ENHANCING LOCOMOTOR LEARNING WITH MOTOR IMAGERY AND TRANSCRANIAL DIRECT CURRENT STIMULATION

A Thesis
by
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Abstract

ENHANCING LOCOMOTOR LEARNING WITH MOTOR IMAGERY AND TRANSCRANIAL DIRECT CURRENT STIMULATION

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Impaired cognitive function, specifically in the prefrontal/executive networks, is strongly associated with age-related motor deficits and fall risk. Neurorehabilitation through motor imagery (MI) training and transcranial direct current stimulation (tDCS) may enhance motor function by inducing neuroplasticity. **Purpose:** To demonstrate the feasibility and observe the effects of MI training on locomotor learning in young, able-bodied populations, as well as the potential added benefit of prefrontal tDCS. **Methods:** A double-blind, randomized, sham-controlled experimental study was performed with 33 young, able-bodied individuals assigned to one of three groups; MIActive (n=12), MISham (n=11), or Control (n=10). All participants walked through a novel, cognitively demanding, 44-meter obstacle course. Following the obstacle course, Active or Sham tDCS was delivered during the MI intervention for approximately 20 mins. In the MIActive group, a current with an intensity of 2.0mA was applied. In the MISham group, a sham stimulation protocol was utilized. The Control group did not participate in the MI training. After the MI

intervention, participants completed the course again. Premotor cortex oxygenated hemoglobin (O2Hb) levels, collected via functional near-infrared spectroscopy (fNIRS), and time to complete the course were assessed before, immediately after the MI training and during a one-week follow-up. Repeated measures ANOVAs were used to determine group (MIActive, MISham, Control) by time (pre, post, 1-week retention) interaction effects for time to completion of the course and ΔO2Hb levels. **Results:** There was a significant group by time interaction effect for time to completion. The MIActive group saw significant improvements in speed from the pre to posttest, posttest to one week follow up, and pre to one week (46s vs. 41s (*p=0.000); 41s* vs. 39s (*p=0.042); 46s vs. 39s (p=0.000*)). MISham had some improvements in time from post to one week (48.6s vs. 45s(*p*=0.033)) and Control had some improvements from pre to one week (39s vs. 36s (*p=0.033)).* A main effect of time and a main effect of group was found in ΔO2Hb. All groups decrease from pre to posttest (*p=0.047*) and the MIActive group was always lower than the other groups (*p=0.012).* **Conclusion:** Participation in a single session of MI training paired with Active tDCS led to learning evidenced by cerebral level adaptations and motor improvements that were maintained for one week after the intervention. Further investigation should be done before applying this result to other populations.

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A big thanks to my parents for believing in me as I set out to obtain my Master's degree, and encouraging me when I decided to take on a thesis project. Your unending support throughout my life has carried me to this point.

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I. Introduction

1.1 Motor imagery and action observation

Sedentary lifestyle, inactivity after injury or surgery, and immobilization has been shown to lead to serious motor and cognitive dysfunctions, which may later lead to motor impairments, including deficient gait performance and fall risk in older adults.[1-3] The motor impairment is, in part, believed to be principally driven by age-related changes occurring at the cortical level.[4] It is increasingly recognized that normal mobility requires higher-level cognitive input from the frontal cerebral circuits such as attention and executive function, and even more so in complex situations such as obstacle course walking.[5] Dysfunction of frontal cerebral circuits are of particular interest because they have broad effects on executive functions that are important to planning, initiating and coordinating both cognitive and motor behaviors in simple and complex environments.[6] In particular, the prefrontal/premotor cortex (PMc,) located just anterior to the primary motor cortex (M1,) is known to be involved in the planning and programming of voluntary movements.[6] Unfortunately, evidence indicates that the PMc and other frontal lobe brain regions are particularly susceptible to age-related dysfunction demonstrated in the compensation related utilization of neural circuits hypothesis.[5,7-10] The PMc is heavily activated during neurorehabilitation exercises[11] and it is the goal of the field to use this information to increase neuroplasticity.

Neurorehabilitation can help to preserve or enhance executive function, however, a considerable challenge for this field is how to produce robust and lasting changes in the brain that support behavioral and motor improvements[10]; these changes can be referred to as neuroplastic changes. Neuroplasticity is defined as the ability of the neural tissue to modify, change, or adapt in both structure and function; young brains are highly plastic and lose

plasticity with age.[12] A cornerstone of neurorehabilitation is motor learning—an enduring change in the ability to perform a motor task due to practice or experience. The aforementioned practice or experience may be from your own physical or mental practice, and may even come from other similar skills where the movement pattern may *transfer* over into the learning of another skill. Better motor learning can accelerate and/or boost the magnitude of functional gains from neurorehabilitation. Mental imagery is one cognitive operation that increases activity in cortical networks, elicits neuroplasticity, and is essential for motor learning.[11,13,14]

Mental imagery is a mental simulation of oneself performing a specific action. There are a few ways in which we can use mental imagery: visual imagery, kinesthetic imagery, and auditory imagery, which draws patient focus towards seeing themselves, feeling their body, or imagining sounds respectively.[13,14] Recent research has shown that kinesthetic/motor imagery (MI) specifically elicits increased corticospinal excitability of the effector limbs [13], exciting the neural pathway from the brain to cause an action. MI has been used to help in learning and retaining motor skills without the necessity of executing those skills, and it has also been shown to elicit significant activity in similar parts of the brain as motor execution, specifically the PMc. [11,14-16]

In the context of motor skill acquisition, video demonstrations are one of the most common instructional methods used to convey information to a learner. This process of observing the relevant actions of another person and subsequently adapting one's own actions is described as action observation (AO).[17] AO is performed by observing a skill, whether virtual or in real life, performed by yourself or someone similar to you. Whilst observing, the individual may also spontaneously imagine themself doing the skill with MI.[13]

AO may elicit a more vivid version of MI, which is a critical aspect of the effectiveness of training.[14,18]

1.2 Monitoring effects of learning through oxygenated hemoglobin

Neuroimaging studies report that a set of common neural structures—dorsolateral PMc and supplementary motor area—are activated during both action production and AO.[11,13,17] Functional Near-Infrared Spectroscopy (fNIRS) has been employed to examine hemodynamic changes, therefore neuronal activation, in these areas in several functional movements and MI of each.[14,16] Research showed that during these tasks, the vividness of MI as reported on a vividness scale was related to the degree of hemodynamic changes and learning of the task.[16] Early learning and execution of motor skills requires high levels of cognitive energy which causes an increase in the flow of oxygenated hemoglobin (O2Hb) for energy in the M1 and PMc. Mental imagery elicits a similar need for cognitive energy, therefore an increase in O2Hb to the PMc.[11] When employed in preliminary studies conducted in our research laboratory, prefrontal hemodynamic changes have been observed with fNIRS through a walking task as well as MI and AO in combination. Greater increases in hemodynamic flow were observed when using fNIRS during a combination of MI and AO than MI alone. Need for, and levels of O2Hb decrease as an individual becomes more familiar with a skill. Because mental imagery elicits similar cerebral neuronal activation as motor skills and aids in the learning of motor skills, O2Hb should decrease after the use of an MI training intervention.

1.3 Enhancing motor imagery: Transcranial direct current stimulation

The loss of motor skill that often accompanies aging can resemble a more novice-like state of motor skill. An area of particular interest in neurorehabilitation is finding a way to

increase the learning effects of MI and AO to improve and reverse the loss of motor skills from aging. One way this may be possible is the use of a low-level, constant-current brain stimulator known as transcranial direct current stimulation (tDCS) which sends a constant electrical signal into the brain to lower the threshold necessary to create an action potential. tDCS can be used to induce positive neuromodulation of frontal and executive circuits to make them more amenable to the "activity-dependent neuroplasticity" that is known to occur with behavioral neurorehabilitation.[19] Lower threshold environments are more conducive for skill learning, because it is easier to fire signals in that area of the brain. tDCS has been shown to induce excitatory effects on brain tissue and provide lasting positive effects in as little as one session.[15,19-22]

The purpose of this study was to evaluate the effects of MI+AO on locomotor learning and the potential added benefit of tDCS through a randomized control trial. The main hypothesis was that the use of tDCS over the PMc combined with MI+AO would enhance motor skill learning evidenced by changes in prefrontal cerebral activation (change in O2Hb levels) at immediate post testing as well as retaining these changes one week later. The secondary hypothesis is that the combination of tDCS and MI+AO will enhance motor skill learning as evidenced by changes in time-to-completion/speed from baseline to immediate post testing and that those changes will be retained at a one-week follow up test.

