THE INFLUENCE OF NUERAL STIMULATION ON THE ACQUISITION AND CONSOLIDATION OF BIMANUAL COORDINATION TASKS

A Dissertation

by

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ABSTRACT

The ability to learn and remember previous events has allowed individuals to thrive and become quite skillful in various ways. Experiences can be encoded, stored, and retrieved after a process called consolidation. The purpose of the two experiments reported was to determine how non-invasive brain stimulation can potentially impact the consolidation processes supporting the formation of motor memories of a rhythmic bimanual motor skill after a short bout of practice. To explore the consolidation process, the experiments manipulated the mechanical degrees of freedom (Collaborators et al.), stimulation-training coupling, and time delay for the retesting period. The results from both experiments indicate that rapid performance improvement occurred as a result of training with concurrent augmented feedback in the form of a Lissajous plot. However, the DoF required to complete the task seems to impact the dependency on visual feedback to detect and correct errors in performance after only a short bout of training. Need something on the fragility of motor memory in experiment 1. When the mechanical DoF was reduced in Experiment 2, participants were able to maintain similar levels of performance during the retest when visual feedback was removed. A key feature of the learning process is identifying how the stability of a pattern changes across practice and the extent that consolidation occurs as measured through the accuracy and stability of a recalled pattern. A novel finding of Experiment 2 is that participants were able to produce the target relative phase after both a 2-hr and 6-hr delayed retest in the absence of visual feedback. This indicates that consolidation processes were establishing a motor memory for the trained action. A main area of interest for both experiments was how the use of non-invasive brain stimulation (tDCS) might affect training performance and the consolidation process of a novel rhythmic bimanual skill. In experiment 1, an effect of stimulation timing (before or during training) was found for early in practice where stimulation before training increased the rate of performance improvements. No effect of stimulation was found for Experiment 2. Previous research has presented conflicting findings regarding the validity of this form of non-invasive brain stimulation, and the present studies do not present any robust findings to help clarify any of these blurry lines.

DEDICATION

This dissertation is dedicated to my beloved wife, Veronica, for her unconditional love and encouragement. After all, there would be no Dr. McCulloch if not for Mrs. McCulloch.

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1. INTRODUCTION

The ability to learn and remember allows one to build upon previous experiences and even predict future outcomes. Not only does this provide a benefit for increased safety and survival on the most basic level, but also allows individuals to thrive and become quite skillful in various ways. The process of forming new memories, such that we can recall something which we have previously practiced or trained with, has been studied for over a century. Memory consolidation is considered the process in which an experience is encoded, stored, and is then retrievable. The concept of consolidation was initially developed over 100 years ago using interference type tasks (Muller & Pilzecker, 1900). In a series of experiments, Muller and Pilzecker demonstrated that newly forming memories of just acquired information could be disrupted by exposure to similar information shortly after training with the original information. It was hypothesized that the underlying processes that support new memory formation are initially *fragile* (unstable) and malleable and then consolidate over a period of time. Since the original observations, extensive research has been undertaken to further determine the time-dependent nature of consolidation and how interference or interventions can impact the consolidation of new memories (Dudai, Karni, & Born, 2015; Robertson, 2012). Widely studied interventions include sleep (Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002), exercise (Robertson & Takacs, 2017), and non-invasive brain stimulation (NIBS) (Chen et al., 2020; McCulloch, Park, Wright, & Buchanan, 2020). Over the last two decades, the use of NIBS as an intervention has been employed often in the field of motor learning. One such NIBS technique, transcranial direct current stimulation (tDCS), has been shown to affect underlying cortical network excitability (Nitsche & Paulus, 2000, 2001). Numerous studies have attempted to determine how this temporary change in cortical excitability can influence the performance and learning of new motor tasks (Carter, Maslovat, & Carlsen, 2015, 2017; Chen et al., 2020; Furuya, Klaus, Nitsche, Paulus, & Altenmuller, 2014; Furuya, Nitsche, Paulus, & Altenmuller, 2013; Gomes-Osman & Field-Fote, 2013; Kim, Kim, & Wright, 2021; McCambridge, Stinear, & Byblow, 2016; Pixa, Steinberg, & Doppelmayr, 2017a, 2017b; Vancleef, Meesen, Swinnen, & Fujiyama, 2016).

In the field of human motor control and coordination, motor tasks or skills can be categorized as various types of actions, such as fine versus gross, discrete versus rhythmic, or uni-manual versus bimanual. These categories are beneficial when designing tasks to reveal the underlying motor, cognitive, perceptual, and neural processes that support the generation of a specific type of action and how those processes may change as a function of the consolidation of newly trained actions. A well-designed task will help to reveal how actions are both similar and unique. For example, performing two tasks separately can be quite easy, such as using one hand to rub the top of your head or to pat your stomach. These are both uni-manual tasks that require the control and coordination of the joints of a single limb. However, rubbing your head and patting your stomach simultaneously is quite difficult before any practice: same actions, but combined into a bimanual task. Why are these two independent tasks more difficult when performed simultaneously? One possible explanation is that the bimanual task requires the control and coordination of more degrees of freedom (limbs, joints, muscles, neural areas) to complete the action compared to the uni-manual tasks.

When studying coordination, it is important to consider the degrees of freedom problem, because for any given motor task there are multiple ways for humans to achieve the same motor goal. In the present work, the DoF problem was explored by asking participants to achieve the same rhythmic bimanual goal with their arms (six mechanical DoF) or their index fingers (two mechanical DoF). As the number of DoF increases, the system must account for more interacting components (joints and muscles) to achieve the task. The combination of DoF changes and brain stimulation will be used in the following experiments to investigate the training and consolidation process of a rhythmic bimanual coordination pattern.

1.1 Bimanual Coordination: Intrinsic Dynamics

Rhythmic motor skills are characterized by repetitive and cyclic motions of the limbs. The dynamic pattern approach to motor control focuses on rhythmic coordination tasks and is centered on the concept of self-organization. This idea of self-organization proposes that behavior is not a result of an internal model or motor program controlling movements, rather the system's DoF (limbs, muscles, nerve cells) self-organizes into stable patterns of coordination. Kelso proposes that biological systems are non-equilibrium systems that are open to interactions with the environment and that coordinative behavior emerges through interactions among the system's DoF and relevant environmental factors/information (Kelso, 1995). This dependence causes patterns of behavior to arise spontaneously as a result of many interacting components. For example, under certain wind conditions and with specific temperatures, a hurricane forms through self-organizing processes into the identifiable counterclockwise rotation pattern of clouds with high winds. There is no central command or controller which instructs the climate to create a hurricane, however, it occurs, nonetheless. Application to human motor control does not imply that memory and perception are not features of the system, application implies how motor, memory, perceptual, and cognitive processes can be approached within the dynamic pattern approach.

Bimanual movements can be performed in various spatio-temporal patterns between the limbs. Two intrinsically stable bimanual patterns, labeled as in-phase and anti-phase, can be performed with little to no training. In-phase is a rhythmic bimanual pattern that typically is defined with respect to homologous muscle activation and symmetric limb movements (Figure

1A). Anti-phase is typically defined with respect to non-homologous muscle activation and asynchronous limb movements (Kelso, 1984; Scholz & Kelso, 1989). To produce the anti-phase pattern, participants need to lag one limb by a half-cycle behind the other limb (Figure 1C). Continuous bimanual tasks are characterized by having repetitive and rhythmic motions which have no obvious beginning or end. The end of one cycle of movement is the beginning of the next and these skills can be stopped at any moment while performing the skill. Relative phase is used as a quantitative measure of the spatio-temporal relationship between the two oscillating limbs in rhythmic bimanual tasks. In-phase is characterized by a 0° relative phase relation with the

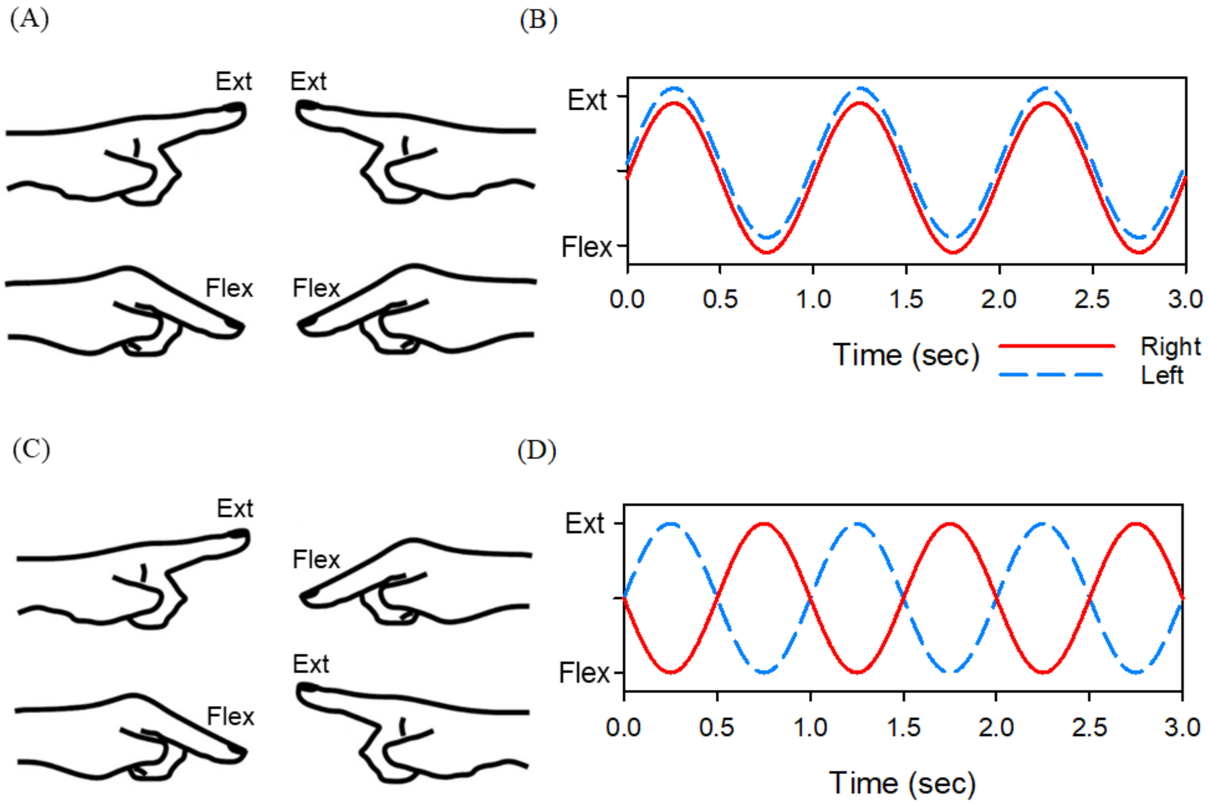

Figure 1. Coordination between the index fingers representing different relative phase patterns. (A) In-phase pattern where the index fingers flex and extend synchronously. (B) Time series representation of in-phase where the left and right fingers flex and extend together. (C) Antiphase pattern where the index fingers alternate flexion and extension. (D) Time series representation of anti-phase where the fingers are alternating flexion/extension actions.

two limbs flexing/extending together in space and across time (Figure 1B), whereas anti-phase is characterized by a 180° relative phase value with one limb flexing as the other extends (Figure 1D). It is well established that 0° and 180° are stable patterns at low movement frequencies (< 2.5 Hz) while in-phase is more stable over a larger range of frequencies (1.5 to > 3.0 Hz) (Buchanan, Kelso, deGuzman, & Ding, 1997; Haken, Kelso, & Bunz, 1985b). However, if movement frequency is increased, an interesting phenomenon occurs. The stability of the antiphase pattern decreases (variability in performance increases) and antiphase transitions to in-phase (Haken et al., 1985b). At higher movement frequencies in-phase maintains stability, and when anti-phase loses stability, the system self-organizes and undergoes a phase transition to the more stable in-phase state (Kelso, Scholz, & Schoner, 1986; Schöner, Haken, & Kelso, 1986). Conversely, in-phase does not spontaneously transition to antiphase at any critical region (Aschersleben et al., 2003; Buchanan et al., 1997).

At the behavioral level, the dynamics (stability, loss of stability) of bimanual coordination patterns are often modeled as minima in potential functions with the motions of the fingers modeled as non-linear oscillators. The Haken-Kelso-Benz (HKB) model, a phenomenological model, focuses primarily on capturing this system level of behavior (Haken et al., 1985b; Kelso & deGuzman, 1988). The relative phase that characterizes the spatio-temporal order among the limbs is represented as an order parameter in these models that defines the stability of the movement patterns and distinguishes the patterns from one another. Relative phase is the primary dependent measure associated with quantifying bimanual coordination in many experiments. Another important variable is the control parameter. The control parameter represents environmental information that can drive or push the system, such as movement frequency, and possibly induce changes in stability of a pattern and transitions between patterns The HKB model is a potential function with stable movement patterns represented as minima (or attractors). The HKB model captures the loss of stability that underlies pattern transitions that occur with a linear increase in a control parameter such as movement frequency. The HKB model defines learning as the stabilization of a new attractor or minima that emerges as a result of self-organizing processes over a practice period (Zanone & Kelso, 1992; Zanone & Kelso, 1997). In other words, over time a newly practiced bimanual pattern consolidates (stabilizes) into a memory, a stable representation of the pattern that can be recalled or reactivated.

A neurological account of the difference in stability of in-phase and anti-phase bimanual patterns has also been formulated. One such reason for in-phase being more stable over a larger range of movement frequencies could be due to neural crosstalk. Conceptually, neural crosstalk accounts aim to explain bimanual coordination findings by understanding the neural pathways and interactions between the command streams in the form of motor commands (copy of) sent to homologous and non-homologous muscles (Cattaert, Semjen, & Summers, 1999; Marteniuk, MacKenzie, & Baba, 1984; Swinnen, 2002; Swinnen & Wenderoth, 2004). The neural crosstalk account posits two independent motor plans existing for each limb and a portion of each motor command is diverted to the unintended limb (Cattaert et al., 1999). Neural crosstalk can occur in the cortical regions (in the supplementary motor area or the primary motor cortex via the corpus callosum) or subcortical areas due to the decussation in the medulla. These connections and interactions cause mutual interference between limbs at different stages of movement planning and organization (Swinnen, 2002). There are also direct and indirect pathways to the spinal cord. The lateral corticospinal tract crosses at the medulla and contralaterally controls precise movements at distal parts of limbs. The ventral corticospinal tract runs ipsilateral through the brainstem and terminates either ipsilateral or contralateral and controls axial and proximal limb muscles (Lemon, 2008; Lemon, Kirkwood, Maier, Nakajima, & Nathan, 2004). This means each hemisphere innervates the contralateral arm, hand, and finger as well as partially innervates the ipsilateral arm (Figure 2). As a result, muscles may receive competing efferent commands from both hemispheres (S.P. Swinnen, 2002). These conflicting commands would explain the fact that anti-phase is less stable than in-phase, and also provide an explanation why performing other bimanual patterns, such as 90° relative phase (Figure 3), requires extensive practice to perform (Lee, Swinnen, & Verschueren, 1995).

