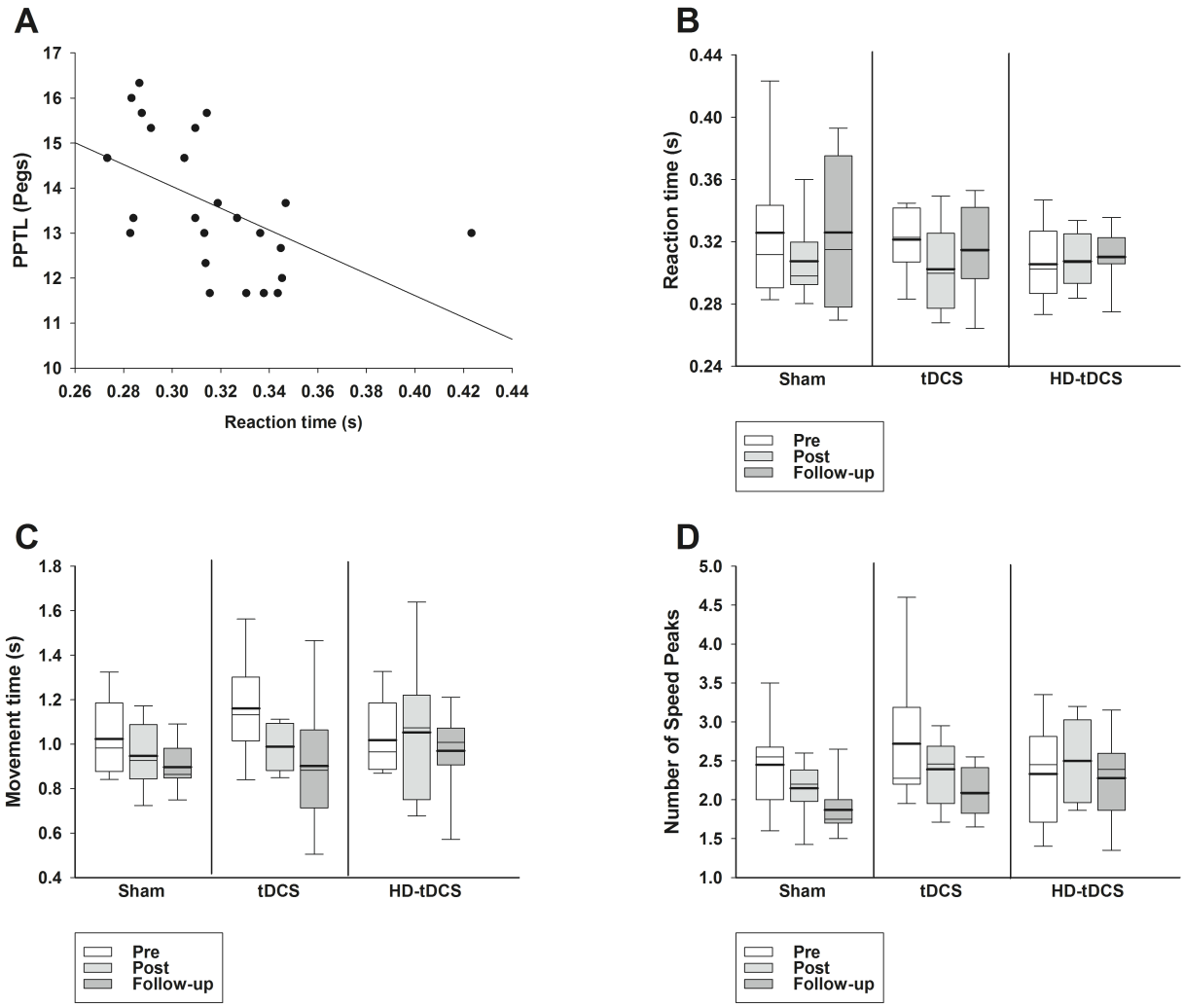

Figure 3.2 *Visually guided reaching with the left hand. A) Scatterplot of baseline left hand Purdue Pegboard Task (PPT_L) score and baseline reaction time. B) Reaction time at pre-training (white), post-training (light grey), and follow-up (dark grey) across the three intervention groups: sham, tDCS, and HD-tDCS. C) Total movement time across the three intervention groups at pre-training, post-training, and follow-up. D) The number of speed peaks across the three intervention groups at pre-training, post-training, and follow-up.*

Kinesthesia

Figure 3 summarizes the kinesthesia outcomes. Baseline IDE (r=-0.544, p=0.006) and IDEv (r=-0.461, p=-0.024) were correlated with age (Figure 3A). Baseline PSRv showed a weak correlation with age (r=-0.399, p=0.053). There was a significant effect of training on IDE (p=0.011), where post-hoc comparisons revealed a decrease in IDE between pre-training vs post-training (p=0.028) and pre-training vs follow-up (p=0.028) (Figure 3B). Thus, the ability to mirror match the direction of movement with the untrained hand in the kinesthesia task improved with training across all groups. There was no significant effect of training on IDEv (p=0.093) (Figure 3A). There was an overall marginal training effect seen in PSR (p=0.093) (Figure 3C). There were no differences in PSR between intervention groups (p>0.542). There was no significant effect of training on PSRv (p=0.721). There was also no interventional (all p>0.207) time (p=0.353) effects on PLR. A significant interaction effect of intervention and training was found (p=0.036). However, there was an overall effect of training within the HD-tDCS group (p=0.005), with a significant increase in PLR from baseline to follow-up (p=0.003). There was no effect of training or intervention on response latency (both p>0.05).