1.4 Aims

- We aim to examine how prefrontal tDCS combined with MI+AO affects learning of a novel motor skill evidenced by cerebral activation. We will examine how cerebral activation changes from baseline to immediate post testing, and if those same changes are retained at one-week post testing.

- We aim to examine how prefrontal tDCS combined with MI+AO affects learning of a novel motor skill evidenced by time-to-completion. We will examine how time-to-completion changes from baseline to immediate post testing, and if those same changes are retained at one-week post testing.

II. Review of the Literature

2.1 Anatomy of the brain: involvement of premotor and motor cortices

Although both are involved with motor tasks, the M1 and the PMc of the brain have been given specific and separate names for their distinct regions.[23] Although both areas contribute to motor execution [23], of particular interest is the PMc. The PMc is involved in motor planning, it is located within the frontal lobe, anterior to M1.[6] The contributions of the PMc are acquisition of motor skills and temporal organization of movement.[24] Therefore, it is responsible for movement planning, directly affecting movement execution.

One way to activate the PMc is through MI—imagining oneself performing a motor execution task while attempting to feel the movements as you imagine them.[13,14] There has been investigation on the most heavily involved cerebral area during MI and it has been concluded that two areas are heavily involved: the PMc, and the supplementary motor area (SMA).[11,14,16] Cerebral activation during MI has been studied primarily by the use of fMRI.[11] A study where participants performed either a motor execution task with their dominant hand, MI of that task, or rested showed that the PMc was heavily activated during mental imagery while the motor area was not activated at all. It is only during motor execution that both areas were activated. This showed that the PMc is heavily active in both situations when the motor area is only active during actual execution, making the PMc a more ideal candidate for brain computer interface (BCI) activation. To test the strength of the results, an epileptic patient who was a candidate for a brain computer interface was given an electrocorticographic BCI centralized over the PMc and was able to perform necessary tasks at 91% accuracy with use of MI.[11] This indicated not only the involvement of the PMc during MI, but the strength of those signals as well.

Another more functional strategy to examine cerebral blood flow is the use of functional near-infrared spectroscopy (fNIRS). fNIRS has been previously employed to find areas of highest activation during a MI task.[16] Results indicated that the supplementary motor area and the PMc were highly active during both motor execution and MI tasks confirming prior literature.[16] It has also been stated that the hemodynamic flow changes evidenced by fNIRs readings are an indication of the degree of motor learning from MI and motor execution tasks.[14]

Finally, the PMc is often targeted in studies utilizing tDCS for skill learning because the M1 is known more for skill execution and has been found less important for mental imagery tasks.[11,15,25] Three separate studies have highlighted the clear activation of the PMc during an MI task and the clear absence of M1 activation during the same task. While the M1 is highly active during motor execution, it is not considered a relevant structure for MI.

2.2 Motor Imagery, Action Observation: neural and hemodynamic responses

It has been shown that MI can elicit activity of effector organ corticospinal tracts known as motor evoked potentials.[13] There is evidence that high levels of motor evoked potentials can be caused by MI for the effector organ and when congruent MI and AO occur with the same organ. AO is suspected to cause unintended MI, leading to similar effects to MI alone.[13] To determine which task showed the highest cognitive demand and activation in the PMc, we conducted a pilot study on six participants where fNIRs data was collected under both AO and MI+AO circumstances. Levels of O2Hb relative to baseline were analyzed to determine the best means at achieving high activations within the PMc. Results from this study indicated no difference between levels of increase of O2Hb during MI and

AO. However, previous pilot data indicated that there was a greater increase in O2Hb during the combined MI+AO task (see figure 1.)

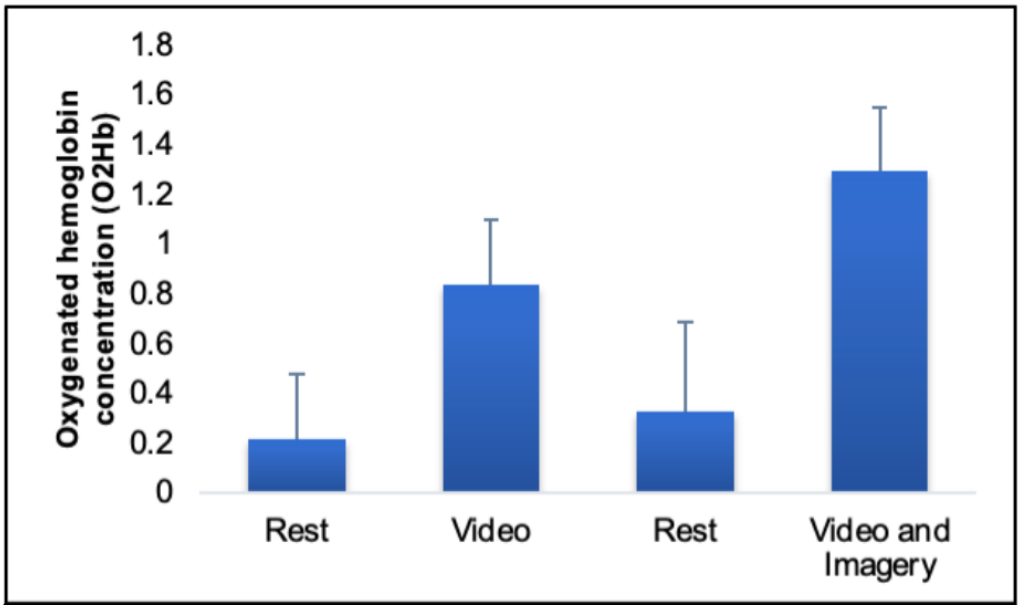

Figure 1. *Change in prefrontal O2Hb concentration during video and video+mental imagery*

Preliminary data examining the difference between prefrontal blood flow during AO, MI combined with AO, and rest indicated a definite increase in prefrontal blood flow from both scenarios as opposed to rest. However, AO in combination with MI elicited a larger increase in prefrontal blood flow, therefore, it is possible a combination of both practices is ideal for producing greater neuroplastic effects.

When performing a skill, motor or cognitive, the brain needs energy to elicit a signal via action potentials, therefore levels of O2Hb during the performance of the skill, or while imagining, will increase to provide oxygen for the task. With learning, the energy level needed to perform the task decreases, and as a result, the hemodynamic flow and subsequent O2Hb levels will decrease.[14] This is an indication that relative O2Hb levels are related to the

degree of learning.[14,16] That is, in early skill execution, the difference between resting- and performance-O2Hb levels will be higher than the difference after some practice. This is most easily and functionally measured via fNIRS.[14,16]

Research has indicated that there is a difference in cerebral activation location between upper and lower extremities.[26] In attempts to confirm the location difference between execution and imagery, as well as right and left hand, and right and left foot, research has indicated that it may be more difficult to monitor lower extremity activation and distinguish between sides with fNIRS.[27] Upper limb imagery shows contralateral activation patterns, although there were differences between left and right sides. Lower limb imagery showed more bilateral and ipsilateral activation.[27]

There is a known difference between the levels of cerebral activation, therefore concentrations of O2Hb, between the younger population and that of the older population. This is known as the Compensation Related Utilization of Neural Circuits Hypothesis (CRUNCH).[5,10] In this case, the aging or dysfunctional brain compensates for a need for cognitive energy in simple tasks by over activating the prefrontal area and under activating in complex tasks. This functional area in which these older adults complete tasks is thus diminished and there is little room for adaptation which can lead to motor deficits.[5,10] Younger adults have a broader range in which they operate for simple and complex tasks, as

shown in figure 2, allowing for better adaptations to task environment and complexity and less likelihood of motor deficits.