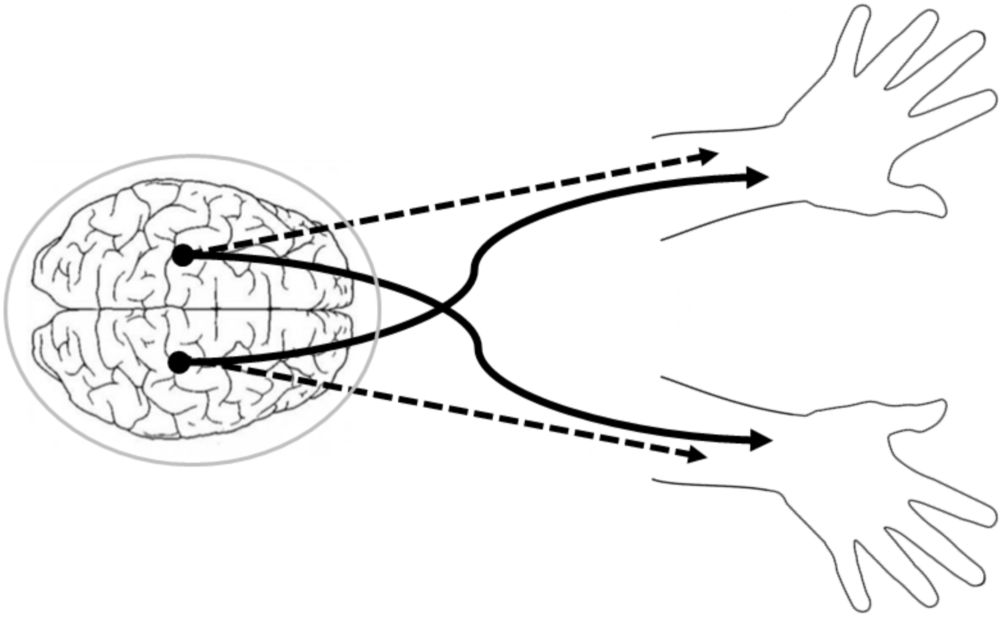

Figure 2. Illustration of efferent motor commands being sent to the contralateral limb (solid-line arrow) and ipsilateral limb (dashed-line arrow) from each hemisphere.

Evidence of neural crosstalk has been shown in a bimanual control task where forces produced by a muscle group (triceps) in one limb resulted in complementary changes in force production in the homologous muscles (triceps) of the contralateral limb (Kennedy, Boyle, Wang, & Shea 2016). In one task, subjects produced symmetric constant forces by pressing their forearms against separate force transducers. An increase or decrease in force by one limb resulted in a corresponding mirrored change in force by the contralateral limb. This relationship was observed regardless of which limb was provided feedback. In a following experiment, participants were asked to produce a varying pattern of forces with one limb (track a sinusoidal wave with a cursor) while the other limb produced a constant force. The limb producing the varying pattern of forces systematically influenced the forces produced by the contralateral limb, even though subjects were asked to keep the limb steady. This demonstrates how uncrossed motor commands may influence muscle activity patterns (interpreted as neural crosstalk) in bimanual tasks.

The in-phase and antiphase bimanual patterns are stable and accurate for a range of movement frequencies and are considered intrinsic to the motor system in that practice or training is not required to produce them. Researchers interested in studying motor learning from the dynamic pattern perspective need to have participants acquire coordination patterns that are not intrinsically stable. A key feature of the learning process is identifying how the stability of a pattern changes across practice and the extent that consolidation occurs as measured through accuracy and stability of a recalled pattern. A relative phase pattern that has been used to study learning from

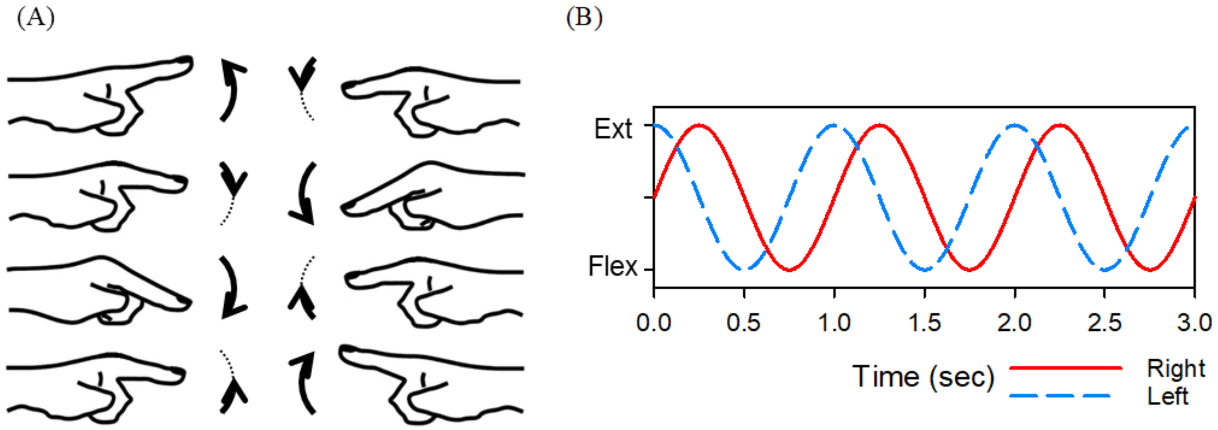

Figure 3. (A) The 90° relative phase pattern requires a quarter-cycle lag between the two index fingers. The solid line arrow (→) indicates the flexion/extension movement of the index finger while the dotted line (**---**) indicates the movement trajectory. (B) A time series representation of the flexion/extension motions for a 90° relative phase pattern with the right finger trailing the left.

the coordination dynamics perspective is a 90° phase pattern (Schöner, Zanone, & Kelso, 1992). This relative phase pattern falls between 0° (in-phase, no lag between limbs) and 180° (antiphase, half-cycle lag between limbs). The behavioral/mechanical requirement within a bimanual task is for one limb to lag the other limb by a quarter-cycle (Figure 3A, B).

1.2 Bimanual Coordination: Learning Dynamics

Augmented feedback is common in all areas of learning and can provide valuable external supplementary information and motivation. For example, a teacher may provide a nod of encouragement to a young student attempting to sound out an unfamiliar word. A baseball coach may review a film of a fastball with a baseball pitcher to discuss mechanics to help the athlete improve their form. Whether the task is one, two, three, or four limbs, feedback can be highly important for learning. Certain bimanual coordination tasks are often hard to perform and require some time investment to learn. Not surprisingly, being able to increase the effectiveness of training and improve skill retention can be highly beneficial. Although providing feedback during training can improve learning, it is not without drawbacks. The guidance effect (Winstein & Schmidt, 1990) predicts that augmented feedback can provide a benefit for motor learning by helping the learner understand their error. If augmented feedback is overused during training, then a dependency can manifest as a result of training conditions and this might reduce the effectiveness of consolidation process that occurs after the end of training. In other words, without available feedback performance is poor, but when feedback is again reintroduced performance improves dramatically (Kovacs, Buchanan, & Shea, 2009a, 2010b).

When training with augmented feedback, it is important to consider presentation timing and type. Augmented feedback can be presented either concurrently or terminally (Abe et al., 2011; Buchanan, 2004; Buchanan, Ryu, Zihlman, & Wright, 2008; Winstein, 1991; Winstein & Schmidt, 1990). Concurrent feedback provides the learner with information that allows them to adjust during a performance. Terminal feedback is presented after a performance and provides the learner with an opportunity to evaluate the information and adjust their strategy when planning the next performance. Augmented feedback can be presented as either discrete or continuous information. Discrete information in rhythmic bimanual tasks is commonly presented as a metronome using lights or auditory tones which define the relative phase pattern to be performed (Tallet, Kostrubiec, & Zanone, 2010; P. G. Zanone & J. A. S. Kelso, 1992; Zanone & Kelso, 1997). Continuous information in rhythmic bimanual tasks is usually presented as some form of visual real-time display providing a flow of information about the current performance allowing learners to adjust their limb motion/strategy and receive immediate feedback (Buchanan, 2004; Buchanan et al., 2008; Kovacs et al., 2009a; Wilson, Snapp-Childs, Coats, & Bingham, 2010). Each type of augmented feedback has its benefits and drawbacks that must be considered when designing a training protocol.

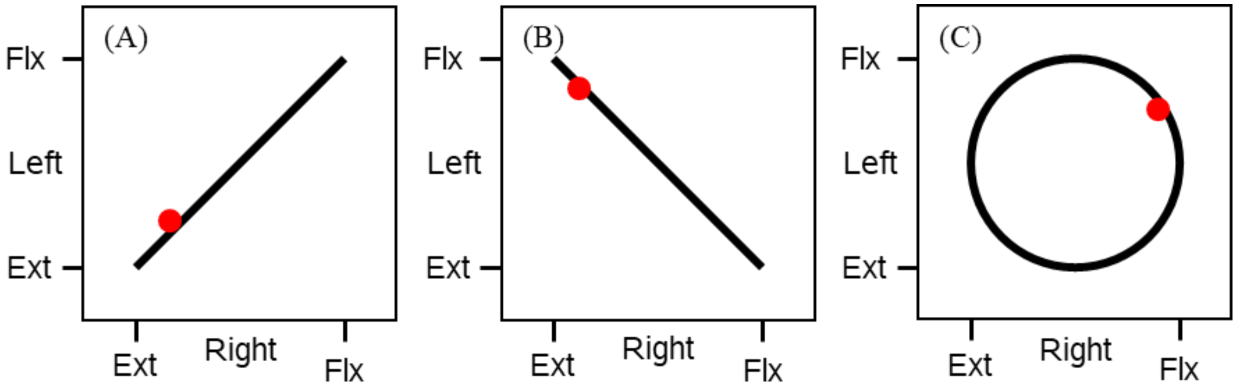

Figure 4. Representation of the Lissajous plot and template displayed to subjects where the *y*-axis represents the motion of the left finger and the *x*-axis represents the right finger. The red dot (●) represents the cursor controlled by displacement of the left and right fingers. A) illustrates the template used to guide the subject to produce the in-phase pattern, flexing and extending symmetrically, while (B) represents alternating flexion/extension which produces antiphase. Figure (C) represents the pattern produced when performing the 90° relative phase pattern.

A popular form of visual feedback display for learning a rhythmic bimanual task is the Lissajous plot (Figure 4). This plot was developed to represent the spatio-temporal relationship between two sine waves regarding their phase and frequency characteristics by Nathaniel Bowditch and Jules Antoine Lissajous in the 1800s. This type of plot has been used in the area of motor control/learning as an augmented feedback training mechanism to facilitate the acquisition of non-intrinsic relative phase patterns such as 90° (Buchanan, 2004; Kovacs & Shea, 2011). The right and left limb displacements are represented on the x-axis and y-axis of the plot, respectively. A Lissajous' template representing the to-be-learned phase and frequency relationship between the two limbs is placed in the plot. A cursor (dot) that can move within the *x*, *y* plane of the plot represents the real-time movement relationship between the two arms (Figures 4A-C). The augmented visual feedback is the motion of the cursor around the Lissajous template. This display provides a representation of the motions of the limbs (several DoF) as a single spatio-temporal relationship of the ongoing coordination pattern.

Ronsse et al. (2010) investigated the effects of visual and auditory feedback on learning and retention. Their task required participants to flex and extend the wrist in a 90° relative phase pattern. The first group received continuous visual feedback of the two-limb displacements via a Lissajous plot. The second group received discrete auditory feedback at movement reversal landmarks (e.g., maximum or minimum flexion). This group was presented with both a high and low tone to distinguish between left and right limb guidance. Analysis of performance results revealed a difference between groups. The visual feedback group was able to learn the task successfully, however, performance deteriorated without feedback at the end of training. This suggests the visual group became dependent on the feedback to perform corrective measures to perform the task. This would explain the performance vulnerability once feedback was removed.

Both groups reached the same peak performance by the end of the training, however, the auditory group improved initially at a slower rate. Interestingly, retest performance for the auditory group was maintained when feedback was removed (Ronsse, et al., 2010). The auditory group performed well with and without feedback and suggests this group consolidated a representation of the required coordination pattern that could be recalled independent of the feedback. The discrete auditory feedback group may have experienced a more challenging learning environment that could have encouraged participants to rely less on external feedback and rely more on proprioceptive information and internal timing (Buchanan & Wang, 2012).

Various studies over the past decade have used the Lissajous plot as a form of augmented feedback to generate a type of ‘rapid tuning’ of the motor system to performing quite difficult tasks. It was hypothesized that the visual information provided through the Lissajous plot and the cursor allowed participants to easily identify their coordination errors and provided an opportunity to adjust their movements in real-time. What had previously been shown to take several days of training could now be trained in a matter of minutes (Kovacs et al., 2009a; Kovacs, Buchanan, & Shea, 2010a). Kovacs and colleagues concluded the Lissajous plot was only able to produce a temporary change in performance as participants were unable to continue to produce the required bimanual pattern when the display was removed. After a short delay (≤ 15 min), participant accuracy and stability degraded without the presence of the Lissajous plot in the retest. It was hypothesized that a dependence on the feedback was developed during training. What was not examined across a series of studies by Kovacs and colleagues was the time scale of the consolidation process. In other words, maybe the Lissajous rapid tuning context can support the consolidation of a trained bimanual motor task, it may just take time for the motor memory to consolidate (Brashers-Krug, Shadmehr, & Bizzi, 1996). A recent study has shown that participants can produce the 90° pattern after Lissajous rapid tuning when extending the retest delay period to 6-hr (McCulloch et al., 2020) instead of 5-15 minutes. Even a 2-hr retest delay was not associated with significant consolidation. McCulloch et al. (2020) argued that a 5-15 minute retest delay was not sufficiently long enough for any consolidation to occur, thereby revealing what appeared to be feedback dependence. The time-dependent nature of consolidation was considered the reason for the difference in memory strength and coordination stability found at the different retest intervals.

1.3 Motor Memory Consolidation

Motor skill consolidation represents the process by which motor skill memories are transformed from an initial fragile state to a more solid or resilient (stable) state. During this period of transformation, memories are especially susceptible to disruption. Proactive interference occurs when older memories interfere with the retrieval of newer memories. This occurs primarily when there is a high similarity between what is to be remembered and the interfering memory. Retroactive interference occurs when new information interferes with the ability to recall previously learned information, such that the interfering activity occurs during the retention interval of the movement we need to remember.

Brashers-Krug (1996) used both retroactive and proactive interference to probe the timeline of a motor skill consolidation period. Subjects moved a handle (attached to a robotic arm) to guide a cursor (presented on a screen) to a series of targets that appeared one at a time. The experiment included an A and B task which manipulated the handle by providing a perturbation force in either a clockwise (Task A) or counter-clockwise (Task B) direction. The control group practiced task A and retested after 24-hrs demonstrating that subjects improved and maintained a significant level of retention for this period after training. The four experimental groups completed a recall of task A at 24-hrs but also practiced task B at one of four time delays: no-break, 5-min, 1-hr, or 4-hrs.