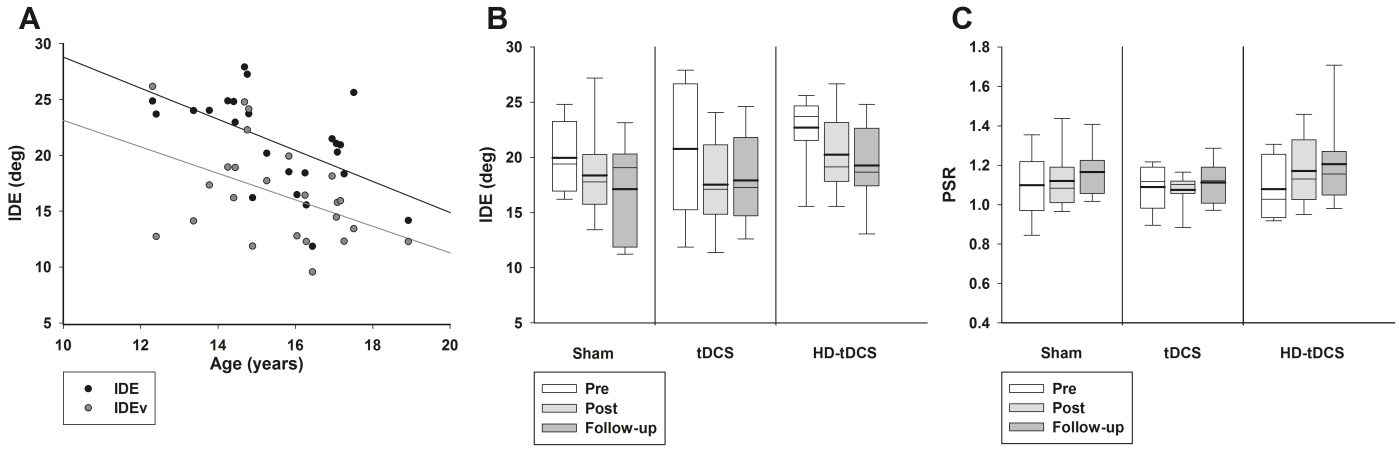

Figure 3.3 *Kinesthesia task. A) Scatter plot of age versus baseline initial direction error (IDE) (black circles) and variability of IDE (IDEv) (grey circles). B) The baseline IDE across the three intervention groups at pre-training (white), post-training (light grey), and follow-up (dark grey). C) The peak speed ratio (PSR) across the pre-training, post-training, and follow-up in the three intervention groups.*

Position matching

Outcomes for the position matching task are summarized in Figure 4. None of the three measures of Var_{xy}, $Shift_{xy}$, or contraction/expansion correlated with age (all $r>0.290$, $p>0.077$). The primary position matching outcome of Var_{xy} correlated with baseline PPT_L ($r=-0.540$, $p=0.006$), where lower variability was correlated with higher (better) PPT_L scores (Figure 4A). Var_{xy} performance did not improve with training ($p=0.687$) (Figure 4B). There was no significant effect of training or intervention group on Var_{xy}, $Shift_{xy}$ or contraction/expansion (all $p>0.05$).

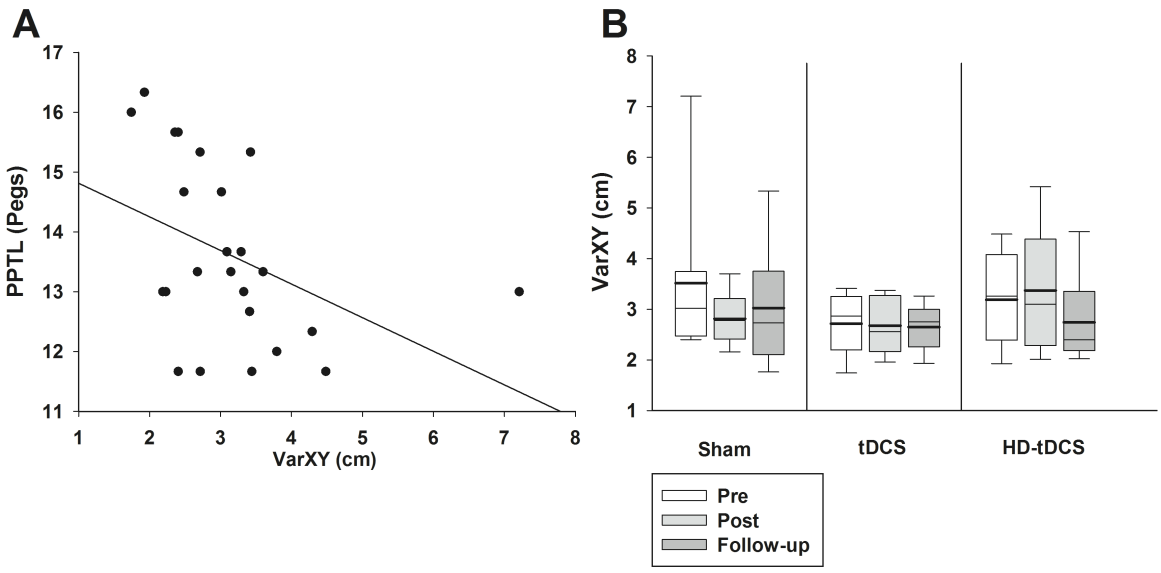

Figure 3.4 *Position matching task endpoint variability (Var_{xy}). A) Scatter plot of baseline Var_{xy} and baseline left hand Purdue Pegboard score (PPT_L). The Var_{xy} across three intervention groups at pre-training (white), post-training (light grey), and follow-up (dark grey).*