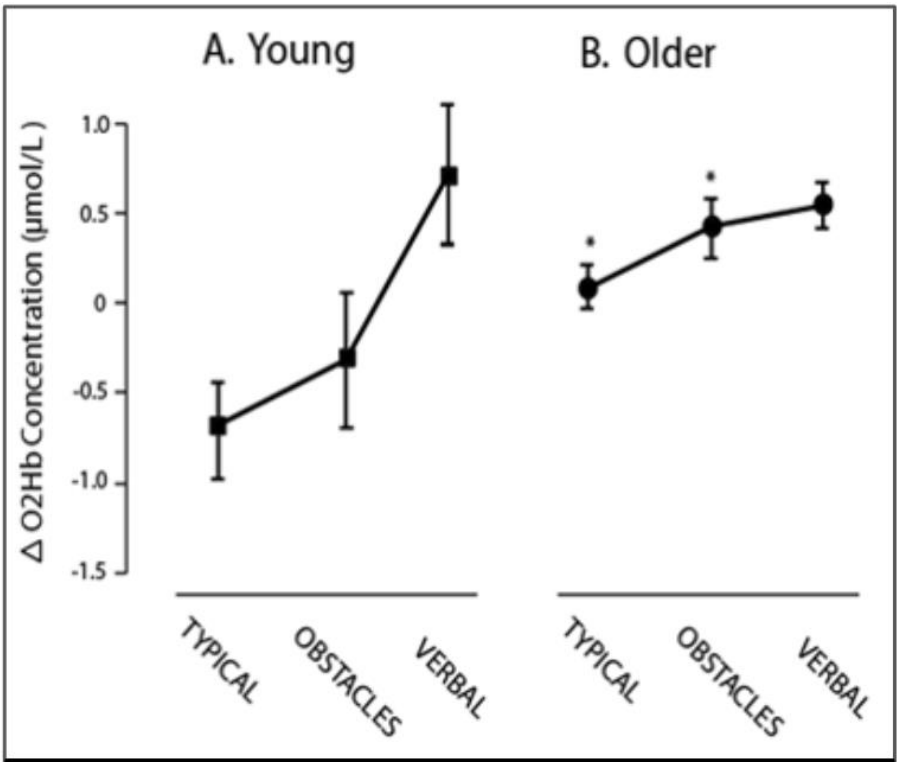

Figure 2. *Preliminary evidence of CRUNCH in younger and older adults*

2.3 Transcranial Direct Current Stimulation: Enhancements in Motor Learning

A major area of interest in neuroscience is the use of transcranial direct current stimulation (tDCS) for learning and rehab. tDCS is considered a painless and non-invasive electrical stimulator making it ideal for broad use. Other similar machines such as transcranial alternating stimulation (tACS) are also considered non-invasive and painless options but have far less safety reports and therefore were not chosen for this study.[28,29] Similarly, repeated transcranial magnetic stimulation is considered to be a non-invasive option which is commonly used in treatments of depression, however the contraindications and side effects are more severe and make it less accessible to the general population.[30] tDCS

has been found to be site-specific[31], indicating that the effects are specific to the area over which it is applied. One study did show that there are specific positive effects with mental imagery training (such as MI and visual imagery) and the placement of tDCS over the M1 and the dorsolateral prefrontal cortex (DLPFC) as compared to other areas such as the right motor area, right premotor area, right supplementary motor area, right cerebellar hemisphere, and right DLPFC[31]. However, another study showed tDCS did not affect learning from mental imagery when placed over M1.[15] In concurrence, another study indicated that tDCS worked best over the PMc.[22] Therefore, the use of tDCS with mental training, such as MI+AO, may be most beneficial when placed on the PMc.

It may be important to consider placement of anode and cathode when using tDCS, as a study has been conducted that noted decreased amounts of learning in participants who received prefrontal cathodal stimulation.[32] Conversely, evidence has shown that the use of anodal tDCS can have positive learning effects when paired with MI, or AO.[22,32] The effects on learning in previous studies have shown that the complexity of the task under consideration matters and that tDCS may have better effects on simple tasks rather than complex.[32] Positive learning effects included both increases in speed of the skill performed, and accuracy with which the learner is able to perform the task.[15,22] Further, a study using anodal tDCS over the DLPFC during a complex motor task showed that there were no mid-session effects from tDCS on a complex motor task. Rather, those who had undergone active anodal tDCS had greater retention effects than other groups.[19] Therefore, anodal tDCS during motor execution may elicit greater retention of learning.

Prefrontal tDCS over the course of multiple sessions, when combined with motor interventions, has shown positive effects on executive functioning as well, indicated by

increased prefrontal activity post-intervention for those undergoing walking rehabilitation [10]. On motor outcomes, there may be some difference in upper and lower limb from tDCS. A meta-analysis reported that combining resistance training and tDCS indicated that the positive resistance training effects are greater on lower limb than on upper limb.[33] Other research reports inconclusive and non-significant evidence for tDCS combined with gait and lower limb rehab.[34]

III. Methods

3.1 Study Design

Participants were enrolled into a 2-session study. Session 1 and session 2 were separated by approximately 7-days and all testing and intervention were achieved in these two sessions. The study was a randomized controlled trial and participants were assigned to one of three groups: MIActive—receiving active tDCS stimulation and participating in a mental training protocol, MISham—receiving sham tDCS stimulation and participating in a mental training protocol, and Control—receiving no stimulation and participating in an irrelevant video-watching task. Participants were randomized via a block design by a member of the team who was not present at collection times. Participants and laboratory technicians were blinded to the stimulation type (active or sham). Independent variables were time (pre, post, and retention trials) and group (MIActive, MISham and Control) and dependent variables were amount of change in O2Hb during performance of a complex obstacle walking course and time to completion of that course.

3.2 Participants and Recruitment

For an alpha=0.05 and a Beta=0.80, 33 participants were recruited and analyzed (MIActive n=12, MISham n=11, Control n=10; Male=6). Participants were young, able-bodied adults between the ages of 18-35 years (23.06 ± 3.05). Exclusion criteria included inability to sit, stand, or navigate obstacles without assistance, as well as deep brain stimulators, having known neurocognitive impairments or head injuries, and sensitivity to tDCS. Sensitivity to tDCS was determined by a standard tDCS contraindication questionnaire

upon arrival and a tDCS sensitivity screen immediately upon beginning the stimulation. tDCS stimulation was aborted if sensitivity was too high upon initiation of the stimulation.

Participants were recruited from the university and surrounding communities by flyers, email, and word of mouth. All participants provided written informed consent before participating in the study as approved by the University Institutional Review Board (IRB-21-0198).

3.2 Experimental Procedure

The experimental design required two testing sessions completed within approximately one week of each other. The aim was for testing sessions to be exactly 7-days apart at the same time of day, but this was not possible in all situations, so the closest option to 7-days was chosen. The first session lasted approximately 2-hours and the second session lasted approximately 30-minutes. Participants were asked not to participate in any intense cognitive or physical activities within 24 hours prior to both sessions.