The no-break, 5-min, and 1-hr groups did not have a significant level of retention for task A at the 24-hrs retest. The 4-hrs group maintained a significant level of retention at the 24-hr retest. Subjects were unable to benefit from previous training with task A, suggesting that learning task B at time points < 4-hrs disrupted the consolidation process of the motor skill for task A (Brashers-Krug et al., 1996). These findings suggest that human motor memory is transformed over time from a fragile state to a more stable state even in the absence of further practice.

Clearly, the time-dependent nature of the motor skill consolidation process is important. Interrupting this process through interference can prevent the accurate recall/formation of a memory. The importance of the time interval for motor consolidation was investigated to explore the role of the primary motor cortex in early motor consolidation (Muellbacher et al., 2002). Repetitive transcranial magnetic stimulation (rTMS) was used to interfere with cortical function and disrupt the consolidation process. For three blocks, participants practiced a ballistic pinching task using their index finger and thumb of the non-dominant hand. The goal was to pace with an external metronome (0.5 Hz) while maintaining a certain pinching acceleration. Applying rTMS to the hand area of the primary motor cortex immediately after practice blocks 1 and 2 canceled the retention of the behavioral improvements. Participants began the next practice block with a similar error as practice block 1. It was determined that the rTMS canceled the behavioral gains from practice but did not affect the ability to improve during subsequent practice. This indicated that rTMS of the primary motor cortex disrupted the process of early motor memory consolidation. In a second experiment, Muellbacher et al. (2002) trained participants on the same pinching task. After one practice block, participants received rTMS after a 6-hr period and then immediately performed a retest. The rTMS failed to interfere with the recall of the performance gain in the pinching task which indicated the gain in performance had consolidated and become resistant to interruption (Muellbacher et al., 2002). The Muellbacher et al. studies demonstrate how the primary motor cortex is involved, on some level, with the early process of motor skill consolidation. These results also support the concept that consolidation is a time-dependent process whereby a memory becomes more resilient over time.

1.3.1 tDCS and Consolidation

Transcranial Direct Current Stimulation (tDCS) is a non-invasive brain stimulation technique that uses low electrical currents to stimulate the brain. At its simplest form, a tDCS device has a positive (anode) and a negative (cathode) electrode. The current generated by tDCS is applied by placing one electrode (either cathode or anode) on the scalp over a target neural region while placing the reference electrode on the scalp over a non-target neural region. Stimulation intensity is measured in milliampere (mA) and is typically applied for several minutes per exposure. For sham stimulation, current is ramped up and back down over several seconds at the beginning and end of the pseudo-stimulation time period to produce the same initial and terminal sensation as commonly experienced with the active condition. Sham stimulation serves as a control condition for active stimulation conditions.

Transcranial direct current stimulation of the motor cortex results in polarity-specific changes of underlying cortical excitability during and for some time after stimulation. Neural modulation that occurs depends on the polarity of the electrode, stimulation duration, and stimulation intensity (Nitsche & Paulus, 2000, 2001). Anodal stimulation has been demonstrated to increase neural excitability whereas cathodal stimulation inhibits excitability. One of the most widely cited studies for this claim is Nitsche and Paulus (2000). This paper included four experiments in an attempt to determine the most appropriate electrode placement, current intensity, stimulation duration, and the strength and duration of the stimulation after-effects. The first study included various electrode placements: occipital cortex, posterior to motor cortex (M1), anterior of M1, ipsilateral M1, contralateral M1, and contralateral supraorbital. The claimed optimal electrode placement was M1 and the contralateral supraorbital area. The next study found that 1 mA of anodal tDCS over M1 for 5 min significantly increased cortical excitability for 4 min after stimulation while cathodal tDCS significantly reduced cortical excitability amplitudes for 3 min. In the concluding studies, it was found that a stimulation intensity of at least 0.6 mA or a minimum of 3 min of anodal tDCS is needed to impact cortical excitability (Nitsche & Paulus, 2000). In another study, these tDCS effects were documented to last up to 90 minutes after the end of stimulation (Nitsche & Paulus, 2001).

Recently, tDCS has been used to somehow enhance the consolidation processes underlying motor memories by increasing the rate of consolidation or the strength of memories at the end of the consolidation period. A study by Tecchio et al. (2010) aimed to determine if increasing excitability of the primary motor cortex by anodal tDCS during the consolidation period of a procedural serial reaction time task (SRRT) induced early consolidation. Participants performed the SRRT with their non-dominant (left) hand and were trained on a combination of random key sequences and repeating key sequences. After training, participants received 1 mA of current for 15 min with the anode electrode over the right primary motor cortex (C4) while the reference (cathode) electrode was placed on the ipsilateral shoulder. The sham condition received 10 sec of stimulation at the beginning and end of the 15 min period. After stimulation, it was found that consolidation of the trained series was enhanced, such that performance improved more between the end of training and the retest for anodal stimulation but not for sham stimulation (Tecchio et al., 2010). It was suggested that anodal tDCS applied to the primary motor cortex after training improves the early consolidation period (15 mins) of procedural learning. It is important to also note, neither anodal nor sham tDCS affected the performance of the random series execution indicating the stimulation benefited the consolidation process and not an overall performance increase in key pressing.

In a more recent paper, Chen et al. (2020) attempted to recreate and expand on Tecchio's findings. Participants experienced either anodal or sham tDCS over the primary motor cortex after practicing an SRTT followed by retest trials 15 min later. Subjects experienced 1 mA of stimulation for 15 min with the anode electrode placed over the right primary motor cortex (C4) while the reference (cathode) electrode was placed on the ipsilateral shoulder. The sham condition delivered stimulation for the first and last 30 secs of the 15 min stimulation period. To expand on Tecchio's study, Chen and colleagues included two additional novel conditions to retest anodal and sham tDCS at 120 min. The expectation was that if anodal tDCS influences the consolidation process which leads to offline gain, the effect should be present at 15 min and 120 min. Their findings revealed an offline gain at both 15 min and 120 min, however, there was no interaction between anodal and sham tDCS (Chen et al., 2020). The lack of a difference between anodal and sham effects somewhat weakens the confidence in the potential for tDCS after training to enhance the consolidation process.

1.3.2 Stimulation Timing

Although the effects of tDCS on learning have been highly studied for uni-manual tasks (Chen et al., 2020; Tecchio et al., 2010), the effects of tDCS on learning novel bimanual tasks have not been the focus of as much research. Current research has yet to provide a decisive best protocol for stimulation-training coupling and target stimulation site for bimanual tasks. To begin, stimulation may be applied immediately before, during, or immediately following training. Research for bimanual learning tasks have used either stimulation before training (Carter et al., 2015, 2017; Furuya et al., 2014; Gomes-Osman & Field-Fote, 2013; McCambridge et al., 2016), or during training (Furuya et al., 2013; Jin, Lee, Kim, & Yoon, 2019; Pixa et al., 2017a, 2017b; Vancleef et al., 2016).

The use of tDCS stimulation before training has produced mixed results for bimanual control and learning tasks. For example, Carter et al. (2015, 2017) found that stimulation before a bimanual control task improved subject performance with anodal tDCS over the supplementary motor area (SMA) compared to cathodal stimulation and sham conditions. In these two studies, participants attempted to produce the antiphase bimanual pattern while maintaining pace with an auditory metronome which increased in frequency in a stepwise manner. The overall aim was to determine if tDCS could prolong the stability of the antiphase pattern by delaying the transition to in-phase. Similarly, in a bimanual asymmetric sequential typing task, stimulation before training was found to increase performance after five days of training when compared to the group that received sham before training. For this multi-day training study, bilateral 1 mA anodal tDCS was used on the left and right primary motor cortex (M1) for 20 min (Gomes-Osman & Field-Fote, 2013). In a metronome-paced bimanual asymmetric sequential typing task, Furuya et al. (2014) found that novices improved performance with stimulation (2 mA, 15 min) whereas experts (skilled pianists) did not show improvement. The target site for stimulation was left and right M1. Although these studies found positive performance effects from tDCS, McCambridge et al. (2016) found tDCS stimulation before training (1 mA, 15 min) had no improvements on a bimanual circle tracing task. The anode and cathode were placed over the left and right M1, respectively. These results are somewhat contradictory to the previous studies mentioned, but McCambridge et al. used a different task (rhythmic circle tracing) than other studies and only tested one montage (right M1 anode, left M1 cathode).

Concurrent tDCS stimulation also seems to produce mixed results as well. Bi-hemispheric high definition tDCS was applied while training with the bimanual Purdue Pegboard Task (PPT). tDCS montages which include multiple anode and cathode electrodes are considered high definition. The anodal stimulation targets were left and right M1 (2 mA for 15 min) and the task was completed with both hands moving in unison or alternating. A significant increase in performance on the PPT was found for completing the task while moving both arms in unison, but not for completing the task with the arms alternating (Pixa et al., 2017b). In a cup stacking bimanual task which also used concurrent high definition tDCS, an interaction of group and time was found for the concurrent anodal tDCS group, but not the sham group, indicating a greater performance gain, but only for one of the two practiced stacking tasks (Pixa et al., 2017a). In another study targeting the impact of anodal tDCS on left M1 and left dorsolateral prefrontal cortex (DLPFC), participants received 2 mA of stimulation for 30 min during training on a bimanual force matching task. Participants were tasked to produce either a static force or trace a sinusoidal wave by pressing their hands against force sensors. Left M1 anodal stimulation was found to increase both force maintenance accuracy and rhythmic alteration of force. However, left DLPFC anodal stimulation only improved force control maintenance (Jin, Lee, Kim, et al., 2019). Although some concurrent stimulation protocols have been shown to affect motor performance, not all research has shown benefits. For example, one study found no benefit of tDCS on bimanual motor performance or change in corticospinal excitability (as measured by MEPs) after stimulation targeting left M1 or left DLPFC (Vancleef et al., 2016). Participants trained using two dials to perform a multi-frequency bimanual Lissajous task while receiving anodal stimulation over four training days. In another study that targeted a more specific population (highly trained individuals: professional pianists), stimulation did not produce any apparent improvement of bimanual motor control processes (Furuya et al., 2013). These results were in direct contrast with a previous study by Furuya et al. (2014) using novice individuals and the same task with stimulation before training.

It is important to note the time differences in the retests between these various studies that have coupled tDCS with bimanual tasks. In the two studies by Carter et al. (2015; 2017), the aim was to improve motor control and stability during performance, so no retest was performed (Carter et al., 2015, 2017). Other research groups looked at the change in performance at a short period after training (less than 15 min) (Furuya et al., 2014; Furuya et al., 2013; Jin, Lee, Kim, et al., 2019; McCambridge et al., 2016). The remaining research groups had subjects perform multi-day training and stimulation with follow-up retests. Gomes-Osman & Field-Fote (2013) used a five-day training schedule paired with tDCS conditions with a one-week follow-up retest. Similarly, Vancleef et al. (2016) incorporated a four-day training paradigm with tDCS and a one-week follow-up retest. In both the cup stacking (2017a) and PPT studies (2017b), Pixa et al. had participants train for three days coupled with tDCS with retests on the fourth day and one week later. None of these studies investigated the specific 4 to 6-hr time period that has been shown to be critical for the initial consolidation process supporting memory formation for aiming tasks and SRRT skills.

To date, no research using bimanual tasks and tDCS has systematically compared before, during, and after training stimulation on motor training or retention. However, a recent study using a uni-manual task attempted to investigate the timing-dependent interactions between tDCS and motor training (Cabral et al., 2015). A simple repetitive abduction-adduction thumb motion was performed and was paired with tDCS either before, during, or after training. The anode electrode was placed over the left M1 (hand area as identified by TMS) and the cathodal reference electrode was placed over the contralateral supraorbital area (1 mA for 13 min). To determine the excitability of the corticospinal tract, motor evoked potentials (MEPs) of the first dorsal interosseous muscle were compared between baseline and post-training/stimulation. Only the before training tDCS produced a significant increase in MEPs (Cabral et al., 2015). This study did not provide or evaluate any performance measures. Further research should be conducted to determine the time-dependent interactions between tDCS and early consolidation of bimanual skills, which is one of the aims of the following research.

1.3.3 Stimulation Montage

In addition to the differences in time coupling for stimulation and training, these studies also varied in target stimulation sites. The most effective target stimulation area has not been determined for bimanual task training. The target sites for current research are the primary motor cortex, the left dorsolateral prefrontal cortex, and the supplementary motor cortex. Stimulation to M1 (hand area) has been the most widely targeted stimulation site for bimanual tasks and has varying results on effectiveness (Furuya et al., 2014; Furuya et al., 2013; Gomes-Osman & Field-Fote, 2013; McCambridge et al., 2016; Pixa et al., 2017a, 2017b; Vancleef et al., 2016). Stimulation of the left DLPFC was found to be both effective and ineffective to improve training (Jin, Lee, Kim, et al., 2019; Vancleef et al., 2016). The SMA may be an ideal stimulation site for bimanual control (not learning/consolidation) tasks as demonstrated by two studies that found promising results (Carter et al., 2015, 2017). Although these studies have used various motor tasks and training protocols, when determining target stimulation sites, it is important to discuss what cortical areas are active during the learning process.

The general cortical networks associated with bimanual coordination tasks were identified as the primary somatosensory cortex (S1), M1, premotor areas, SMA proper, cerebellum, cingulate motor area, and DLPFC (Debaere, Wenderoth, Sunaert, Van Hecke, & Swinnen, 2004; Puttemans, Wenderoth, & Swinnen, 2005; Remy, Wenderoth, Lipkens, & Swinnen, 2008). Two studies using functional magnetic resonance imaging (fMRI) investigated the activation of the cortical network for bimanual skills and how these areas within the network changed throughout training (Debaere et al., 2004; Remy et al., 2008). Debaere et al. (2004) and Rémy et al. (2008) trained participants to produce the 90° relative phase pattern over four days of training. Participants flexed and extended their wrists while using a Lissajous plot to learn the new coordination pattern. Similarities of cortical activation across training were observed between the studies. For example, both groups found the right DLPFC and the right dorsal pre-motor cortex (PMDc) had greater activation at the beginning of training compared to the post-training scans. Debaere and colleagues also found the right ventral pre-motor cortex (PMVc) was more active during early compared to late training. Concerning greater activation at the end of the training, Debaere et al. found the left PMDc had greater activation, suggesting a shift from the right to the left PMDc as the motor skill is learned.