Object hit

Figure 5 summarizes the outcomes for the object hit task. There was a significant correlation between baseline total hits and age (r=0.428, p=0.037) where older participants hit more balls. There was a modest correlation between baseline PPT_L score and total hits (r=0.385, p=0.062), where higher PPT_L scores correlated with more objects hit. There was no significant effect of training or intervention group on total balls hit (all p>0.05). There was an overall training effect on the number of balls hit with the left hand at post-training (p=0.041) (Figure 5A). However, post-hoc comparisons did not demonstrate a difference between time points. A Benjamini-Hochberg procedure was performed to further examine post-hoc comparisons and there was a significant difference between pre-training and follow-up (p=0.017) and post-training compared to follow-up (p=0.033). Baseline total hits with the right hand were correlated with age (r=0.410, p=0.046). There was no training effect on number of balls hit with the right hand (p=0.131) (Figure 5B). A significant intervention effect at post-training was identified (p=0.038) where post-hoc analysis suggested that the HD-tDCS group hit more balls with the right hand at post-training compared to sham (p=0.032).

The hand hit bias marginally shifted towards 0 over training demonstrating an increased use of their left hand, regardless of laterality index measured at baseline (p=0.072) (Figure 5C). There was a significant difference between intervention groups for hand hit bias (p<0.027) at both post-training and follow-up where the sham group had a smaller bias compared to the HD-tDCS

group (p=0.024 and p=0.027, respectively). Baseline left hand speed correlated with baseline PPT_L score (p=0.407, r=0.048), where faster movements correlated with higher PPT_L score. Left hand speed did not increase over training (p=0.214) or across intervention groups (all p>0.692). Untrained right hand speed also did not change over training (p=0.747). There was however an overall difference in right hand speed across intervention groups at post (p=0.044) and follow-up (p=0.005) where post-hoc comparisons identified a significant increase from pre- to post-training in the HD-tDCS group compared to sham (p=0.044). At follow-up, the HD-tDCS group had higher right hand speed compared to sham and tDCS groups (p=0.007 and p=0.040, respectively). There was also a significant training effect on hand speed bias at post-training (p=0.046) and follow-up (p=0.038) where the HD-tDCS group showed higher hand speed bias at both training points compared to sham (p=0.049 and p=0.033, respectively).

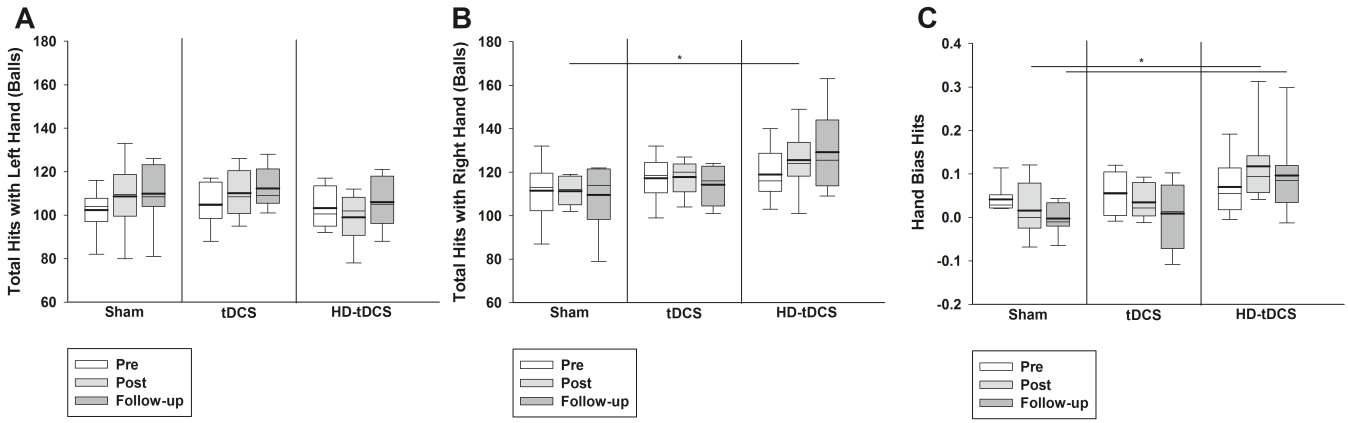

Figure 3.5 *Object Hit task. A) The number of total hits with the left hand and B) number of total hits with the right hand across the three intervention groups at pre-training (white), post-training (light), and follow-up C) The hand bias hits across the three intervention groups at pre-training, post training, and follow-up. *p<0.05*

3.5 Discussion

We quantified changes in sensorimotor function induced by tDCS-enhanced motor learning in children using robotics. We demonstrated coherence between baseline function and improvements in the trained task and multiple robotic measures. Improvements in specific components of visually guided reaching and kinesthesia were observed with motor learning across intervention groups. Intervention-specific effects were not as clear. Our findings demonstrate the ability of robotics to explore motor learning and its modulation by non-invasive neurostimulation in children.