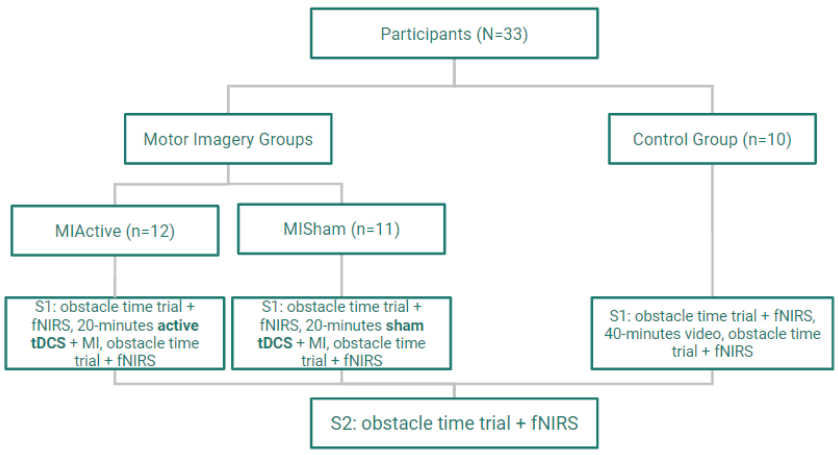

Figure 3. *Procedural flow of methods. S1= session 1; S2= session 2*

3.2a Initial Questionnaires and Pre-Intervention Set-Up

Upon arrival, participants completed a tDCS inclusion criteria form to ensure the safety of the administration of tDCS. Any participants who did not meet the inclusion criteria were not administered tDCS. A trained lab technician then fit the participant with an fNIRS headpiece (Octamon, Artinis Medical Systems, Nijmegen, The Netherlands) over the prefrontal cortex. The headpiece was tightened so that it did not bring discomfort to the participant but would not move or fall off during the collection. The device was connected to the computer via Bluetooth using the Artinis Medical Systems Oxysoft data collection software. Each optode was assessed for proper connection before instructions were given on the obstacle course task. Instructions for the baseline measurement and the obstacle course were provided, as well as one visual demonstration of the obstacle course by the primary investigator.

To establish baseline levels of O2Hb, participants were seated in a chair with their back supported and facing a blank wall at which they were asked to focus their gaze. Participants were instructed to remain still and count to 30 out loud at a minimum of a 30-second pace, focusing on each number as they said it aloud. The baseline level was used to calculate change in O2Hb (ΔO2Hb) during the complex walking course by this equation:

(Walking O2Hb concentration) – (Resting O2Hb Concentration) = ΔO2Hb

Once baseline fNIRS testing was completed, the participant stood on the starting point of the obstacle course and was free to begin walking.

3.2b Pretest

The obstacle course consisted of a series of agility hurdles creating obstacles along a 44-meter walking course (see Figure 4. and Figure 5.) This length was chosen due to the amount of space available in the laboratory. The hurdles were set at three different heights to allow for variation of obstacles. The distribution of hurdles was randomized in a way that was not predictable—some very close to each other and others further apart. The obstacle course was created based on previous investigations to increase cognitive effort and provide a novel task to participants.[10,35] Participants were instructed to perform the course at the quickest and most efficient walking pace possible without kicking or knocking over any hurdles. They were also informed that a lab technician would time them from the heel strike of their first step to the time they returned with both feet back to the start/finish point. Participants performed a timed trial through the obstacle course with a baseline O2Hb collection prior to each trial for a total of three baseline collections with three corresponding obstacle course trials. Whether the participant made an error during a trial was noted (errors included kicking, knocking over or missing a hurdle, or going the wrong direction). Once three course trials were completed, the fNIRS device was removed and participants moved on to the pre-intervention portion of the collection.

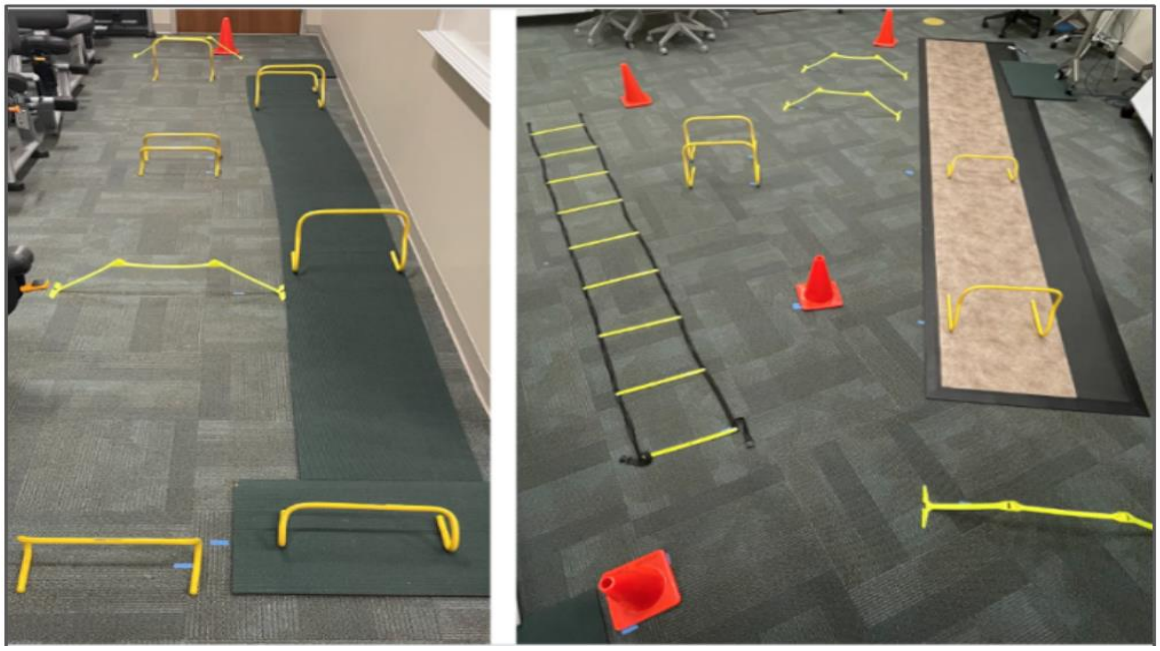

Figure 4. *Images of actual complex walking course*

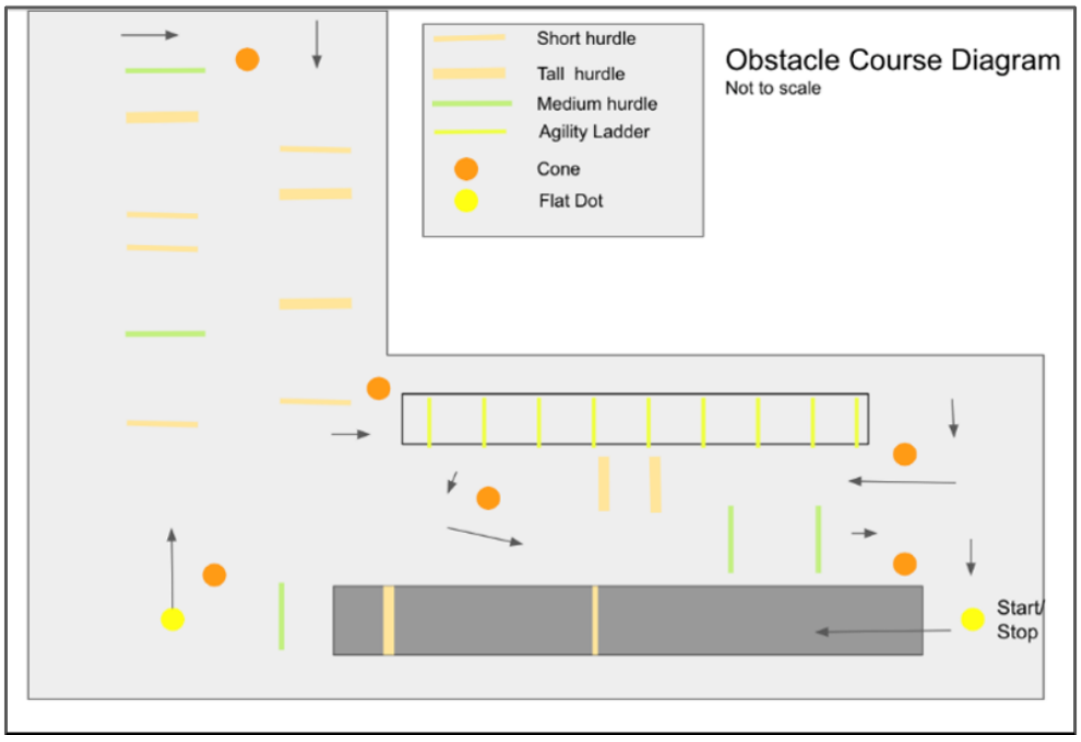

Figure 5. *Diagram of a 44-meter complex walking course. Arrows denote walking direction*