In a similar study, Puttemans and colleagues trained participants to produce a multifrequency (2:1) bimanual coordination pattern and used fMRI to examine the cortical network across several training days (Puttemans et al., 2005). The protocol included a pre-training scan, 4 days of training, a mid-training scan, 4 more days of training, and a post-training scan. This study intended to over-train participants to the point of automaticity, therefore it offers more depth to how the landscape changes throughout training. Similar cortical activation patterns were observed in the previously mentioned studies. The right ventral pre-motor cortex activity was greater for pre-training compared to mid-training, similar to the Debaere et al. (2004) findings. Interestingly, the SMA was highly activated in both the pre- and mid-training scanning sessions but significantly decreased at the post-training scan suggesting that extended practice no longer requires greater activation of this area. Regarding the primary motor cortex (M1), there was a temporary increase in activation such that activity was greater at mid-training compared to both pre- and post-training scans. Of the identified active cortical areas, the M1, SMA, and DLPFC have been targeted for stimulation in current tDCS literature.

When determining the stimulation montage for the following two experiments, two cortical areas were the focus of concern. As identified through fMRI studies (Debaere et al., 2004; Puttemans et al., 2005; Remy et al., 2008), M1 and SMA are two active cortical areas that are important for the planning and execution of bimanual tasks. Therefore, the montage to be selected should provide the greatest likelihood that stimulation current affected both these motor areas in both the left and right hemispheres. The stimulation montage used in the proposed studies was determined through modeling comparisons of montages used in current literature. Three montages were modeled using HD-Explore (Soterix Medical Inc.) and are presented in Figure 5A-C. This software has been used to model current flow in similar studies using uni-manual tasks (Ballard, Eakin, Maldonado, & Bernard, 2021; Kim et al., 2021). The target sites for stimulation are shown in the far-left image and were placed using the 10-20 EEG system. This first montage (Figure 5A) was used to target the SMA during motor training. The field intensity is greater in the frontal lobe and appears to have little to no direct impact on the primary motor cortex. Figure 5B models the contralateral montage which targets left M1 for anodal stimulation. This montage appears to have an asymmetric field intensity dispersion between hemispheres. Due to electrode placements, the left hemisphere was modeled to have a higher field intensity more caudal compared to the right hemisphere. There also appears to be an imbalance between the field intensities for M1 and the SMA between hemispheres. The third model (Figure 5C) uses bi-hemispheric stimulation targeting the left and right M1. This modeling indicates a symmetric field intensity between hemispheres

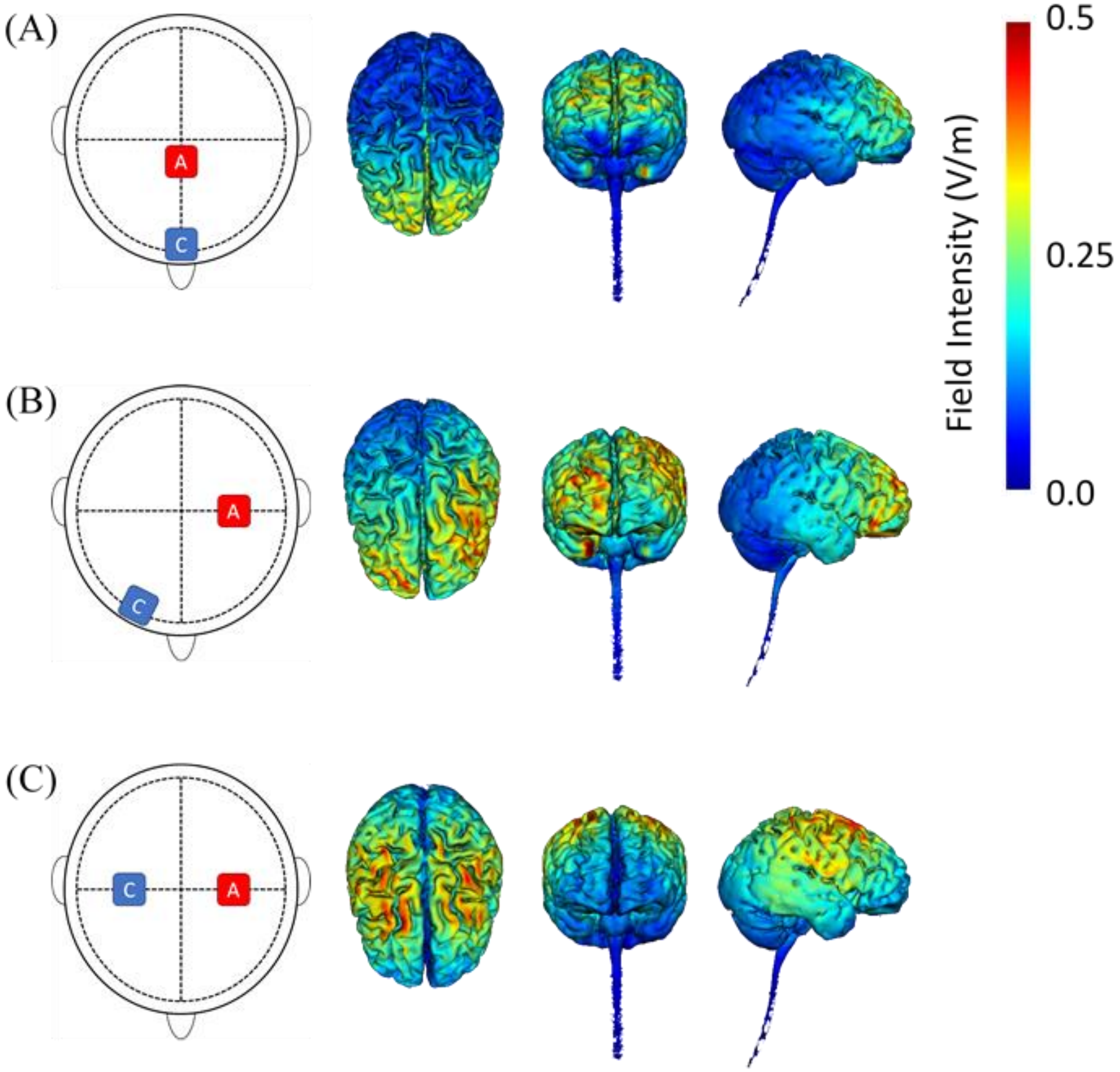

Figure 5. tDCS electrode placements and 3-D modeling of the current field intensity with HD Explore (Soterix Medical Inc.). The electrodes are identified as the red square for the anode and the blue square for the cathode. The images from left to right are the electrode placements according to the 10-20 EEG system, the dorsal view, frontal (rostral) view, and right side (sagittal) view of the modeled brain. (A) This montage targeted the SMA by placing the anode over FCz and the cathode on the FPz. (B) The left M1 is targeted with the anode placed over C3 and the cathode placed contralateral supraorbital, on Fp2. (C) Bi-hemispheric stimulation targeting the primary motor cortex where the anode is placed over C3 and the cathode is placed over C4.

and appears to have high field intensities around M1 and SMA. It is noteworthy that the modeling software produces highly similar field intensity models for both the left-anode montage and the right-anode montage, only the current flow direction was reversed. The bi-hemispheric montage was chosen for the proposed experiments because of the high field intensities displayed in the target neural areas (M1 and SMA) and the symmetric dispersion between hemispheres. This montage has been used in previous bimanual studies (Furuya et al., 2014; Furuya et al., 2013; McCambridge et al., 2016).

1.4 The Consolidation Process of Bimanual Learning

Research has shown that many procedural skills show resiliency to interference after a 4-hr window from the end of training to a retest of the trained skill (Brashers-Krug et al., 1996; Press, Casement, Pascual-Leone, & Robertson, 2005; Robertson, Pascual-Leone, & Miall, 2004). This reduced susceptibility represents the consolidation process from a fragile state to a stable state of a newly formed motor memory. Consolidation can emerge as either memory stabilization or off-line learning depending on the relationship between the end of training and retest performance (Robertson et al., 2004). Memory stabilization is characterized by the end of practice performance being equal to the retest performance, such that no significant forgetting has occurred (Brashers-Krug et al., 1996; Goedert & Willingham, 2002). Off-line learning emerges as a significant increase in performance at retest compared to the end of training (Press et al., 2005; Walker et al., 2002). However, consolidation emerging as memory stabilization or offline enhancement after the 4-hr window identified as important for uni-manual has only recently been investigated using bimanual tasks.

A recent study by McCulloch et al. (2020) examined the time scale of the consolidation process for a rhythmic bimanual task. One aim was to determine if consolidation of a rhythmic bimanual task takes place in the form of memory stabilization or off-line learning after the 4-hr window. The experiment consisted of an initial training session followed by an interference/retest session. Subjects were asked to flex and extend their index fingers to produce the 90º relative phase during the training session (10 trials, 5 min) and a 45º relative phase pattern in the interference session (10 trials, 5 min). Subjects were provided a Lissajous plot for augmented feedback during both sessions. After the interference task, subjects rested for 2 min and were asked to perform the 90º relative phase pattern without the Lissajous plot. The 45º pattern was intended to serve as a retroactive interference task for the 90º pattern recall test. To explore the time-dependent nature of the consolidation process, participants returned for the interference/retest session at either a 2-hr or 6-hr delay after the end of training. These time delays were chosen to probe the resiliency of the motor memory during and after the critical 4-hr window. The findings revealed a form of memory stabilization of the 90º pattern for the 6-hr delay group; performance at the end of the 90º training session was similar to the retest performance, even in the absence of augmented feedback. Subjects in the 2-hr delay group performed significantly worse on the 90º pattern during the retest trials compared to the end of the training session (McCulloch et al., 2020). This would suggest the initial encoding of the motor memory for the 90° bimanual pattern was still fragile at 2-hrs and subject to interference by training with the new 45° pattern, similar to the initial encoding of other procedural skills (Brashers-Krug et al., 1996; Muellbacher et al., 2002). The fragility of this motor memory decreased over time through the consolidation process which emerged as memory stabilization at 6-hrs. An important finding of this study was that the time scale for rhythmic bimanual motor memory consolidation is similar to uni-manual serial and discrete tasks.

Providing augmented feedback during motor skill training can improve learning outcomes, but it is not without drawbacks. As previously discussed, the guidance effect (Winstein & Schmidt, 1990) predicts that augmented feedback can provide a benefit for motor learning by helping the learner understand their error. However, if augmented feedback is overused during training, then a dependency can manifest and influence the consolidation processes that occur after the end of training. In other words, without the availability of the feedback performance is poor, but when feedback is again reintroduced performance improves dramatically (Kovacs et al., 2009a, 2010b). It is important to note that Kovacs et al. (2009, 2010b) only retested 15 min after training. A key difference between the Kovacs et al. (2009, 2010b) and McCulloch et al. (2020) studies is the time delay between the end of training and the retest. Results of the Kovacs' experiments indicate that using a Lissajous plot as augmented feedback creates a dependency, whereas McCulloch et al. found memory stabilization at 6-hrs after training. This would indicate that subjects were able to form a new motor memory not dependent upon the augmented feedback as a form of error detection. Performance deteriorated at 2-hr which supports the hypothesis that the Kovacs et al. (2009, 2010b) retest at 15 min was not sufficiently removed from the end of training to determine if a stable motor memory was beginning to form.

1.5 Hypothesis

Two experiments were conducted to explore the time-dependent nature of the consolidation process for rhythmic bimanual coordination tasks. To probe this consolidation process, the experiments manipulated the mechanical degrees of freedom, stimulation-training coupling, and time delay of the retesting period. In Experiment 1, subjects experienced tDCS either before or during training on the 90° pattern and retested at 6-hrs. To perform the rhythmic bimanual task, participants manipulated two handles by flexing/extending their whole arms (six mechanical degrees of freedom: shoulders, elbows, wrists). All participants were expected to show performance improvement during the training trials. It was anticipated that participants who received tDCS would demonstrate an increase in motor performance, compared to the sham groups, which would last at least until 6-hr post-training. This prediction of improved performance with tDCS is consistent with several studies (Carter et al., 2015, 2017; Furuya et al., 2014; Gomes-

Osman & Field-Fote, 2013; Jin, Lee, Kim, et al., 2019; Jin, Lee, Oh, Celeste Flores Gimenez, & Yoon, 2019; Pixa et al., 2017a, 2017b).

For Experiment 2, subjects received before training tDCS and retested the trained 90º pattern at either 2 or 6-hrs. To reduce the degrees of freedom from the first experiment (from six to two), participants flexed/extended their index fingers at the metacarpophalangeal joint to produce the rhythmic bimanual task. All participants were expected to learn the task over the training period. Previous work has shown the time delay between training the 90° pattern and the retest is critical since the 90° trained pattern was resilient to interference after a 6-hr delay but not after a 2-hr delay (McCulloch et al., 2020). It was expected that performance would stabilize or potentially be enhanced at the 6-hr retest, and performance would be poor at the 2-hr retest. tDCS was expected to enhance the consolidation process and potentially improve performance compared to the sham groups. This would be evident by maintained or improved performance at the 6-hr delay retest. The 2-hr-delay group is expected to have diminished performance compared to the end of training. However, if tDCS has an impact at this time point during the consolidation process then performance maintenance may be evident when compared to the sham group at the 2-hr delay.

2. EXPERIMENT 1: METHODS

2.1 Participants

A total of 96 participants were recruited through the Motor Neuroscience pool in the Department of Health and Kinesiology at TAMU. The participants were randomly placed into six groups (Table 1). Participants were young adults (M_{age} = 21 ± 1.7 yrs.; 66 Female, 30 Male; 85 right-handed, 6 left-handed, 5 mixed-handed) and free of any neuromuscular disorders which inhibit upper limb movement or sensation. The experimental procedure, consent form, and all questionnaires were approved by the Human Subjects Interval Review Board at TAMU. All participants consented to participate in line with the Helsinki Declaration.

Table 1. Experimental groups: Stimulation Montage and Stimulation Timing.

	Stimulation Montage		
Stimulation Timing	Left-Anode	Right-Anode	Sham
Before	N = 16	N = 16	N = 16
During	N = 16	N = 16	N = 16

2.2 Task and Experiment Setup

2.2.1 Bimanual task

Sitting in an upright and comfortable position, participants grasped two vertical handles and slid them along a fixed track mounted on a desk (Figure 6A). The task required abduct-adduct motions of their whole arms in the horizontal plane parallel to the tabletop (Figure 6B). Augmented visual feedback was displayed via a Lissajous plot on a computer monitor placed directly in front of the participant (approximately 60 cm) at eye level. The horizontal displacement of the right and left arms was mapped to the *x*-axis and *y*-axis of the template in the Lissajous plot, respectively.