Robotics have been a vital tool to understand motor learning in adults including defining specific metrics such as the corrective responses important in motor learning of goal direct reaching [242]. Long latency responses are important in corrective movements and have been shown to be correlated with reaching errors during learning [243]. Motor learning requires both feedforward and feedback models. Feed forward models utilize state estimation of limb position and are highly involved in goal directed reaching [244]. Feedback mechanisms are modulated by GABA interneurons in the spinal cord, and ablation of these neurons involved in proprioceptive afferents result in forelimb oscillations in reaching [245]. Robotics can also be utilized as a training tool in motor learning [236]. Robotic applied force fields can alter movement trajectory and assess forces of corrective motions [246]. We believe our results add a novel component to these diverse explorations of motor learning mechanisms by expanding into the realm of noninvasive neuromodulation in pediatrics.

The correlations we observed between KINARM metrics and PPT performance at baseline supports the use of this robotic tool in studying the mechanisms of motor learning in children performing this task. The KINARM robot is a validated, well-studied, objective measure of sensorimotor function. Previous studies have demonstrated that the re-test reliability of visually guided reaching [154,175], kinesthesia [161], and position matching [155,175] tasks is strong. The re-test reliability of the object hit may be more variable, as re-test reliability is high in adults, [169] but a learning effect is seen in pediatric population [175]. To overcome learning effects on the object hit task, we opted to add a practice session at the beginning of the KINARM assessments, which was not included in the analysis. Given the strong re-test reliability of our KINARM assessments, we postulate that that the sensorimotor changes we observed are not related to a learning effect on the KINARM. Rather, these changes can be attributed to hand dexterity training or a combination of training and intervention.

Robot technologies have also been utilized in tDCS studies in adults and pediatric disease populations. A case study of an adult participant with unilateral spastic CP found that reaching accuracy on a robotic task was improved when conventional anodal tDCS was applied over multiple sessions combined with robotic therapy [247]. Another single session trial of children with CP receiving ipsilesional M1 tDCS or sham, combined with functional training, examined changes in spatiotemporal variables associated with upper arm reaching movement [248]. This study described a reduction in total and returning movement durations in both the paretic and non-paretic limbs in the tDCS group but not the sham controls. Here, we observed that after five days of hand training, overall reaching movements were faster and less corrective movements were made. In our study, only a modest number of specific effects possibly related to tDCS

intervention were suggested. This finding may be due to our participants having intact sensorimotor function, where it may be difficult to detect small functional changes. This is in contrast to studies of clinical populations such as CP, which have pronounced sensorimotor deficits that may be more sensitive to change.

Few tDCS motor learning studies have examined sensorimotor functional correlates. One pilot study of five adult stroke or traumatic brain injury participants applied bihemispheric tDCS paired with upper extremity physical therapy, and examined effects on PPT scores and the KINARM visually guided reaching and object hit tasks [249]. Findings suggested possible effects on path length ratio and the miss bias of the object hit task at post-training compared to baseline. However, the same study did not report changes in PPT scores, which may be due to the study design that focused on gross motor training. Our study identified a marginal shift in hand hit bias across training but not a change in the miss bias over training or intervention. The observed shift in hand hit bias may be attributed to the intensive non-dominant hand training that participants underwent, which may have assisted in improving left hand function. Taken together, there is a clear need to synergize both measurement and training tasks to understand the meaning of alterations in robotic measures of sensorimotor function.

Motor learning is not a unidirectional process but rather requires constant sensory signals to inform the motor system. A controlled study of children and adults with CP performing fine motor training through piano playing for four consecutive weeks demonstrated an improved ability to sense and perceive local vibrations [250]. Another study found that a motor learning paradigm involving velocity-dependent force fields improved proprioceptive estimates of hand

position in space [70]. These studies support our findings that hand dexterity training can alter proprioceptive function. The object hit task also required visuospatial attentiveness. We demonstrated improved bimanual motor ability including a possible shift in hand hit bias, where both hands were used more equally. Interestingly, despite showing enhanced motor learning, the HD-tDCS group showed a significant increase in hand hit bias towards the right hand. Whether this finding is due to changes in visuospatial attention or motor function requires further study. As well, these effects suggest that HD-tDCS may have differential effects on sensory motor function, highlighting a need for mechanistic studies in adults and children.

The results obtained here contribute novel data to the growing, but limited, field of non-invasive neuromodulation in children. Unlike long-standing adult evidence, the proof of principle study which showed that conventional tDCS of motor cortex can enhance motor learning in healthy school-aged children was only recently completed [195]. Also, unlike much of the adult evidence to date, these findings have recently been replicated within the larger trial on which the current study is based, demonstrating that both conventional and HD-tDCS can enhance motor skill acquisition [231]. Safety data for tDCS applications in children is increasingly established but still represents a very small proportion of the published evidence [7,251]. The KINARM data presented here adds to and further reinforces the favourable safety profile of tDCS in the developing brain by demonstrating no decreases in detailed metrics of sensory and motor function within a controlled study.

Our study explored possible sensory and motor effects of anodal tDCS and HD-tDCS targeting M1. In addition to motor control, M1 may also be involved in aspects of proprioception, such as

position sense and kinesthesia. Neuroimaging studies have demonstrated activations during kinesthesia-related tasks in Brodmann areas 4a, 4p, 6 and SMA during kinesthetic illusions [252]. Brodmann area 2, which is more classically involved in kinesthesia, was also activated. Such studies have demonstrated that kinesthesia is associated with motor areas and robust connections exist throughout the frontal and parietal cortex, all of which may have been influenced by tDCS and, less likely, by HD-tDCS. Our inability to identify any large effects of tDCS or HD-tDCS on kinesthesia may relate to multiple factors.