3.2c. Pre-Intervention Protocol

Participants who were selected as part of an MI group (MIActive or MISham) began questionnaires required for the intervention. The first questionnaire administered was the movement imagery questionnaire 3rd edition (MIQ-3)[36] which is utilized to assess an individual's ability to vividly visualize and kinesthetically imagine different tasks. The MIQ-3 is a 12-item questionnaire in which participants are instructed to perform a motor task and subsequently imagine themselves doing that task in one of the three forms of mental imagery (first person visual, third person visual, and kinesthetically.) The questionnaire involves 4 movements repeated 3 times throughout—one time for each version of mental imagery. Upon completion of the mental task, participants gave a rating on a scale from 1-7 (1=very hard to see/feel, 7=very easy to see/feel) indicating how difficult or easy it was to mentally perform. The MIQ-3 has been shown to be a valid measure of one's ability to mentally perform a task and the effectiveness of observational learning based on their ability.[37]

Following the MIQ-3, participants were fit for the tDCS using the international 10-20 system of brain electrode placement. This is a standardized protocol used to ensure precise placement of electrodes. Electrodes were inserted into sponges and placed on a forehead strap (Soterix EASYStrap: Frontal) at the precise location of F3 (placement of cathode) and F4 (placement of anode) determined by the 10-20 system of measurement. Sponges were dampened with saline solution (0.9% sodium chloride) at 4 mL per side per sponge.

Once the strap was placed on the participant's forehead and electrodes were connected to the tDCS machine (Soterix, 1x1 tDCS-CT clinical trial stimulator, New York) connection was examined to ensure it was high enough to effectively stimulate brain tissue. Each participant was assigned a unique code which was randomized for active or sham

stimulation. Upon entering the code, participants were provided with a tDCS sensitivity questionnaire. The questionnaire addressed fourteen symptoms (four of which were pseudo symptoms) that may be caused by tDCS stimulation such as itching, burning, and tingling. Participants responded with a number on a scale of 1-10 (1=absent, 10=present and severe). Scores at or above a 5 for the ten true symptoms were considered a contraindication for tDCS stimulation. Additionally, the total score was calculated by adding all fourteen symptom scores together. The use of the sensitivity questionnaire allowed determination of possible differences in symptoms across MISham and MIActive groups as the initial 1-2 minutes of tDCS, regardless of group, causes a sensation across all participants. If sensitivity symptom levels were at an acceptable range, participants were then instructed on the intervention protocol.

3.2d. Intervention

The participants received a 20-minute session of tDCS at 2-mA (MIActive) or a 20-minute session of sham tDCS (MISham.) During this 20-minute tDCS session, the participants watched a video sequence consisting of an individual completing twenty walking trials (twenty video clips—each clip represents one trial.) Instructions given prior to watching the video were as follows:

> *Please watch the following video. You will see someone walking over the course you just completed, which will be repeated several times. Place your focus on each foot as it approaches each obstacle and imagine performing the task yourself during the entire period of the video. Periodically there will be instructions presented on the screen to help you refocus and to instruct you to focus on different aspects of*

the video or your own imagery. I will follow up at the halfway point to ensure you remain focused on the task. Now try to relax as you watch the video.

Participants sat comfortably with their backs supported and watched the videos at normal play speed with a short break (50-seconds) every five clips. Both MIActive and MISham watched the same video at the same speed. Total MI+AO training time was approximately 20-minutes, consistent with the stimulation duration. At 10-minutes of stimulation the following prompt was given by the researcher:

This is a reminder to continue to do your best focusing on the task at hand. You have reached the halfway mark and should now be participating in kinesthetic imagery of the obstacle course. Remember to attempt to feel and see yourself achieving the movements as vividly as possible throughout the course.

Prior research indicated that 15 to 30-minutes of observation was appropriate for observational learning protocols to observe improvements.[20] Video perspective (see figure 6.) was chosen based on previous research as well which suggested that third-person perspective may lead to stronger memory representations and better retention compared to first person views.[38,39] Clips were shown on a computer screen with minimal distractions surrounding the screen and desk.

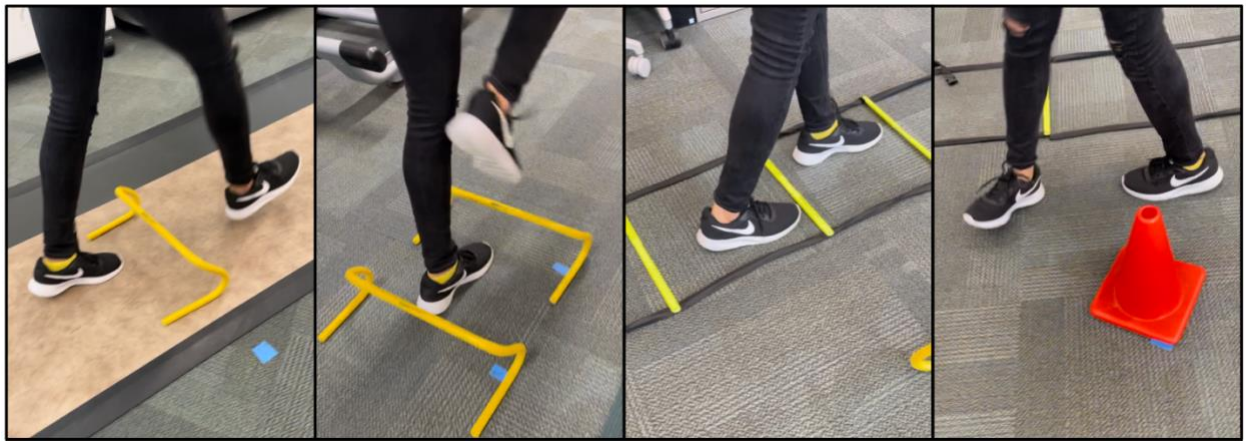

Figure 6. *Stills from the video utilized during the mental training protocol as an example of the point of view chosen*

Immediately following the cessation of stimulation, participants were given the tDCS sensitivity questionnaire again to assess if and to what degree symptoms had changed over the duration of stimulation. The electrodes were removed and participants were offered a break before continuing with the post-intervention data collection.

The control group participated in an irrelevant task for equal duration of the MI group's pre-intervention and intervention phase. The control participants were seated at the same computer to watch a 40-minute clip of a Bob Ross painting video.[40] This video was selected because the nature of the painting movements in the video did not reflect the nature of the movements in the obstacle walking course. The 40-minute duration accounts for the amount of time it took the MI groups to complete questionnaires, get fitted for tDCS and watch the 20-minute MI video. Participants were instructed to relax and focus on the video rather than attempting any mental imagery.

3.2e Posttest

After the intervention, the participants performed the obstacle course in a manner identical to the Pretest utilizing the fNIRS to measure cerebral activation. Instructions were

provided in the same manner as the Pretest, but the primary investigator did *not* provide a visual example of course completion.

3.2f Session two—all groups

The second session consisted of a one-week posttest which ran identical to the pretest and posttest of the initial visit utilizing the fNIRS to measure cerebral activation. The participant was instructed in the same fashion as the day-one posttest trial. Participants were dismissed after the third walking trial.

3.3 Statistical Analysis

Times of the three trials at each test were averaged to observe any learning effects during the obstacle course. A 2-way factorial ANOVA with a between subjects factor of group (3 levels) and a within subjects factor of time (3 levels) was used. For ΔO2Hb, the ANOVA was used to determine the effect of the MIActive intervention on changes in cerebral activation (ΔO2Hb) from Pretest to posttest to one week following the intervention. For time-to-completion, the ANOVA was used to determine the effect of the MI+AO/tDCS intervention on changes in motor skills from pretest to posttest to one week following the intervention. A t-test was used to analyze any differences in the MIQ-3, and the tDCS sensitivity for each group.

IV. Results

4.1 Participant Recruitment & Demographics

After a quality control check on all data and omission of some data as mentioned in the methods section, data from 33 participants were analyzed. Thirty-eight participants were enrolled, data from five participants were omitted as a result of missing follow-up appointments or attending a follow-up appointment too far out from the initial testing day. Additionally, some of those participants were missing data from the right side of the frontal lobe (See figure 7.)