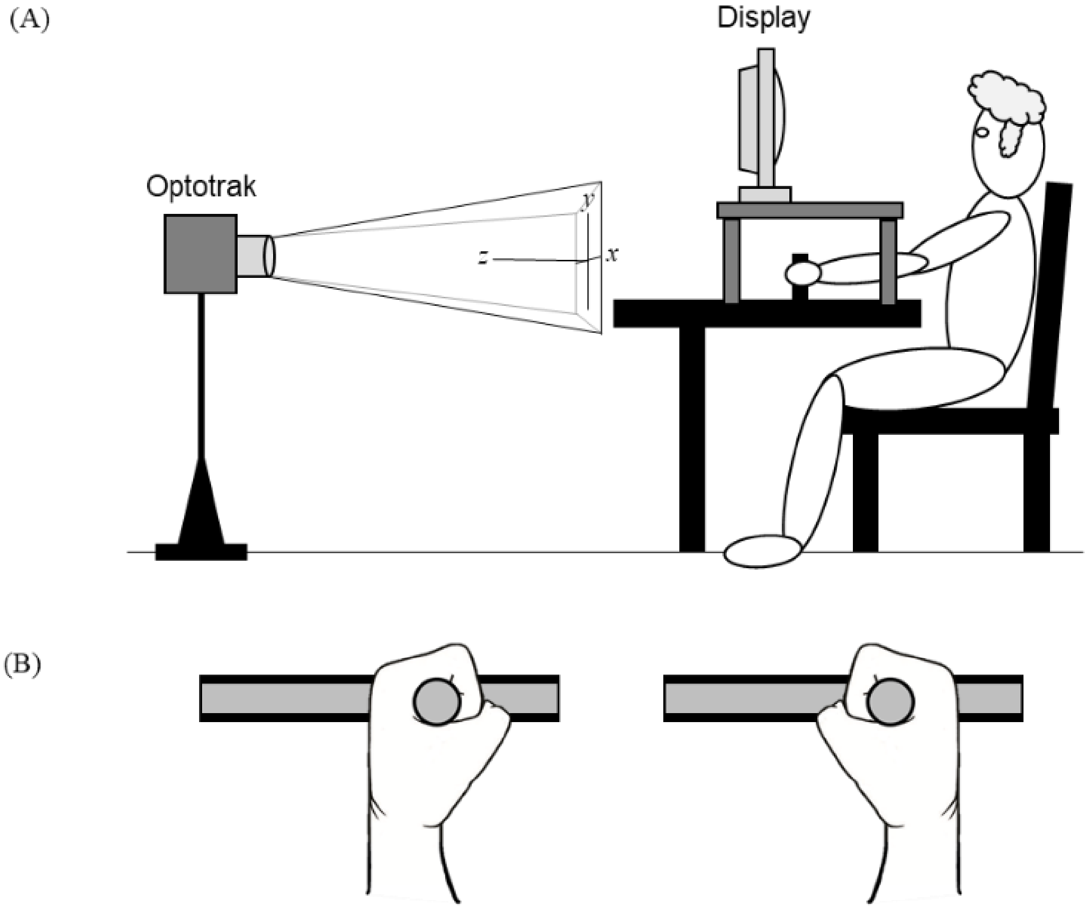

Figure 6. (A) Experiment set-up showing participant sitting at the desk grabbing the vertical handles. Visual display was placed on the hood which restricted the vision of the arms to the participant. (B) Dorsal view of participant grasping the vertical handles fixed to the sliding track on the table.

For the familiarization trials, participants received verbal instructions and used visual feedback provided by the Lissajous plot to perform 0° and 180° patterns (Figures 1 and 4). The bimanual training pattern was a 90° relative phase pattern also defined with the Lissajous plot (Figures 3 and 4). The handles the participants grasped were under a hood which removed vision of the participant's limbs (Figure 6A).

2.2.2 tDCS

This experiment investigated the training and consolidation process of the motor memory for a rhythmic bimanual coordination task over a 6-hr retest interval. A 1×1 low-intensity transcranial electrical stimulator system (Soterix Medical, New York, NY) was used to deliver direct current stimulation to modulate cortical excitability either before or during training of the bimanual coordination pattern. The 2-mA current was transferred by an anodal and cathodal conductive rubber electrode (5 × 5 cm) resulting in a maximum current density of 0.08 mA/cm. The electrodes were placed in sponges soaked in 5 mL of a 0.9% saline solution. The center of the anodal and cathodal electrodes was placed over one of two target areas identified using the 10-20 EEG placement system: 1) C3 anode and C4 cathode (Left-Anode), or 2) C3 cathode and C4 anode (Right-Anode). As modeled by the HD-Explore software (Soterix Medical Inc.), M1 and SMA should have a heightened field intensity as a result of the stimulation montage (Figure 5C). Previous research has used similar montages with bimanual tasks (Furuya et al., 2014; Furuya et al., 2013; McCambridge et al., 2016). The sham condition was pseudo-randomly chosen as one of these two combinations. The electrodes were placed at a minimal distance of 6 cm to decrease the probability of shunting current through the scalp (Rush & Driscoll, 1968). In the active stimulation condition, the stimulation lasted for 21 minutes at an intensity of 2 mA with a ramping up and down over a 30-second interval. In the sham condition, the current ramped up and back down over a 60-second interval to reach a peak of 2 mA at the beginning and end of the same 20-minute period to produce a similar initial and terminal sensation as used with the active condition.

2.2.3 Experimental Procedure

The experiment required participants to partake in two separate sessions separated by 6 hours. Session 1, the training session, lasted between 60 and 85 minutes while session 2, the retest session, lasted between 15 and 30 minutes. Every training-retest trial lasted 20 seconds and was followed by a 20-second rest. In the first session, participants completed six familiarization trials using the Lissajous plot as feedback: three in-phase trials, three antiphase trials. After performing the familiarization trials, the tDCS electrodes were secured to the participant's scalp. Stimulation was applied either before or during the 20 training trials (T1-T20) depending on the experimental group (Figure 7). The before training stimulation groups waited in a relaxed position while abstaining from any movements for the 20 min stimulation period. After stimulation, the 20 training trials began. The during training stimulation groups began the stimulation after a 20 min rest period and performed the 20 training trials concurrently with stimulation. The participants used the Lissajous plot and the provided template as a guide during the learning trials. After completing the trials, the session ended, and participants completed the post-survey questionnaire as an opportunity to report any adverse side effects or sensations experienced due to stimulation.

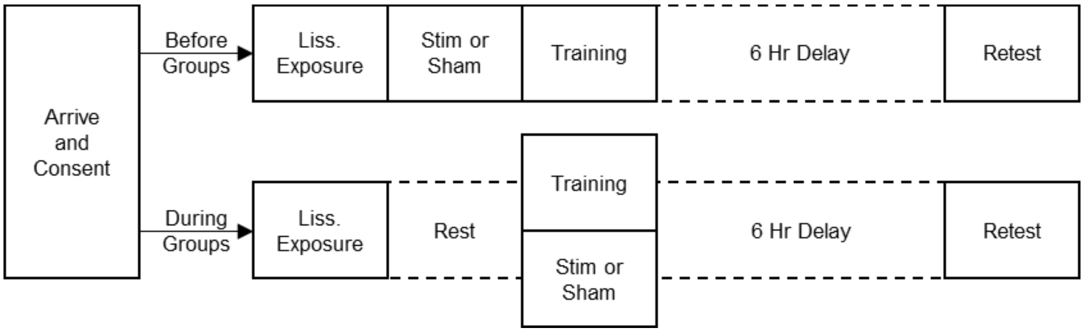

Figure 7. Experimental timeline. Participants were separated into either Before Training (top) or During Training (bottom) stimulation timing. After consent and passing the screening process, participants completed the initial exposure to the Lissajous plot. Then, depending on the group, participants either rested for 20 min and began the stimulation and training trials or began the stimulation while passively sitting and begin training after the completion of stimulation. Following the session was a 6-hr delay before the retest trials.

After a 6-hr delay, the participants returned to the lab to complete eight more trials (R1-R8). In the retest session, participants were asked to perform the 90° coordination pattern from memory without any feedback (Lissajous plot) for four 20 sec trials (R1-R4). Then participants were given the Lissajous plot to complete the last four 20 sec trials of the session (R5-R6). The retest trials provide an estimate of the strength of the motor memory for the trained 90° pattern after the 6-hr consolidation interval.

2.3 Data Collection and Analysis

The Optotrak Certus 3D camera system (Northern Digital, Inc.) was used to record the motion of two infra-red LEDs attached to the vertical handles. The camera was positioned approximately two meters from the participants' hands and approximately one meter parallel to the floor (Figure 6A). The markers were sampled at 100 Hz with 2000 data points collected per trial. All dependent measures were calculated using Matlab 2014b (The Mathworks, Inc.). The *x*-axis time series of the markers were dual-pass filtered (Butterworth) with a 10 Hz cutoff.

2.4 Performance Measures

Performance was evaluated by calculating the relative phase of the hand motions (Figures 1-4). The horizontal displacement motion of the handles was represented by the *x*-axis in the *XYZ* plane of the Optotrak system. This axis was used to compute continuous individual phase angles (θ_i) for each arm. The *x*-axis time series for each arm (dx_i) was differentiated to produce a velocity signal (dx_i/dt_i). The displacement and velocity time series were normalized to the range -1, 1 on a half-cycle basis (Varlet & Richardson, 2011) before computing the individual phase angles for the left (θ_l) and right (θ_r) arms: $\theta_i = tan^{-1}\,[dx_i/(dx_i/dt_i)]$. To evaluate performance improvements in the task, continuous relative phase ($\boldsymbol{\phi}_C$) was calculated by subtracting the left arm phase angle from the right arm phase angle, $\phi_c = \theta_r - \theta_l$ (J. P. Scholz & J. A. Kelso, 1989). The $\boldsymbol{\phi}_C$ time-series data points were transformed (circular) to unit vectors in a two-dimensional plane. Two performance measures were calculated using the $\boldsymbol{\phi}_C$ time series. A time-on-task estimate represented as a distribution of relative phase calculated based on nine 20° bins across the range 0 ° to 180 °. This measure was calculated as the percentage of points of the $\boldsymbol{\phi}_C$ time series

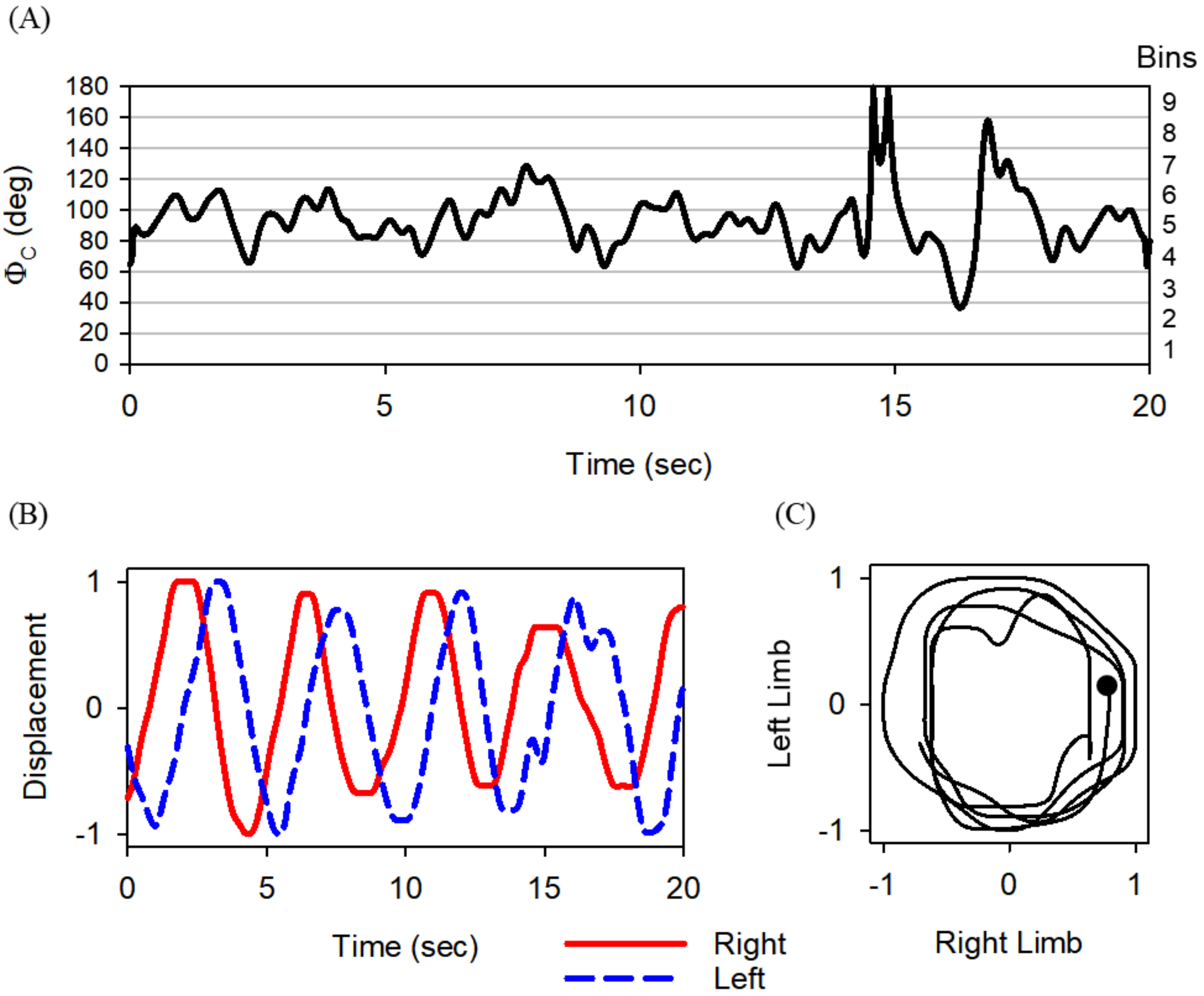

Figure 8. (A) Continuous relative phase for a single 90º pattern training trial. Horizontal reference lines are the 9 bins of 20°. (B) Left and right limb displacement time series and (C) a Lissajous plot of a single trial during the 90º pattern training session.

which fell within each of the nine bins (Figure 8a). This measure provides an estimate of performance accuracy. The second measure, root mean square error (RMSE), was calculated as the square root of the difference between every point (i) in the ϕ_{Ci} time series and the target relative phase value (T = 90º) for a trial = $\sqrt{\sum_{i=1}^{n}(T-\phi_{Ci})^2/n}$. The RMSE provides a performance accuracy measure linked to the training pattern. Both measures were used to evaluate training and the extent of consolidation that emerged after training. Relative phase variability ($\boldsymbol{\phi}_{SD}$) was computed through the circular transformation of the $\boldsymbol{\phi}_C$ time series and is used to evaluate the stability of coordination.

A peak picking routine was used on a cycle-by-cycle basis to calculate cycle duration (msec) for each finger individually. The individual cycle durations were averaged and converted to frequency (Freq) in Hz. The peak picking routine also calculated movement amplitude on a cycle-by-cycle basis for each finger and the individual amplitudes were averaged across all cycles to provide a mean amplitude (Amp) per trial.

2.5 Statistics

The data means from the performance measures ($\boldsymbol{\phi}_{SD}$, RMSE, Freq, and Amp) from the initial training session were analyzed using Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (T1-20) ANOVAs with repeated measures on Trial design. To further analyze the training session, the distribution of the $\boldsymbol{\phi}_C$ time series for the first, seventh, and last trials were analyzed using a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (T1, T7, T20) × Bin (B1, B5, B9) ANOVA with repeated measures on Trial and Bin design. These three trials were chosen to represent the beginning of training (T1), the point in training where most participants began to plateau or maintain performance (T7), and the end of training (T20). Bins 1, 5, and 9 were chosen as these bins contain in-phase (Bin 1: 0°), antiphase (Bin 9: 180°), the intrinsically stable bimanual patterns, and the target relative phase (Bin 5: 90°).