Our study examined correlations between baseline KINARM sensorimotor function across age groups and motor function. In both healthy control and stroke-affected adults, visually guided reaching and position matching task variables have been correlated with PPT [164]. In healthy children, however, PPT score may not correlate with Var_{xy} or $Shift_{xy}$ [170], suggesting age-specific differences. This previous finding is inconsistent with our work, where we showed that baseline Var_{xy} was significantly correlated with baseline PPT_L. We identified a correlation between baseline reaction time and baseline PPT_L, with no correlation to the remaining variables. A pediatric study of healthy controls and concussion patients found non-dominant hand PPT scores correlated with reaction time, initial distance ratio, and path length ratio in the visually guided reaching task [174]. This study also reported a correlation between non-dominant hand PPT scores and hits with their non-dominant hand in the object hit task. We did not identify such a correlation with non-dominant hand PPT scores and baseline total hits with the left hand. Therefore, our study was able to reproduce some but not all correlations of previous studies.

Previous work by our lab suggested that perinatal stroke populations show dysfunction in position sense, kinesthesia, and visually guided reaching [170–172]. The application of tDCS paired with motor therapy as a possible treatment to improve sensorimotor function in this clinical population was supported by two early clinical trials [94,97]. The addition of detailed behavioral outcomes has been suggested as an important outcome in the design of such childhood disability trials as they move forward [199]. Our results here support the feasibility of this approach while also helping to define the potential and limitations of the ability of such measures to demonstrate intervention-induced change.

Our study has important limitations. Our ability to fully define the effects of tDCS and HD-tDCS on robotic outcomes may have been restricted by our modest sample size, which was powered on the primary clinical outcome [231]. Our age range spans a group of children and adolescents that are developmentally unique, possibly contributing to substantial variability in response. Our study excluded younger children, who are also in need of investigation. The differential effects of tDCS on various age groups are poorly understood. We had a comprehensive and consistent protocol with multiple set breaks to minimize fatigue effects, but these cannot be excluded and likely varied across subjects. There are other known factors that may dictate responsiveness to brain stimulation that we could not control including sleep, experience, and genetics.

In conclusion, robotics can quantify task-specific sensorimotor functions before and after motor training and neurostimulation interventions in children. Hand motor training may be mediated by specific improvements in elements of visually guided reaching and kinesthesia. Although tDCS

and HD-tDCS can enhance such motor learning, the robotic sensorimotor correlates of such neuromodulation may require more powerful studies to be defined.

3.5 Data Availability

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

3.6 Conflicts of Interest

The authors have no conflict of interest to report.

3.7 Funding Statement

This work was supported by the Canadian Institutes of Health Research.