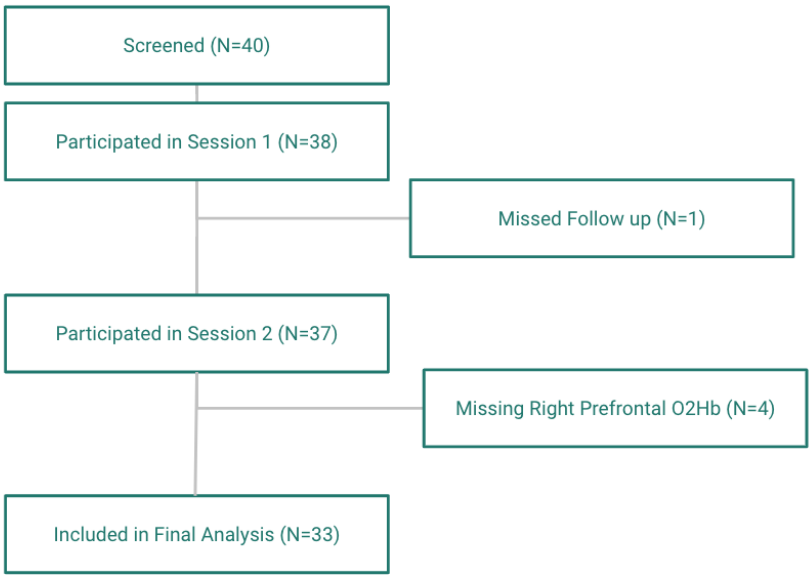

Figure 7*. Consort diagram for participant recruitment and analysis*

Table 1. *Participant demographics and average days between session 1 and session 2*

	N	Age	Follow up (days)
MIActive	12	22.42 ± 1.98	7 ± 0
MISham	11	22.36 ± 2.06	6.91 ± 0.30
Control	10	24.1 ± 4.04	6.8 ± 0.42
Total	33	23.06 ± 3.05	7.18 ± 1.07

4.2 Time-to-Completion

After determining the data was normally distributed, a repeated measures ANOVA was used to determine if there were group by time interactions for time-to-completion of the obstacle course. Sphericity was violated, so a Huynh-Feldt correction was used. This revealed that there was a significant group by time interaction effect ($F(3.45, 51.746)= 4.322$, $p=0.006$, $\eta^2=0.224$). An LSD pairwise comparison post hoc was run to determine the differences (see Table 2.) The MIActive Group had significant differences at all time points while the MISham and Control groups had differences from posttest to retention and from pre to posttest respectively (see Figure 8.)

Table 2. *Summary of group and trial time to completion and speed which is inversely related. Error scores are reported as the number of people in each group that had an error during that trial. This table denotes the group by time interaction effects of time to completion.*

Group	**Session**	**Time (sec)**	**Speed (m/s)**	**Error (n=yes)**
MIActive	Pre	45.80±8.46	0.98±0.16	7
	Post	39.88±3.69*	1.10±0.10	7
	Week	38.13±4.80†@@	1.16±0.15	6
Control	Pre	38.73±8.94	1.19±0.32	8
	Post	36.78±8.67	1.26±0.34	9
	Week	35.57±9.14@	1.31±0.38	5
MISham	Pre	46.92±6.17	0.95±0.14	7
	Post	46.35±4.49	0.95±0.10	10
	Week	44.82±3.99†	0.98±0.09	5

** Significant interaction effect from pre to post p<0.001*
@ Significant interaction effect from pre to week p<0.05;@@ p<0.001
† Significant interaction effect from post to week p<0.05

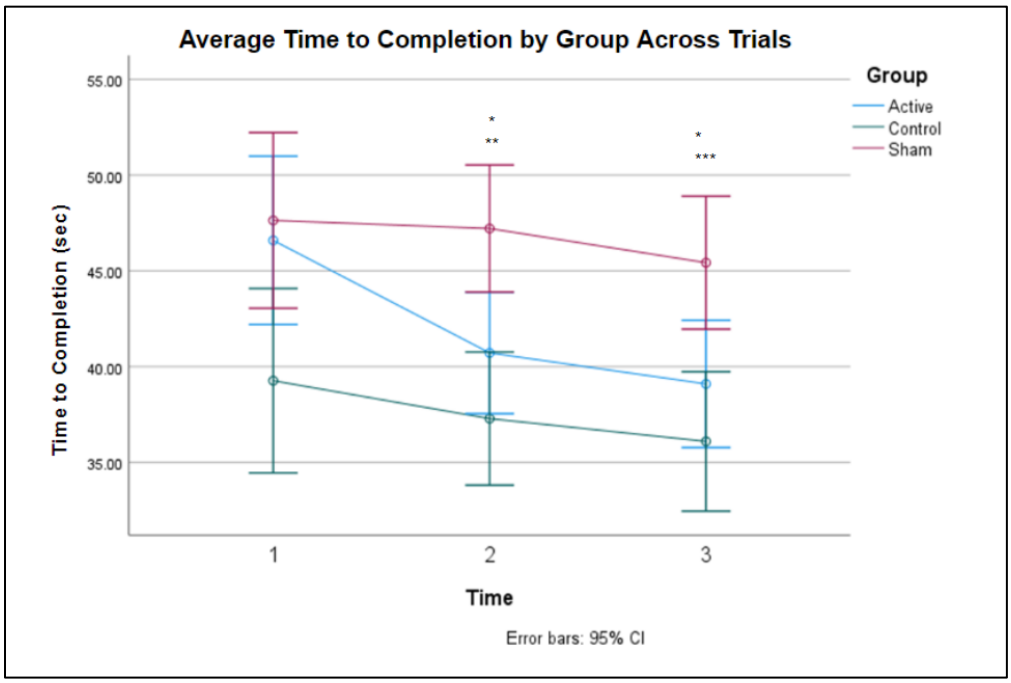

Figure 8. *Time-to-completion across all trials, pretest (1), posttest (2), and retention test (3) of a 44-meter complex walking course.*
**MIActive had a significant decrease in time from Pre to Post, Post to Retention, and Pre to Retention.*
***Control had a significant decrease in time from Pre to Posttesting.*
****MISham had a significant decrease in time from Post to Retention testing.*

4.3 Change in Oxygenated Hemoglobin

After determining the data was normally distributed for ΔO2Hb, repeated measures ANOVA was used to determine if there were significant group by time interaction effects. There was a main effect of time ($F(2,60)=4.431$, $p=0.013$, $\eta^2=0.129$) and a main effect of group ($F(2,30)=5.198$, $p=0.012$, $\eta^2=0.257$.) An LSD pairwise comparison post hoc was run to determine the differences (Table 3.) All groups had a significant decrease from pretest to posttest ($p=0.013$) and the MIActive group was lower than all groups at all times (Figure 9.) The test of within subjects effects showed a medium effect size ($\eta^2=0.82$.)