The data means from retest performance ($\boldsymbol{\phi}_{SD}$, RMSE, Freq, and Amp) were analyzed with a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (T20, R1-8) ANOVA with Trial as a repeated measure. This was to compare the end of training performance (T20) to the four retest trials without visual feedback (R1-4) and the four retest trials (R5-8) where participants were reintroduced to the visual feedback. To further analyze the retest session, the distribution of the $\boldsymbol{\phi}_C$ time series was analyzed three ways. First, a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (T20, R1-4) × Bin (B1, B5, B9) ANOVAs with repeated measures on Trial and Bin design were performed. This analysis compared the end of training (T20) to the four retest trials sans visual feedback (R1-4). Next, a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (T20, R5-8) × Bin (B1, B5, B9) ANOVAs with repeated measures on Trial and Bin design were performed. These ANOVA compared the end of training with the retest trials where visual feedback was re-introduced. Finally, a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (R1-8) × Bin (B1, B5, B9) ANOVA with a repeated measure of Trial and Bin was performed. Here, the eight retest trials were compared to examine consolidation/retention based on the availability of visual feedback.

For all ANOVAs, post hoc comparisons were performed using a Bonferroni test ($\alpha = 0.05$) when appropriate. The results are presented in two sections: the initial exposure to the 90º pattern in the training session, and then retest and consolidation of the 90º pattern.

2.6 Results

To provide a qualitative measure of each trial's performance, a visual inspection was conducted with the $\boldsymbol{\phi}_C$ time series and limb displacement time series to categorize each trial in one of four ways: in-phase, antiphase, 90°, or roaming. The distribution of this classification is presented in Table 2. For each trial, the count for each category is displayed as well as the percent of occurrence within each trial. Displayed are training trials T1 and T20 and retest trials R1-8 which are separated by stimulation montage. Trials were categorized as In-phase or Antiphase

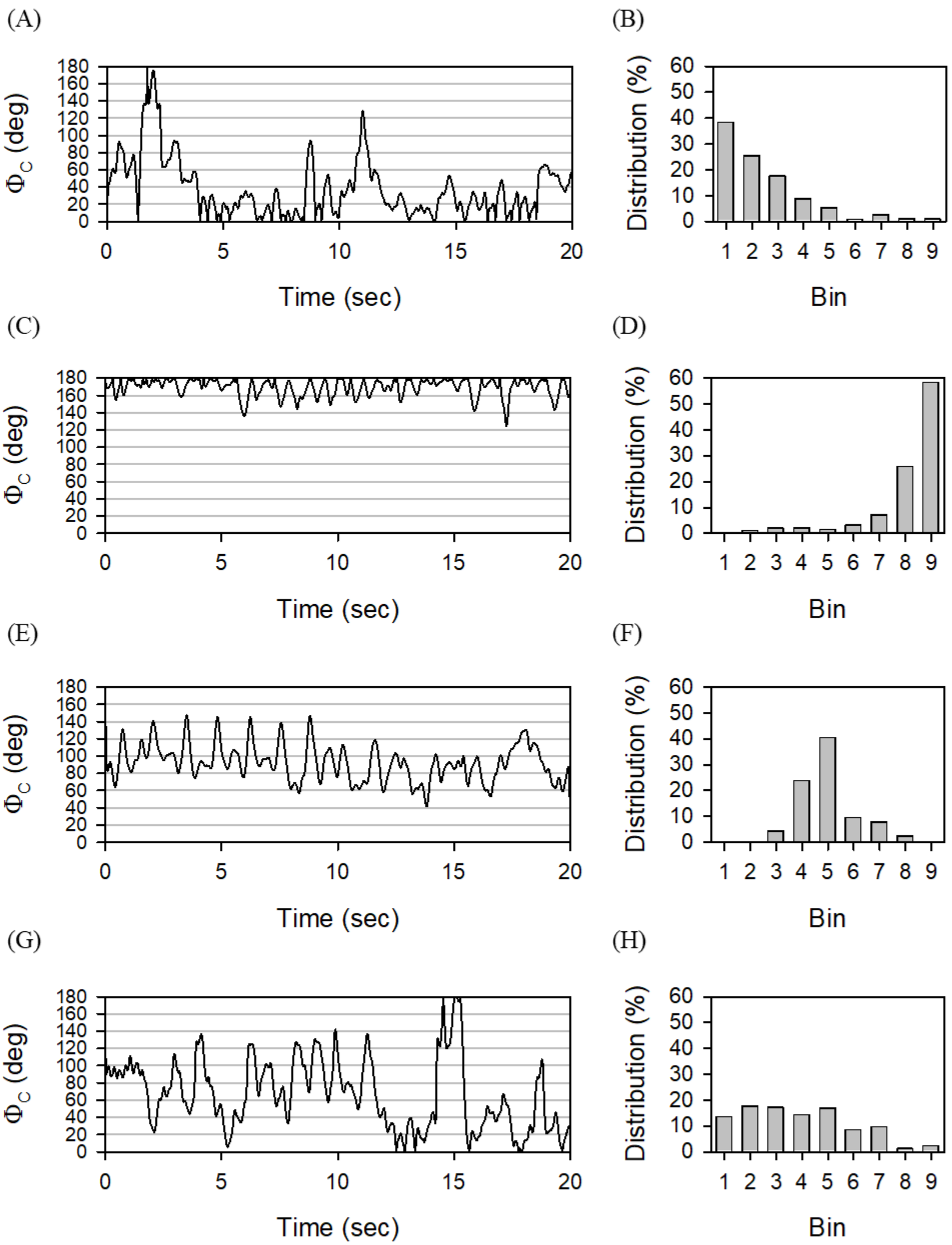

Figure 9. Figures A, C, E, G are single trial examples of relative phase for each of the categories. Figures B, D, F, H are the corresponding relative phase distributions. (A, B) A single trial was categorized as in-phase where more time was spent performing at or close to 0° relative phase. (C, D) A retest trial was categorized as antiphase where more time was spent at or near 180° relative phase. (E, F) A retest trial where the participant spent more time at or near 90° relative phase. (G, H) A single retest trial was categorized as roaming since the trial was not characterized by a single dominant peak in the relative phase bin.

when performance was centered on 0° and 180°, respectively, and participants maintained the pattern throughout the trial. See Figure 9A-D. Trials categorized as 90° maintained a constant center around 90° relative phase and did not wander outside of this target (Figure 9E-F). Roaming was considered any performance which did not maintain one pattern (0°, 90°, 180°) or which seemed to have no prevailing pattern emerge during the trial (Figure 9G-H).

Based on the visual inspection, participants improved from T1, where a 90° relative phase was dominant in roughly 50% of trials, to T20, where 90° was the dominant phase in 98% of trials. An interesting difference emerges between the Stimulation Timing groups during retest trials R1-4. Participants who received tDCS during training performed antiphase during the retest without visual feedback more often than participants who received tDCS before training. It also appears that participants' performance was classified as roaming more often for the tDCS Before group compared to the tDCS During groups. During the last four trials, where visual feedback was reintroduced, participants mostly performed 90° relative phase (> 90% trials overall).

Table 2. Visual evaluation of each trial to categorize performance.

Stimulation Timing	Relative Phase	T1	T20	R1	R2	R3	R4	R5	R6	R7	R8
During	Inphase	**2**	**0**	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
N = 48		4%	0%	4%	2%	0%	0%	0%	0%	0%	0%
	Antiphase	**2**	**0**	**17**	**17**	**17**	**16**	**0**	**0**	**0**	**0**
		4%	0%	35%	35%	35%	33%	0%	0%	0%	0%
	90 deg	**27**	**48**	**11**	**13**	**15**	**16**	**45**	**46**	**47**	**47**
		56%	100%	23%	27%	31%	33%	94%	96%	98%	98%
	Roaming	**17**	**0**	**18**	**16**	**16**	**16**	**3**	**2**	**1**	**1**
		35%	0%	38%	33%	33%	33%	6%	4%	2%	2%
Before	Inphase	**0**	**0**	**2**	**2**	**2**	**2**	**0**	**0**	**0**	**0**
N = 48		0%	0%	4%	4%	4%	4%	0%	0%	0%	0%
	Antiphase	**1**	**0**	**6**	**8**	**6**	**6**	**0**	**0**	**0**	**0**
		2%	0%	13%	17%	13%	13%	0%	0%	0%	0%
	90 deg	**24**	**46**	**16**	**16**	**15**	**18**	**42**	**45**	**45**	**45**
		50%	96%	33%	33%	31%	38%	88%	94%	94%	94%
	Roaming	**23**	**2**	**24**	**22**	**25**	**22**	**6**	**3**	**3**	**3**
		48%	4%	50%	46%	52%	46%	13%	6%	6%	6%

2.6.1 Training session: Initial exposure to 90° relative phase

This section presents the training session results where participants practiced the 90° relative phase. First, performance data was analyzed across all twenty training trials. Then we analyze the continuous relative phase distribution by comparing training trials T1, T7, and T20.

For all groups, performance accuracy and stability increased in producing the 90° relative phase pattern over the training session. Analysis of RMSE revealed a main effect of Trial ($F_{(19,1710)} = 66.371$, $p \leq 0.001$, $\eta_p^2 = 0.424$) and a Stimulation Montage × Stimulation Timing interaction ($F_{(2,90)} = 3.585$, $p = 0.032$, $\eta_p^2 = 0.074$). A Bonferroni adjustment for the main effect of Trial revealed a significant decrease in error across training trials T1 through T8 (*ps* ≤ 0.001) (Figure 10B). Further analysis of the interaction found that the Left-Anode tDCS before the training group performed with less error than the sham before the training group ($p = 0.035$) (Figure 11B).

The analysis of $\boldsymbol{\phi}_{SD}$ revealed a significant increase in performance stability over the training session with a main effect of Trial ($F_{(19,1710)} = 58.103$, $p < 0.001$, $\eta_p^2 = 0.392$) (Figure 10A). Bonferroni post hoc tests revealed significant decreases in $\boldsymbol{\phi}_{SD}$ for each of the first six training trials (*ps* ≤ 0.001). The ANOVAs also found a Stimulation Montage × Stimulation Timing interaction ($F_{(2,90)} = 3.823$, $p = 0.025$, $\eta_p^2 = 0.078$) and a Stimulation Timing × Trials interaction ($F_{(19,1710)} = 2.018$, $p = 0.041$, $\eta_p^2 = 0.022$). The Bonferroni post hoc analysis of the Stimulation Montage × Stimulation Timing (Figure 11A) interaction revealed that the group which received Right-Anode stimulation during training performed with greater variability than the group which received Right-Anode stimulation before training ($p = 0.006$). Further analysis of the Stimulation Timing × Trial interaction revealed that the groups which received tDCS during training, compared to the groups which received tDCS before training, performed with greater variability during

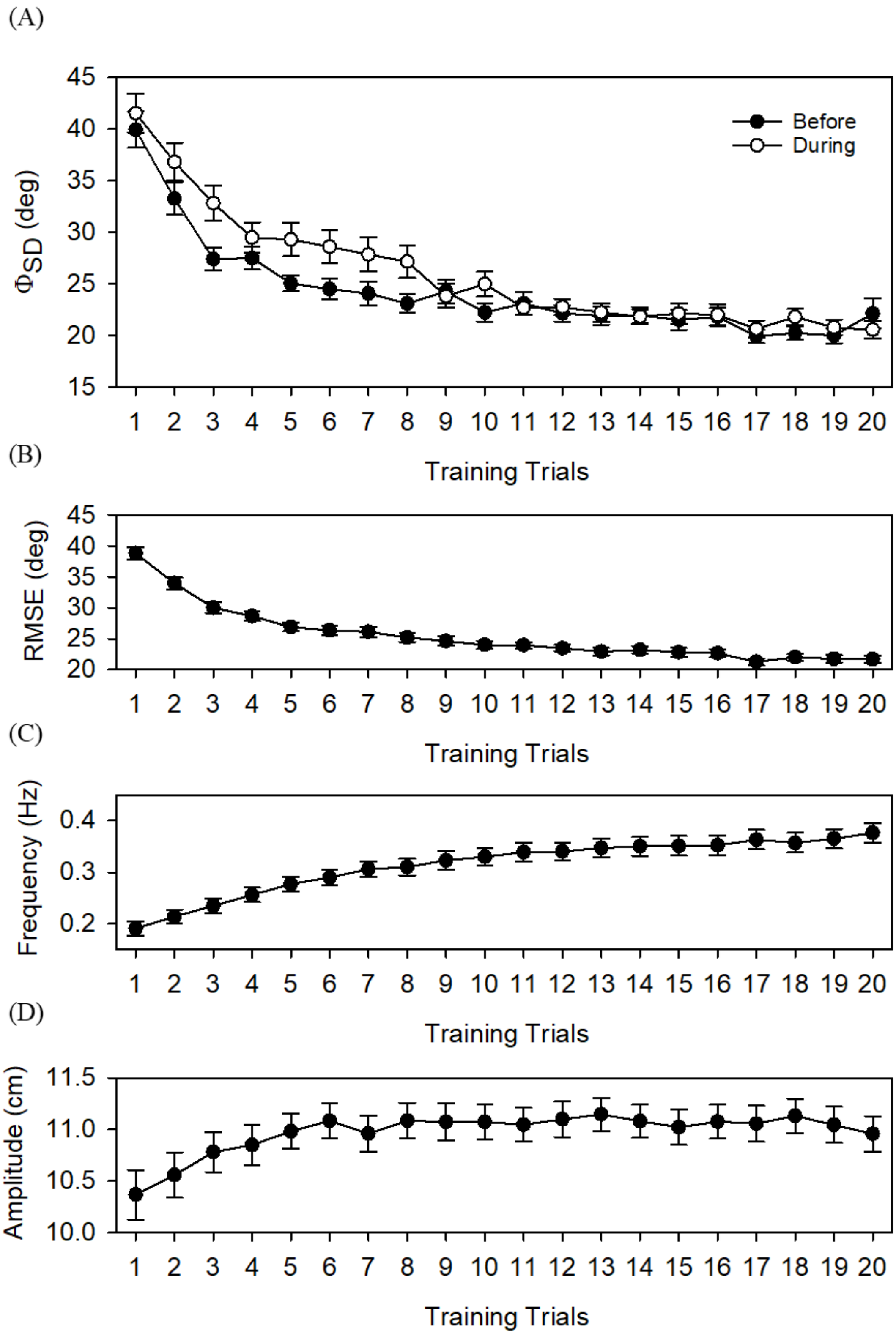

Figure 10. (A) The standard deviation of the relative phase is plotted across trials by stimulation timing. The root mean square error (B), movement frequency (C), and movement amplitude (D) are plotted across all training trials. The *error bars* represent one standard error of the mean.

training trials T3, T5, T6, and T8 ($ps \leq 0.035$). A three-way interaction was found for $\boldsymbol{\phi}_{SD}$, however, post hoc analysis revealed no significant differences ($ps > 0.05$).