3.8 Supplementary Material

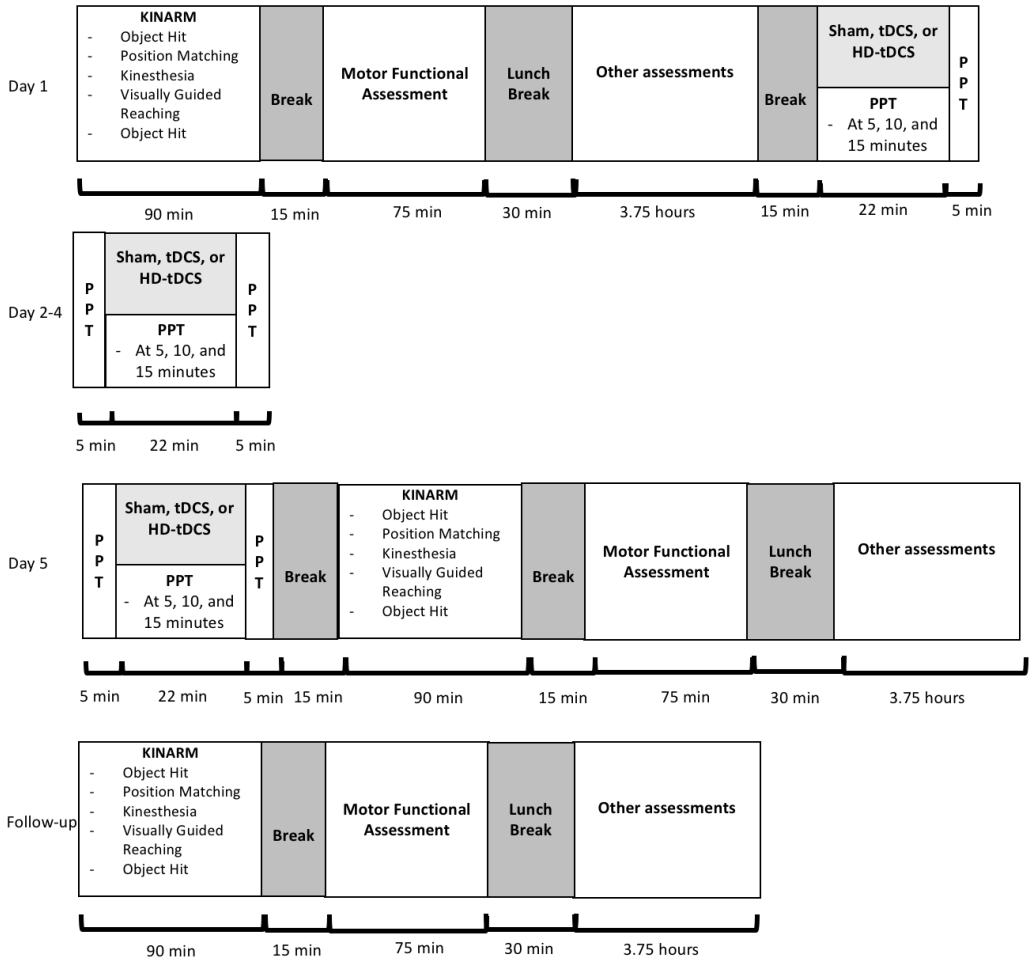

Figure S3.1. *Trial design*

Chapter 4 - Conclusion

4.1 Summary of Main Findings

Here we investigated the effects of tDCS and HD-tDCS on motor learning and sensorimotor function in healthy children. Our study was the first to systematically apply HD-tDCS in a pediatric population, providing novel evidence of the safety and tolerability of HD-tDCS. Our findings suggest comparable enhancement of motor learning with HD-tDCS and conventional tDCS montages. We observed no decline of other motor or neurocognitive functions, contributing to growing evidence of safety and favorable tolerability of direct-current stimulation in children. We further demonstrated the ability of robotics to quantify sensorimotor changes associated with motor training and neurostimulation in children and adolescents. We observed improvements in specific elements including visually guided reaching and kinesthesia in the trained arm. Our findings advance neurostimulation applications and the measurement of their effects in children, including those with disabilities such as cerebral palsy.

4.2 The effect of tDCS and HD-tDCS on motor learning

We demonstrated that both tDCS and HD-tDCS increased the rate of motor learning as assessed by the PPT. Retention testing suggested that this improvement in skill was retained up to six weeks later. In contrast, training alone demonstrated skill decay after six weeks. This difference in retention of skill between intervention groups was not previously described [96] and may reflect differences in the training dose between comparable studies.

Despite both methods passing weak current through the brain, the patterns of electric field induced by tDCS and HD-tDCS differ. The targeting of tDCS over M1 has been shown to produce diffuse cortical effects, with current maxima lying between the anode and cathode, rather than directly under the electrodes [33,92]. In contrast, the HD-tDCS has current maxima more focused under the active electrode [28,34,92] which may lead to relatively more targeted stimulation. Despite the differences in electric field patterns induced by these unique montages, very similar motor learning improvements were observed in our study. This finding may be explained by the hypothesis that stimulation of M1 is crucial in enhancing motor learning. While HD-tDCS was considered as more focal, the conventional tDCS montage applied likely stimulates other cortical areas such as the premotor cortex, SMA, and primary somatosensory cortex. That both almost certainly stimulate M1 and produced nearly identical effects suggests that such stimulation may be sufficient to enhance motor learning. Another hypothesis proposes that diffuse stimulation of larger networks, possibly involving fontal regions, may be necessary for conventional tDCS improvements in motor learning. This idea of diffuse stimulation of networks is supported by current modeling studies [92] but not our findings. Future studies are warranted to investigate what specific regions of the brain must be stimulated to enhance motor learning. Advances in HD-tDCS technology may facilitate such studies.

This trial replicated and validated a previous similar investigation by our team, which applied the same tDCS montage, current strength, and training task [96]. Unlike the previous study, we also investigated the novel effects of HD-tDCS in children, where we demonstrated enhanced motor learning. The learning curves of the anodal conventional tDCS groups of these two studies showed similar trends though overall we observed smaller effect sizes than the previous study.

This finding may be due to the AMPED study sham group outperforming the previous study's sham group with increased variance between subjects. In combination, the AMPED study and previous work further demonstrated that tDCS and HD-tDCS targeting the M1 can enhance motor learning in children. Validation studies of neurostimulation have been rare and are an important step to responsibly advance the field. We hope our efforts will ultimately facilitate the advancement of pediatric non-invasive brain stimulation efforts.

The field of determining inter-individual differences in the susceptibility to tDCS and other non-invasive neurostimulation effects is poorly defined but increasingly emphasized [18]. Baseline skill may be an important factor in understanding the effects of anodal tDCS on an array of tasks. For example, one study of anodal tDCS targeting the right dorsolateral prefrontal cortex found that novice musicians benefited from active stimulation compared to expert musicians that had hindered performance with active stimulation [253]. Another recent study examined the effect of tDCS on surgical skill learning in medical students and found that low-skill performers receiving tDCS improved tumor resection ability more than both the low-skill participants receiving sham and the high-skill performers receiving tDCS [229]. This study supported our findings that baseline skill may influence the extent of motor skill enhancement seen with tDCS. The low performer group in our study showed significantly enhanced motor learning with tDCS and HD-tDCS compared to sham, whereas our high performer intervention groups appeared to show no effects of stimulation whatsoever. This finding is novel in pediatric neurostimulation research and further study is needed to determine if baseline skill dictates response to stimulation. This dichotomy suggests that basal levels of skill should be considered in the design of future trials.