Table 3. *Summary of group and trial change in O2Hb. This table denotes the main effects of time and group in change in O2Hb.*

Group	Session	ΔO2Hb
MIActive	Pre	0.08 ± 0.48
	Post	-0.52 ± 0.66*
	Week	-0.53 ± 0.47
Control	Pre	0.56 ± 0.47@@
	Post	0.20 ± 0.827*@@
	Week	0.12 ± 0.64$$
MISham	Pre	0.22 ± 0.14*
	Post	-0.19 ± 0.84@
	Week	0.31 ± 1.03@

** Significant main effect of time from pre to post p<0.05*
@ Significant main effect of group—MIActive is significantly lower p<0.05; @@ p<0.01

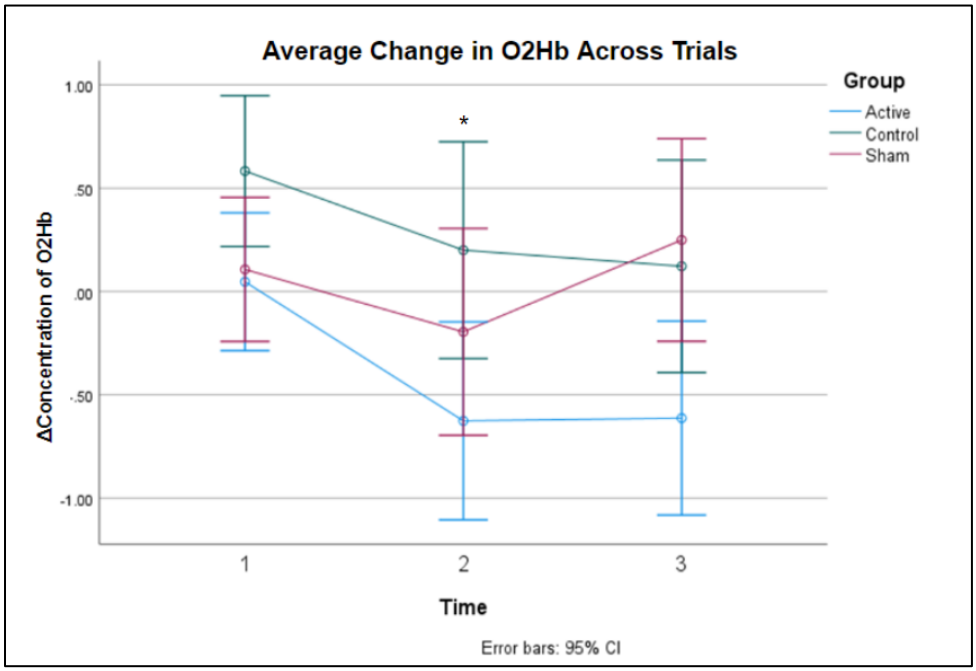

Figure 9. *The average change in O2Hb levels during a 44-meter complex obstacle course task at the pretest (1), posttest (2), and retention test (3). MIActive was lower than all groups at all times.*
**Significant main effect of Time—all groups decreased in O2Hb from Pretest to Posttest (p= 0.013)*

4.4 MIQ-3 and Sensitivity Questionnaires

MIActive and MISham were the only groups who completed the MIQ-3 and the tDCS Sensitivity Questionnaire, this data was irrelevant to the control group. An independent samples t-test was run to determine if there were differences between groups on MIQ-3 scores and tDCS sensitivity. There were no differences between the MIActive group and the MISham group on Kinesthetic Imagery, External Imagery, and MIQ-3 total scores (see table 5.) Further, results on the tDCS Sensitivity screen were not statistically different between groups for pretest but the MIActive group experienced significantly lower sensitivity at the posttest (see table 4.) Both MIActive and MISham groups were able to correctly guess which stimulation they had about 52% of the time.

Table 4. *Pre-Intervention Survey data: relevant scores from MIQ-3 and tDCS Sensitivity.*

	External Imagery (avg / 28)	***Kinesthetic Imagery (avg / 28)***	***Total MIQ-3 (avg / 84)***	***Total pre-tDCS Sensitivity (avg / 140)***	***Total post-tDCS Sensitivity (avg / 140)***
MIActive	21.33	22.92	68.5	19.00	16.83
MISham	22.73	21.77	67.59	21.64	20.82
t-test	*p=0.37*	*p=0.56*	*p=0.80*	*p=0.211*	*p=0.045**

*Significant difference between groups in sensitivity levels after tDCS stimulation.

Table 5. *Participant guesses on which stimulation they had after the intervention. 82.6% of participants guessed active. 52.2% of participants were actually active, while 47.8% were sham. 52.2% of participants guessed correctly.*

Group	*Guessed Active*	*Guessed Sham*	*Total*
MIActive	**10**	**2**	**12**
MISham	**9**	**2**	**11**
Total	**19**	**4**	**23**

V. Discussion

This study examined how prefrontal tDCS combined with mental training of MI+AO affected the learning and performance of a complex obstacle walking course. Learning and performance were examined by changes in time-to-completion over multiple trials and whether those changes were maintained at one-week testing. Learning was additionally examined by calculating changes in O2Hb from rest to walking state for pre and post testing and one-week retention testing. Findings indicated that the combination of MI+AO and 2.0 mA tDCS stimulation for 20-minutes was effective at improving motor outcomes and that it may have some effect on cerebral activation evidenced by changes in O2Hb over time. Results are strengthened by the confirmation that there were no differences between abilities to participate in mental imagery between groups.

5.1 Motor Adaptations

According to these results, a non-motor intervention of 20-minute active tDCS combined with MI+AO was a feasible means of producing motor adaptations across all time points. A non-motor intervention is only practical if any changes on a cerebral level further result in positive motor outcomes and enhancements to skill performance as well. Results indicated that the MIActive group immediately reduced their time-to-completion after a single session (45.80±8.46 sec → 39.88±3.69 sec; $p<0.001$). Additionally, the MIActive group held on to those motor improvements and further improved their time-to-completion significantly at the one-week retention test (39.88±3.69 sec → 38.13±4.80, $p=0.042$). The overall change in time was very significant for this group as well, (45.80±8.46 → 38.13±4.80, $p<0.001$). These results indicate that the multi-layered approach of active tDCS

brain stimulation in combination with mental training was effective at producing motor outcomes.

The same cannot be said for mental training alone (MISham) which had no change from pretest to posttest and some change from post to one week testing, however nothing of significance in the overall pre to retention testing (see table 2. & Figure 8.) Further, the occurrence of potential online learning (control), was slim and only produced significant effects from pretest to one week retention (see table 2. & figure 8.) This is in agreeance with results summarized in two recent meta-analyses that indicated the use of MI and AO in rehabilitation of lower limb injuries proved effective in improving function only when adjunct to traditional rehab.[41,42] These results suggest that mental training alone is not effective enough to elicit and maintain significant motor outcomes, rather the multimodal approach of a mental training protocol with a physical rehabilitation, or the use of a mental training protocol with cerebral stimulation is necessary to promote positive motor outcomes.

Prefrontal cortex stimulation with tDCS is aimed at application to the executive circuits that inform decision making and motor planning. Whether the stimulation and subsequent ease of action potential creation allows better integration of the neural circuits involved in sensory input, motor planning, predicting possible outcomes, or a combination of all of the above, we cannot know without more conclusive functional imaging. It is also important to note that the 10-20 system of measure was intended for precise application of stimulation to the PMc (F3 and F4), however there can still be some field effect from tDCS which may stimulate other areas as well. One study stated that placement of electrical stimulation over F3 and F4 resulted in broad field effects in the prefrontal cortex.[43] Therefore, motor outcomes may be a result of stimulation to the DLPFC—responsible for

executive functioning, response selection, and working memory.[44] Further, a combination of field effects to other prefrontal areas that are responsible for attention, habit forming, and spatial memory—medial prefrontal cortex [45]—could likewise add to the resulting motor outcomes from this intervention. Exact changes on a cerebral level that may have led to these improvements cannot be pinpointed without further research and functional imaging.

The motor adaptation finding applies to many situations, and most importantly, those suffering from motor dysfunctions due to aging, sedentary lifestyle, inactivity after injury or surgery, and immobilization. Recovery time and time spent in physical therapy, occupational therapy, and neurorehabilitation post-surgery or injury can range from weeks to months, costing valuable time, money, and resources. Often, patients are unable to begin rehab immediately after a procedure or injury, further there is a limited amount of physical work an injury sight is able to take at one time. As opposed to mental training alone, the finding of motor adaptations in response to MI+AO and tDCS indicates that there may be a way for patients to begin rehab early and have positive physical outcomes prior to beginning traditional rehab. This intervention may also be used as a form of pre-rehabilitation for those who are at risk for a fall or injury due to functional capacity as illustrated by the CRUNCH hypothesis or those who have movements disorders such as Parkinson's.