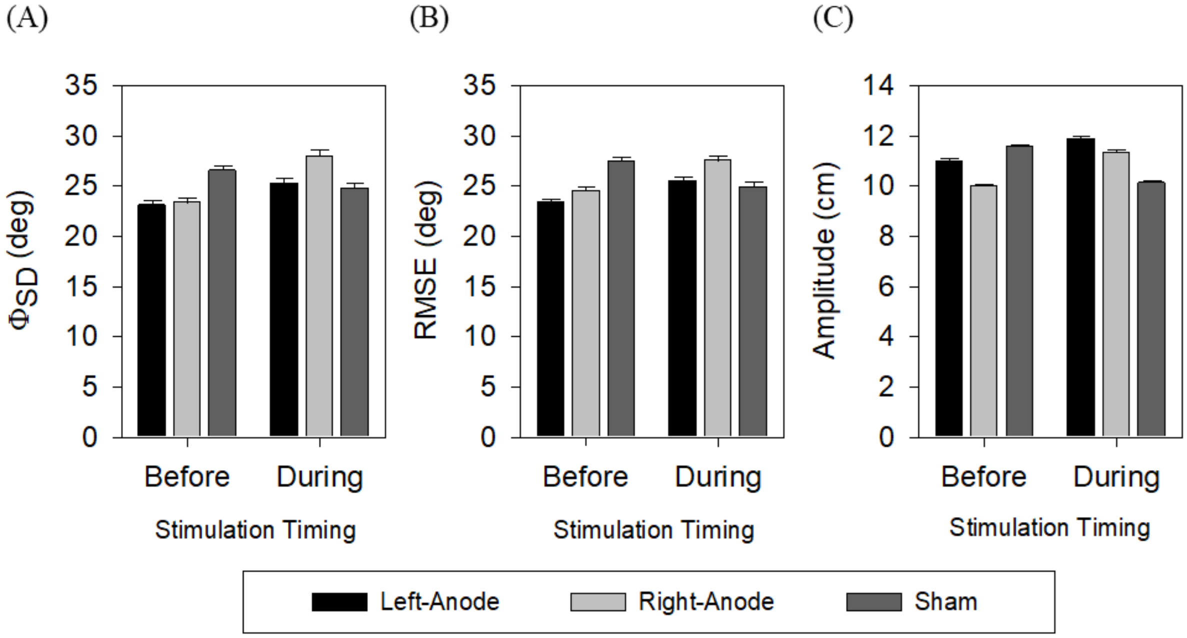

Figure 11. Plotted are the Stimulation Montage × Stimulation Timing interactions for the standard deviation of relative phase (A), root mean square error (B), and movement amplitude (C) measures for the training session. Each *error bar* represents one standard error of the mean.

The ANOVA of the Freq data revealed main effects of Stimulation Timing ($F_{(1,90)} = 4.917$, $p = 0.029$, $\eta_p^2 = 0.052$) and Trial ($F_{(19,1710)} = 52.472$, $p \leq 0.001$, $\eta_p^2 = 0.368$). Participants who received tDCS before training moved at a faster frequency (*Mean* = 0.347 Hz, *SD* = 0.168) than the groups which received stimulation during (*Mean* = 0.280 Hz, *SD* = 0.170) training ($p = 0.029$). Overall, frequency increased across training (Figure 10C). The post hoc analysis of Trial revealed a significant increase across trials T1 to T11 ($p \leq 0.001$). Movement amplitude was analyzed across the training trials. The ANOVA revealed a main effect of Trial ($F_{(19,1710)} = 4.917$, $p \leq 0.001$, $\eta_p^2 = 0.052$). Amplitude in training trials T1 and T2 was significantly smaller than the remaining training trials ($ps \leq 0.010$) (Figure 10D). A Stimulation Montage × Stimulation Timing interaction ($F_{(2,90)}$

$= 8.591$, $p \leq 0.001$, $\eta_p^2 = 0.160$) was found for movement amplitude (Figure 11C). Participants who received sham stimulation before training performed with greater amplitude than the sham during training group ($p = 0.006$). Conversely, the groups which received Right-Anode tDCS during training had greater amplitude than the group which received Right-Anode stimulation before training ($p = 0.010$). No other main effects or interactions were found for movement amplitude across the training trials.

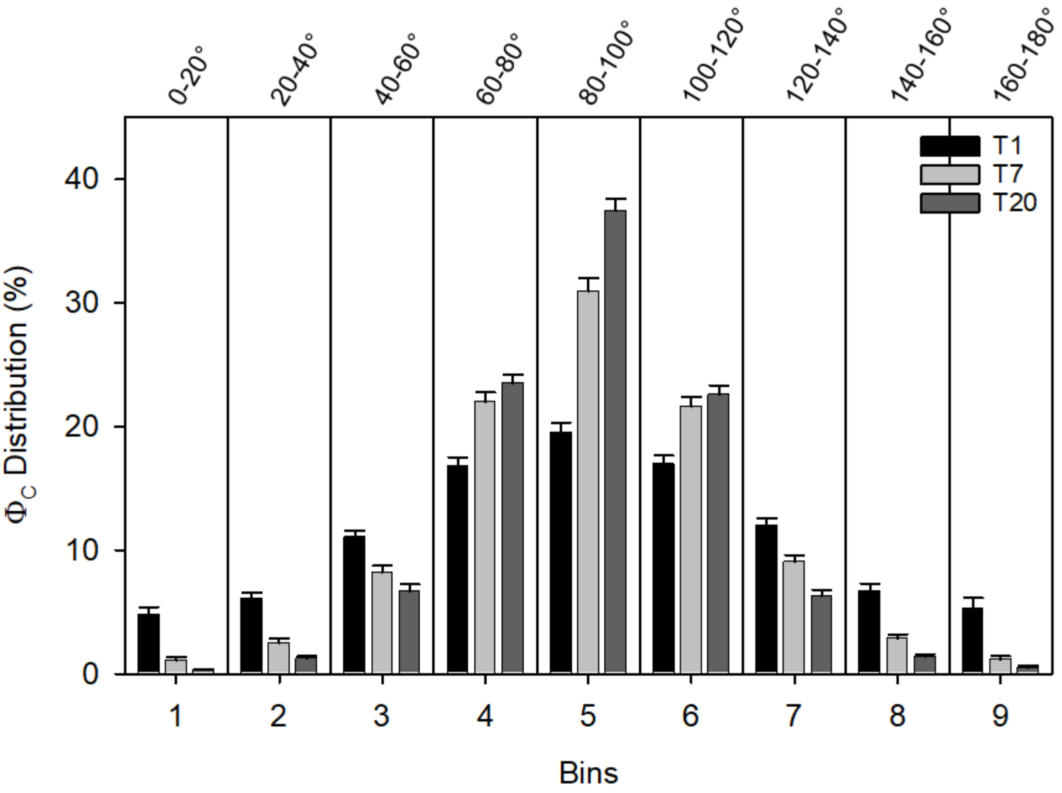

Figure 12. Plotted is the distribution of continuous relative phase for training trials T1, T7, and T20. Each bin represents a range of 20° from 0° to 180°. The three training trials plotted were chosen to represent the beginning of training (T1), the point in training when most participants began to plateau their performance (T7), and the end of training (T20). Each *error bar* represents one standard error of the mean.

To further analyze the training session, the continuous relative phase distribution was compared for training trials T1, T7, and T20. The ANOVA revealed main effects of Bin ($F_{(2,180)}$ =

1074.297, $p \leq 0.001$, $\eta_p^2 = 0.923$) and Trial ($F_{(2,180)} = 21.171$, $p \leq 0.001$, $\eta_p^2 = 0.190$) (Figure 12). A post hoc analysis of the Bin effect revealed that participants spent more time in Bin5 (80° to 100°) compared to Bin1 and Bin9 ($p \leq 0.001$). The ANOVA also revealed a significant Trial × Bin interaction ($F_{(4,360)} = 106.655$, $p \leq 0.001$, $\eta_p^2 = 0.542$) (Figure 12). Participants spent more time in Bin1 (in-phase) and Bin 9 (antiphase) during trial T1 than during T7 and T20 ($ps \leq 0.001$). The post hoc analysis also revealed that participants spent the most time in Bin5 during T20 compared to T1 and T7 ($ps \leq 0.001$) and they spent the least amount of time in Bin5 during T1 ($ps \leq 0.001$).

2.6.2 Retest and consolidation of the 90° pattern

In this section, performance measures ϕ_{SD}, RMSE, Freq, and Amp were compared across training trial T20 and retest trials R1-8.

The RMSE analysis revealed a main effect of Trial ($F_{(8,720)} = 209.004$, $p \leq 0.0001$, $\eta_p^2 = 0.699$). The post hoc analysis of RMSE revealed that participants performed with less error during T20 and R5-8 compared to R1-4 ($ps \leq 0.001$). Also, the first retest trial when visual feedback was reintroduced (R5) had greater error than trials R6-8 ($ps \leq 0.001$). The ANOVA also revealed two interactions, Stimulation Timing × Trial ($F_{(8,720)} = 4.437$, $p = 0.005$, $\eta_p^2 = 0.047$) and Stimulation Montage × Trial ($F_{(16,720)} = 4.029$, $p \leq 0.001$, $\eta_p^2 = 0.082$). The post hoc analysis revealed that error increased in R1-4 compared to T20 and R5-8 regardless of stimulation montage or stimulation timing ($ps \leq 0.001$). A stimulation montage difference was found for the groups which received Right-Anode tDCS such that performance error in R5 was less than R1-4, but significantly more than R6-8 ($ps \leq 0.042$) (Figure 13B). Additionally, the group which received tDCS before training performed with greater error in R5 than R6 and R7 ($ps \leq 0.012$) (Figure 13C).

Analysis of ϕ_{SD} found a main effect of Trial ($F_{(8,720)} = 67.894$, $p \leq 0.0001$, $\eta_p^2 = 0.430$). The post hoc analysis revealed a difference between retest trials R1-4 and T20, R5-8 ($ps \leq 0.001$)

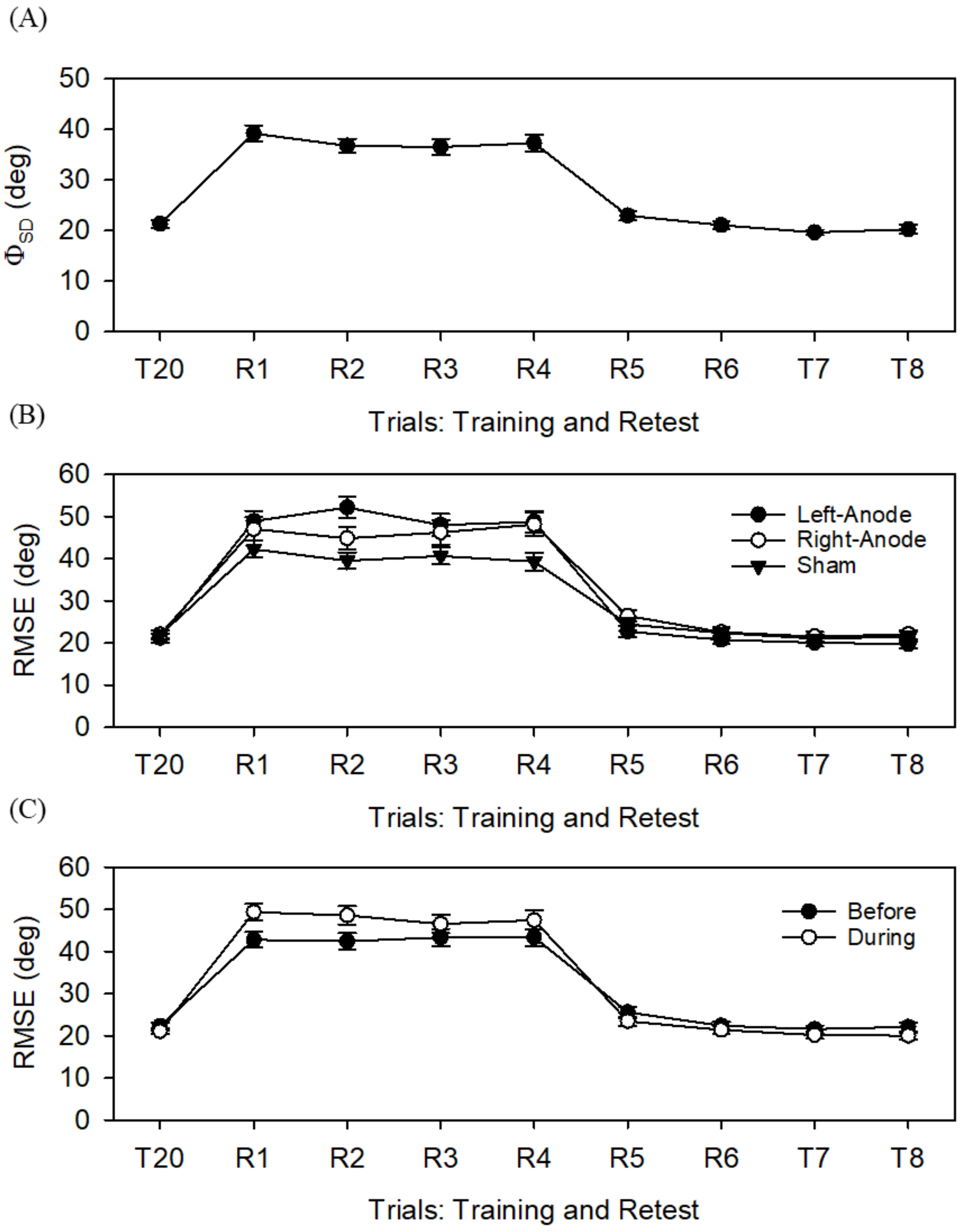

Figure 13. Plotted are the standard deviation of relative phase (A), and RMSE (B, C) for training trials T20 and retest trials R1-8. Plots B and C are the Stimulation Montage × Trial and Stimulation Timing × Trial interactions for RMSE. Each *error bars* represent one standard error of the mean.

(Figure 13A). Performance during the four retest trials without visual feedback had greater variability than the end of training and when compared to performance when visual feedback was reintroduced. The first retest trial when visual feedback was reintroduced (R5) had greater variability than one of the later trials, R7 ($p = 0.022$).