4.3 Safety of tDCS and HD-tDCS in a pediatric population

We demonstrated that both conventional tDCS and HD-tDCS was feasible, safe, and tolerable in healthy school-aged children. These findings align with larger safety data in adults and children [7]. We did not observe any scalp damage, seizure activity, or abnormal cognitive states. The use of neurophysiological testing as a safety outcome was novel in pediatric tDCS research and a valuable adjunct to the first study of HD-tDCS in children. We found no decline in neurocognitive function across the three intervention groups as anticipated. We also did not observe any decrease in hand function on either side, as measured by the PPT and JTT. A decrease in performance on these tasks may have been an indicator of unexpected modulation of motor networks. Using the KINARM robot, we also did not observe decrease in detailed tests of sensorimotor function across groups. The HD-tDCS group demonstrated an increased right hand-hit bias in the object hit task however this group did not show any consistent decrease in left hand function.

Current modeling studies have suggested that when identical stimulation montages were applied, children experienced stronger electric field strengths at the neuronal level [92]. This finding has implications for the safety of tDCS and HD-tDCS application in children, in which stimulation parameters are often based on adult studies. Adult investigations have commonly applied a standard of 1 to 2mA currents. Recently, an adult stroke study applied 4mA bihemispheric tDCS in a single session and reported no adverse events. In a pediatric population, commonly applied current strengths may lead to the induction of electric fields that surpass those seen in the strongest currents applied in adults. We cannot rule out that electric fields induced in pediatric populations may induce electric fields comparable to those of 4mA tDCS, the strongest applied

in adult trials with dedicated safety outcomes. Additional rigorous safety studies are necessary to examine changes in complex neurological processes, as well as long-term investigations of the effect of tDCS and HD-tDCS in the developing brain.

4.4 Robotic assessment of tDCS and HD-tDCS enhanced motor learning

The findings of our study suggest that robotics can assess changes in sensorimotor function following a multi-day neurostimulation-paired motor training paradigm in children. Robotics have previously been used as a training task in a variety of motor learning paradigms. Force field paradigms allow for movement to be unexpectedly altered, which induces adaption over a short timescale [236]. Robot-assisted motor training with force fields can demonstrate movement adaptation and feedforward control of movement [246]. Mechanical perturbations in goal directed reaching have also demonstrated the importance of online feedback responses and adaption [243]. There have been multiple studies exploring the use of robotics as an outcome measure to explore changes in motor learning, however pediatric literature is limited. While robotics hold potential to examine sensorimotor changes associated with motor learning, other sensory assessing modalities can also be used. A study examined the effect of fine motor training, through piano playing, in children and adults with CP and demonstrated improved sensory perception [250]. Our study further demonstrated the utility of robotics to quantify sensorimotor changes resulting from fine motor training in children.

Robotics have been used as a training tool in trials of non-invasive brain stimulation-paired motor learning. Robotics have been combined with tDCS in chronic adult stroke cases for upper

limb rehabilitation [254]. There has been promising results of clinical improvements when tDCS is applied concurrently to robotic therapy, compared to robotic therapy alone [255–257].
Our small number of specific effects associated with active stimulation may be explained by a variety of factors such as the modest power of our convenience sample, functional changes, the specific anatomical regions stimulated, or other elements. The use of tDCS and robotics has also been utilized in clinical populations such as children with CP and have shown improved reaching accuracy on robotic reaching tasks over single sessions [248]. In our study, we did not observe a significant benefit of tDCS or HD-tDCS on reaching, despite a larger dose of training compared to previous investigations. This finding may be due to a difficultly in detecting small functional changes in healthy children, who already have high function, in contrast to pronounced deficits observed in the CP population.

Lesion-to-function studies utilizing robotics as an outcome measure have also identified specific regions of the brain involved in proprioception. The posterior parietal cortex, transverse temporal gyrus, and arcuate fasciculus have demonstrated a possible involvement in position sense function [142]. The networks involved in position sense ability are complex however this previous study highlighted possible targets for non-invasive brain stimulation. Our tDCS and HD-tDCS montage may not have been optimized to reach these areas, limiting the changes seen in position sense. Current modeling of tDCS and HD-tDCS demonstrate that a low amount of diffuse current may be reaching the posterior parietal region [92] but this stimulation may be too weak to contribute to functional improvements.

Not surprisingly, different brain regions are responsible for specific deficits in aspects of kinesthesia function [258]. Robotic assessment paired with neuroimaging in adult stroke identified the bilateral post-central and supramarginal gyri are involved in interpreting movement direction. In our study, we saw improvements in initial direction error with training, regardless of intervention. Current modeling can also inform such understanding. Previous work has demonstrated that motor cortex tDCS and even HD-tDCS electric fields may reach more remote locations such as the supramarginal gyrus [92], however it is unknown how cortical excitability may change in this region. Likewise, it is unknown how simply stimulating this region relates to functional changes, as the relationship between neuronal polarization by tDCS and neurological function is complex. Our sham and tDCS groups showed marginal improvements in path length ratio. In contrast, the HD-tDCS group had an increase in path length ratio (the participants' matched movement length was longer than the robots) from baseline to follow-up. The previously described studies suggest that impairments in path length ratio were associated with lesions in the right superior and middle temporal gyri, anterior insula, supramarginal gyrus, and bilateral parietal opercula. Current modeling of M1-targeting HD-tDCS shows that induced-electric field stimulate the supramarginal gyrus and anterior insula. It has not been described how cortical excitability may be affected by HD-tDCS in these regions, however this may contribute to why we observe a decreased ability to optimize path-length ratios. Robotics are a valuable tool for assessing sensorimotor functional changes associated with motor learning and tDCS. Our results are encouraging for future studies to examine further sensorimotor changes resulting from motor learning in children. Integrating advanced imaging to trials such as ours may further inform additional anatomical or functional specificity of mechanisms of motor learning and its enhancement with tDCS.