5.2 Cerebral Level Adaptations

ΔO2Hb is reflective of the level of cognitive energy necessary to perform the skill and is expected to decrease as neural adaptations occur with learning in healthy populations.[14] Although statistical analysis revealed a significant decrease from pretest to posttest in all groups, it can be speculated that MI+AO and active tDCS was an overall effective tool for learning and creating some level of neural adaptations as there was a

medium effect size in the analysis of group by time interaction (η^2=0.82). When examining figure 9. it is clear that there may be some difference in the way the MIActive group responded to the training from the way the MISham group responded, however this difference went undetected in statistical analysis. Another study has shown what also appears to be a difference between the ΔO2Hb response to active tDCS and sham tDCS appeared descriptively but not statistically.[10] One study using tDCS in combination with a motor task showed differences in hemoglobin responses that were statistically significant between active and sham.[46] It is possible that there is a difference between groups that is not detected statistically and may better be detected with the analysis of another variable related to blood flow.

The use of average levels of O2Hb across the collection period was not the only output that could have been analyzed; deoxygenated hemoglobin (HHb), total hemoglobin, the difference between O2Hb and HHb (HbDiff) and maximum and minimum of each parameter are all options as well. The HbDiff may provide more information on the oxygen uptake of the cerebral tissue, while ΔO2Hb alone signifies an increase in flow to that area with increased cerebral activation.[47] Additional reasons that may explain the lack of difference between groups here is the level of focus on the intervention itself. Whether or not someone was fully focused on the MI intervention may have affected the degree of adaptation as a result. Further investigation will be done into these measures as this may result in a better explanation of the trend of ΔO2Hb evident in figure 9.

We speculate that the O2Hb dynamics after the intervention are indicative of underlying neural adaptations, therefore a need for less cognitive energy and oxygen demand. This result may be of particular interest to the population described in the CRUNCH

hypothesis. The decrease in cerebral activation seen in the young population with the MI+AO and tDCS intervention may indicate an increased functional range during a complex motor task. However, evidence has shown that younger adults have a broader range for adaptation to tasks of increasing demand.[5,9,10] Whether this particular intervention allows for the same increase in functional range for older adults and aging brains as it appears to have done in this population requires further investigation. Additionally, exploration using fMRI or other similar means could provide insight into the specific neural adaptations occurring with the use of tDCS during and after interventions using this investigation's paradigm.

The retention result evident in the MIActive group is useful information as long term learning and neuroplastic changes are beneficial for training and overloading learning at subsequent intervention points. Maintenance of training adaptations between training sessions is an ideal mechanism for neurorehabilitation as well as sport and athletic training. Each time a rehab patient or athlete attends a training session the hope is that movement or strength can be overloaded and progresses linearly for proceeding visits. If regression back to baseline is evident at each consecutive training session, as it was in MISham (See Figure 9.) it becomes difficult to learn and build on a skill effectively. In addition, it is beneficial to know that tDCS may allow for a lower floor within an individual's functional range and may induce a lasting effect on that functional range. In this study, active tDCS adjunct to MI+AO training seemed to induce neuroplastic learning effects that lead to decreased concentration of O2Hb during a complex task from pre to post testing, and allowed for maintenance of those changes at retention testing. Further investigation is necessary to determine if there was a true difference in cerebral adaptations with the active tDCS protocol as is descriptively present in the resulting figure (Figure 9.)

Although statistical analysis indicated no significant change from post to baseline for all groups, it is clear that there was a difference in the way the MISham group responded to the training, as their levels of O2Hb returned to at or above baseline levels at the one week follow up (Figure 9.) Therefore, the mental training intervention alone may not be enough to produce long term significant effects on O2Hb concentrations and walking speeds in new skills. A previous study did show an increase in learning of skills with mental training alone, however, this was predominantly on small movements such as finger tapping and ball rolling, and were not broad full body gross motor tasks.[14] The results of this investigation indicated that mental training alone on a novel gross motor task was not enough to induce significant cerebral changes. Additionally, the above study was also lacking in retention data. It would have been beneficial to see the outcome of a one-week test to see if improvements remained or declined at one week from mental training alone. Further investigation into other variables in fNIRS data may allow a more conclusive result on the difference between MIActive and MISham that we already see as a trend. This descriptive trend however points to what we suspect to be true, that mental training alone is not enough to elicit long term changes in cerebral activation.

5.3 Questionnaires

The MIQ-3 is a verbal assessment tool that allows researchers to assess an individual's ability to imagine things kinesthetically and visually from two perspectives. An individual's ability to vividly imagine a scenario directly affects the usefulness of mental training in that manner.[14] The MIQ-3 was used as a means to assess any potential differences between groups, and as a means to assess the potential success of mental training on an individual basis. Of particular interest were Kinesthetic Imagery and the External Visual

Imagery sections as well as the total scores. Upon analysis, there were no significant differences found between the MIActive and MISham group scores achieved on the MIQ-3 (see table 4.) Individually, no participant scored at a level indicating a lack of vividness and concern for the effectiveness of mental training. Results indicated that between the two groups, both had an equal chance of success with mental training, as the MIQ-3 has been shown to demonstrate that better imagers have greater use of observational learning.[37] Therefore, differences in group's motor and cerebral adaptations were not affected by their ability to train using the mental training protocol.

An additional test that was utilized to ensure both MI groups were similar was the tDCS sensitivity questionnaire. Both groups experienced similar levels of sensitivity at the beginning of intervention, however the MIActive group showed significantly lower levels of sensitivity than the MISham group after the intervention (p=0.046) (see table 5.) Even though there was a difference in the sensitivity levels between the two groups after the intervention, only about half were able to correctly guess which group they were in, and there was a trend for most participants to guess Active (see table 5.) Therefore it is likely that this difference did not affect the blinding of the study. Thus, it can be concluded that all groups were equal in ability to participate in the mental training intervention, and that participants were not able to accurately guess their protocol based on symptoms—neither of these things seemed to have an effect on the outcome of this study.

5.4 Limitations

Without further investigation we cannot be sure that this intervention would have the same positive effect on an older population, or an older, impaired population, as differences already exist in the levels of activation noted by CRUNCH. Furthermore, there is no way to

know that this intervention involved the ideal parameters necessary to attain our goals without further investigation. Subsequent research may try the same intervention with a shift in duration, intensity of tDCS, or multiple sessions to determine the most effective dosage for the outlined purposes.

Some noises within the lab may have been distracting during the mental training protocol, as well outside noises that could not be controlled. It may be ideal to have the participant do the intervention in a completely separate sound proof room with the auditory prompts added into the video itself or with the use of noise cancelling earphones—this may increase a participants maximal level of focus during the intervention. For the control group, it is possible that the choice of control video was not ideal for controlling training effects. Although the choice of video was based on the movements occurring within the video, we cannot be certain that another task would not better mitigate the chance of a participant imagining the obstacle course or movements.

VI. Conclusion

The multimodal, non-motor intervention, using a 20-minute motor imagery and action observation training paired with 2mA of tDCS to the premotor cortex effectively improved walking speed and decreased concentrations of oxygenated hemoglobin during a novel complex motor task in young healthy adults. Further research is needed to determine the applicability of this protocol in achieving the same outcomes in other populations. The same protocol should be attempted in older and impaired populations, and with adjustments to the intensity and duration of the protocol to determine the best possible means of achieving motor outcomes with a non-motor intervention. Finally, additional investigation should be done on hemodynamic changes to establish more precisely the possible group differences in cerebral activation.

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Vita

Hope Elizabeth Gamwell Muscarello was born in Charlotte, North Carolina, to Richard and Cindy Gamwell. She graduated from high school, Barnabas Home School, in May 2015. In the August of 2015, she entered Wingate University to study Exercise Science, and in June 2019 she was awarded the Bachelor of Science degree. In the fall of 2021, she accepted a research assistantship in Exercise Science at Appalachian State University and began study toward a Master of Science degree. The M.S. is expected to be awarded in May of 2023.

Ms. Gamwell became Mrs. Gamwell Muscarello in June of 2022. She has been involved in coaching competitive gymnastics for 10 years and intends to have continual involvement in the sport and implementation of science principles to the sport. After graduation, she plans to reside in Hickory, North Carolina with her husband.