4.5 Limitations

Our findings suggest that motor learning was improved by tDCS and HD-tDCS in healthy school aged children but there were inherent limitations that must be considered in the interpretation of our results. Importantly, we were limited by our informed but modest sample size and this may have decreased our ability to fully define the efficacy of, or any potential differences between, tDCS and HD-tDCS, as well as robotic-assessed sensorimotor outcomes. Our trial was powered to demonstrate changes in the primary trial outcome, change in PPT_L score but we encountered higher than expected variance and performance in the sham group with implications for both the primary and secondary sensorimotor function analyses. An important limitation of motor learning studies involving repeated motor training was a possible skill ceiling effect that may be reached. Once this ceiling is reached, no further improvements may be observed with additional practice, limiting the continued effects of tDCS on motor learning. The ceiling effect of PPT has not been well-established but our learning curves suggested ongoing gains even at day five. Participants performed the PPT_L a total of 81 times throughout the study. The non-dominant hand was used to try to diminish the possibility of reaching a skill plateau. We have had success using PPT as the main motor outcome in tDCS motor learning in the past [96], however it is important to note that almost half the number of repetitions was performed.

Another important limitation relates to fatigue. We had an extensive and consistent protocol with multiple set breaks to try to diminish possible fatigue effects. The KINARM assessment was completed at the beginning of the day to try to diminish mental fatigue. In addition to fatigue, there are known factors that may lead to tDCS variability such as common polymorphisms in the

BDNF gene that adds heterogeneity to our sample [38]. Our study did not genotype individuals, therefore a portion of our sample may have shown differential responses to tDCS and motor learning. Other limitations pertaining to secondary outcomes include that the SRTT has not been commonly applied in children in the past to measure implicit motor learning [193,259,260]. Children may have a hard time focusing for the task's duration but there are 30 second breaks after each trial. We have had success using SRTT in our previous study who had a younger median age [96]. The KINARM also has some limitations. The KINARM manipulates the shoulder and elbow joint, therefore limiting the ability to extrapolate results to complicated 3D movements that include fine motor skills in the hand which are clearly involved in some components of the PPT. The KINARM also operates exclusively in the horizontal plane, limiting its ability to assess the wrist and intrinsic hand function. However, previous adult and pediatric studies have demonstrated that the KINARM is more sensitive in quantifying sensorimotor function change compared to traditional clinical measures [154,155,157,170,171].

4.6 Study Implications

This study has direct implications for the use of tDCS and HD-tDCS in motor learning in children. It provided further safety data in healthy, typically developing children to inform practice in clinical populations such as pediatric CP. This study demonstrated a possible effect of intense motor training improving aspects of visually guided reaching and kinesthesia function. Robotics have defined kinesthesia and visually guided reaching deficits in children with CP [171,172] and our findings may be important in motivating further trials children with CP to combat these deficits. We did not observe an intervention effect to improve sensorimotor

function. However, there may be utility for tDCS and HD-tDCS in CP where there is pronounced sensorimotor deficits that may be more sensitive to change.

4.7 Future directions

In the future, larger studies dedicated to examining possible robotic sensorimotor changes associated with tDCS and HD-tDCS enhanced motor learning in children are needed. It is crucial to further optimize tDCS and HD-tDCS protocols while exploring the mechanism of action in pediatrics. This step is necessary to advance the therapeutic application of tDCS and HD-tDCS in children with CP and other motor disabilities. The use of robotics as a training tool may be an important next step in children. The application of tDCS and HD-tDCS during robotic tasks such as visually guided reaching or force-field adaptation may allow for further sensorimotor motor gains in both control and clinical pediatric populations. Future studies involving EEG may be an important tool in further understanding the effect of tDCS in the developing brain. The use of this tool may be vital in characterizing post-synaptic activity in the brain and has utility in examining tDCS effects in children with Autism Spectrum Disorder [261]. The use of cerebellar tDCS to improve motor function in CP is a developing field [262]. The use of this montage to enhance motor coordination in larger studies of healthy and CP affected children is warranted.

There are many complex factors affecting an individual's capacity for tDCS enhanced motor learning, as observed in our low and high performers' susceptibility for neurostimulation-enhanced motor learning. Others have demonstrated that non-responders to tDCS have a decreased capacity for motor learning, however there was no difference in cortical excitability changes found between responders and non-responders [263]. Further studies need to be

dedicated to understand the possible neurophysiological variability between individuals and this variability's effect on neuroplasticity [38]. A future direction would be to examine BDNF polymorphisms in the context of neuroplasticity in children, which may alter their response to tDCS.

Computational modeling is a crucial tool in understanding strength of electric field differences between adults and children, as well as differences between children at differing developmental stages. This field of research is expanding and there are multiple factors that may contribute to differences between participants, such variations in grey and white matter and CSF volume, which change throughout development [27,92]. There is a need to characterize inter-individual differences to therefore optimize a more individualized approach to tDCS application.

In conclusion, tDCS and HD-tDCS can safely enhance motor learning in healthy children. Motor training on the PPT may be accompanied by specific functional sensorimotor changes. Our findings promise to advance stimulation protocols and outcome measures to advance therapeutic options for children with motor disabilities such as cerebral palsy.

4.8 References

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Sensorimotor robotic measures of tDCS and HD-tDCS enhanced motor learning in children

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Sensorimotor robotic measures of tDCS and HD-tDCS enhanced motor learning in children

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