Movement frequency was analyzed and found a main effect of Trial ($F_{(8,720)} = 16.564$, $p \leq 0.001$, $\eta_p^2 = 0.155$) and a Stimulation Montage × Trial ($F_{(16,720)} = 2.984$, $p = 0.015$, $\eta_p^2 = 0.062$) interaction. Movement frequency was greatest at R8 (*ps* ≤ 0.034) followed by R7 (*ps* ≤ 0.023). Movement frequency was lowest during R1 and R2 which was significantly slower than R6-8 (*ps* ≤ 0.019). For the Stimulation Montage × Trial interaction, the post hoc analysis found differences between trials for the Left-Anode groups. Retest trials R1-4 moved slower than trials R6-8 (*ps* ≤ 0.028) but not R5. Interestingly, retest trials R5 and R6 were significantly slower than R7-8 (*ps* ≤ 0.016). There was no difference between trials for the Right-Anode and Sham stimulation montages. Analysis of the movement amplitude (Mean = 6.90 cm, SE = 0.060) revealed no main effects or interactions.

To further analyze the retest trials, the continuous relative phase distribution of training trial T20 and retest trials R1-4 where participants performed without visual feedback was compared. The ANOVA revealed a main effect of Bin ($F_{(2,180)} = 89.612$, $p \leq 0.001$, $\eta_p^2 = 0.499$) (Figure 14). The ANOVA also revealed three interactions: Bin × Stimulation Montage ($F_{(4,180)} = 5.256$, $p \leq 0.001$, $\eta_p^2 = 0.105$), Bin × Stimulation Timing ($F_{(2,180)} = 6.611$, $p = 0.003$, $\eta_p^2 = 0.068$), and Bin × Trial ($F_{(8,720)} = 102.829$, $p \leq 0.001$, $\eta_p^2 = 0.533$). The Bonferroni post hoc analysis of the Bin × Stimulation Montage interaction found differences between bins for each montage (Figure 15A). Participants who experienced Left-Anode and Right-Anode tDCS spent more time doing 90° and antiphase compared to in-phase (*ps* ≤ 0.001). However, participants who experienced

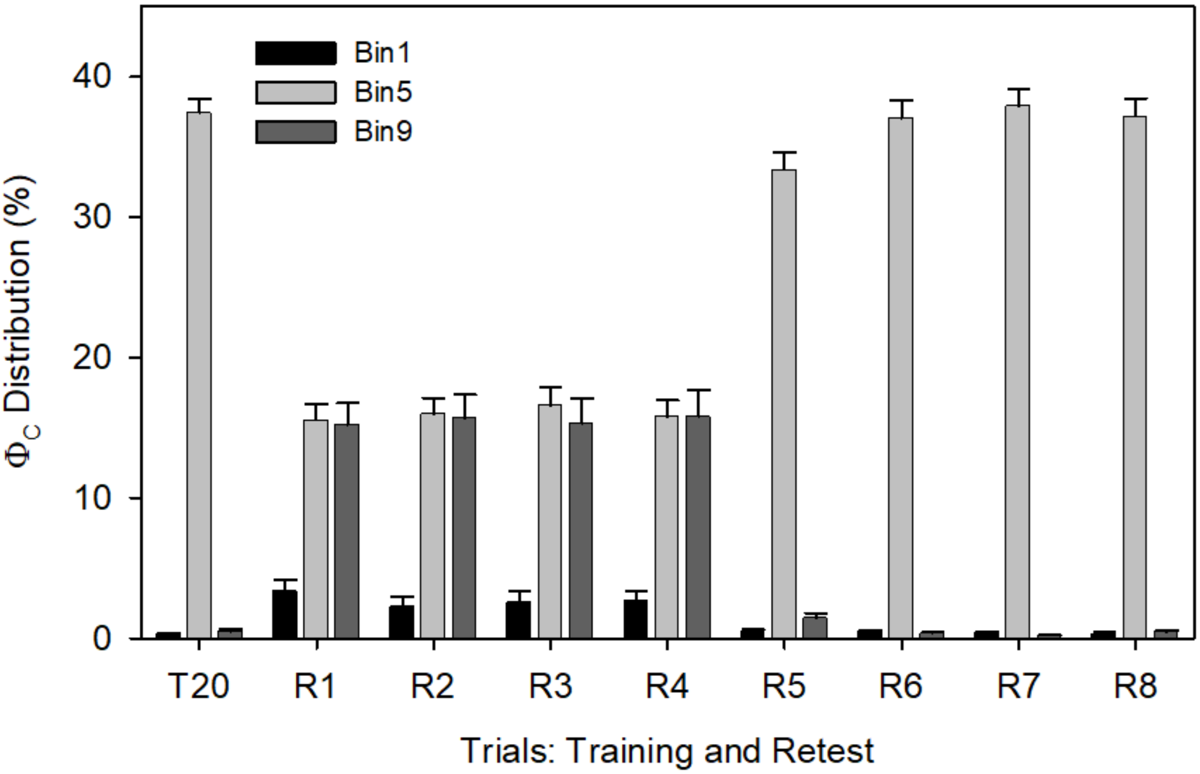

Figure 14. Plotted are Bin1, 5, and 9 for training trial T20 and retest trials R1-8. Bin1, 5, and 9 represent the percent of time spent performing the relative phase patterns 0°, 90°, and 180°, respectively. T20 is the performance at the end of the training session. R1-4 is the retest when visual feedback was removed while R5-8 is the retest when visual feedback was reintroduced. Each *error bars* represent one standard error of the mean.

Sham tDCS spent more time doing 90° compared to in-phase and antiphase ($ps \leq 0.001$). A difference did emerge in Bin9, where the groups which experienced Left-Anode tDCS spent more time doing antiphase than the sham stimulation groups ($p \leq 0.001$). For the second interaction, Bin × Stimulation Timing, the post hoc analysis found a difference in Bin9 (Figure 15B). Participants who experienced tDCS before training spent less time performing antiphase than the groups which experienced tDCS during training ($p = 0.007$). Differences were found for both Before and During tDCS where participants spent more time in Bin9 than Bin1 ($ps \leq 0.002$) and more time in Bin5 than Bin1 ($ps \leq 0.001$). For the last interaction, Bin × Trial (Figure 14), the post hoc analysis found differences within bins and trials. During T20, all participants spent more time performing 90° than in-phase and antiphase (*ps* ≤ 0.001). During retest trials R1-4, all participants spent more time doing 90° and antiphase than in-phase (*ps* ≤ 0.001). The post hoc analysis also found a trial difference within bins. Participants spent more time in Bin1 and Bin9 during retest trials R1-4 than training trial T20 (*ps* ≤ 0.034). Participants also spent more time at 90° during T20 compared to R1-4 (*ps* ≤ 0.001).

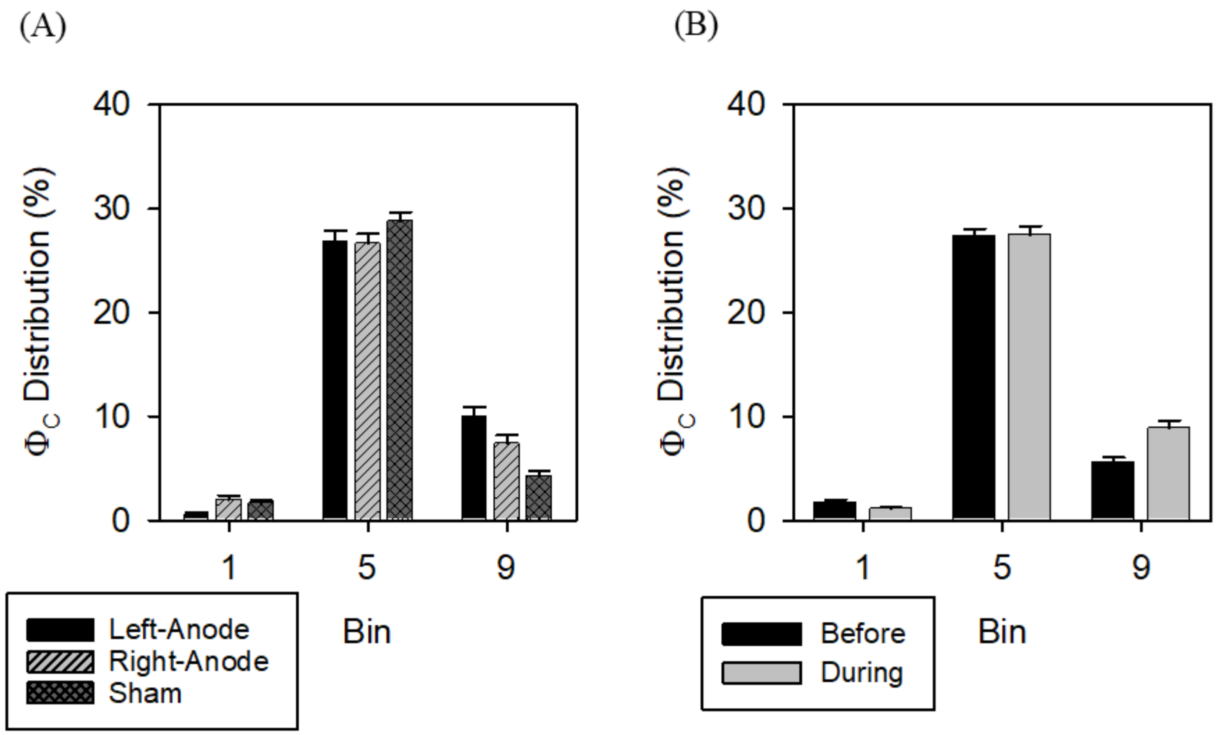

Figure 15. Plotted are the distribution of the continuous relative phase data for the (A) Stimulation montage × Bin and (B) Stimulation Timing × Bin interactions. Each *error bars* represent one standard error of the mean.

For the last four trials of the retest session, participants were reintroduced to the visual feedback. We compared T20 and R5-8, for the distribution of the continuous relative phase (Bins1, 5, and 9). The ANOVA revealed main effects of Bin ($F_{(2,180)}$ = 1197.125, $p \leq 0.001$, $\eta_p^2 = 0.930$) and Trial ($F_{(4,360)}$ = 53.121, $p = 0.014$, $\eta_p^2 = 0.036$), and also an interaction for Bin × Trial ($F_{(8,720)}$

$= 7.657, p \leq 0.001, \eta_p^2 = 0.078$). The Bonferroni post hoc analysis for the main effect of Bin found that all participants spent more time in Bin5 than Bin1 and Bin9 (*ps* ≤ 0.001). Analysis of the main effect of Trial revealed that participants spent more time in Bin1, 5, or 9 during retest trial R7 than during R5 ($p = 0.019$). For the Bin × Trial interaction (Figure 14), the post hos analysis found that performance retest trial R5 spent significantly less time in Bin5 than all other trials (*ps* ≤ 0.011) and spent more time in Bin9 compared to retest trials R6-8 (*ps* ≤ 0.015). For all trials, participants spent the most time in Bin5 compared to Bin1 and Bin 9 (*ps* ≤ 0.001), however, during R5 participants also performed antiphase (Bin9) more often than in-phase (Bin1) ($p = 0.020$).

Finally, we wanted to compare all retest trials to analyze retest performance without visual feedback compared to the reintroduction of feedback. Analysis of the retest trials R1-8 for the distribution of the continuous relative phase (Bins1, 5, and 9) found the main effect of Bin ($F_{(2,180)} = 315.553, p \leq 0.001, \eta_p^2 = 0.778$) and interactions of Bin × Stimulation Montage ($F_{(4,180)} = 3.990$, $p = 0.007, \eta_p^2 = 0.087$) and Bin × Trial ($F_{(14,1260)} = 129.335, p \leq 0.001, \eta_p^2 = 0.590$). Overall, participants spent more time in Bin9 than Bin1 ($p \leq 0.001$) and the most time in Bin5 (*ps* ≤ 0.001). The Bonferroni post hoc analysis for the Bin × Stimulation Montage interaction found Left-Anode, Right-Anode, and Sham tDCS groups spent more time in Bin5 than Bin1 and Bin9 (*ps* ≤ 0.001). Additionally, for the Left-Anode and Right-Anode tDCS groups, participants performed antiphase more often than in-phase (*ps* ≤ 0.001). For the Bin × Trial interaction (Figure 14), the post hoc analysis found a difference between Bin5 and Bin9. Participants spent significantly less time performing 90 ° during retest trials R1-4 compared to R5-8. Although participants performed 90° more often in R5 compared to R1-4, they spent less time in Bin5 during R5 than R6-8 (*ps* ≤ 0.031). Overall, participants performed antiphase more during R1-4 than in R5-8 (*ps* ≤ 0.001) and spent more time performing antiphase in R5 than during R6-8 (*ps* ≤ 0.043). Participants spent more time performing 90° and antiphase than in-phase in retest trials R1-4 ($ps \leq 0.001$). However, participants spent more time performing 90° than in-phase and antiphase during retest trials R5-8 ($ps \leq 0.001$).

3. DISCUSSION

The purpose of experiment 1 was to explore the time-dependent nature of the consolidation process for rhythmic bimanual coordination tasks. To probe this consolidation process, the experiment manipulated the stimulation-training coupling for tDCS and practice. For the training session, all participants improved performance of the 90° relative phase as evident by the decrease in $\boldsymbol{\phi}_{SD}$ and RMSE. A difference did emerge between stimulation timing for the $\boldsymbol{\phi}_{SD}$ measure early in training were participants who receive tDCS before training reduced their variability at a faster rate before plateauing compared to groups that received tDCS during training. Participants increased their movement frequency and movement amplitude before maintaining a comfortable movement speed and amplitude. The distribution of the continuous relative phase revealed that participants performed in-phase and antiphase more often at the start of training than later during training. Also, participants were able to maintain the 90° relative phase more often as a result of training. This rapid training improvement is consistent with previous work using the Lissajous training protocol (Kovacs et al., 2009a; McCulloch et al., 2020).

The first four retest trials were performed without visual feedback and overall the same level of performance as at the end of the training was not found. However, even without visual feedback, some participants were still able to perform the target relative phase, while others were drawn to antiphase or roaming. The observation of attraction to 180° and sometimes the ability to achieve 90° suggests that the motor memory is still fragile or not stable. If the motor memory for 90° is weak, theory predicts attraction to nearby stable relative phase patterns such as 0° and 180° (Schöner et al., 1992; P. G. Zanone & J. A. S. Kelso, 1992). When participants were not performing the 90° relative phase without feedback, there was a greater attraction to favor antiphase compared to in-phase. The reintroduction of visual feedback allowed participants to immediately improve their performance to or near the same level as the end of training. The improvement across R5 and R6 compared to T1 and T2 suggests that the same aspect of task-based memory was available to rapidly tune the pattern again, possibly a visual-spatial representation linking the feedback to the arms' motion. There appears to be a slight stimulation timing difference in the early parts of training the rhythmic bimanual motor skill, but no real differences emerged between stimulation montages that suggest stimulation increased the rate of consolidation.

Performance without visual feedback was not comparable to the end of the training, so we cannot conclude that consolidation in the form of memory stabilization occurred for all participants. Performance during the retest without visual feedback was quite worse than hypothesized, one reason for this may have been the number of mechanical DoF required to perform the motor task. For Experiment 2, we reduced the number of mechanical DoF by switching to index finger flexion/extension. There was a minimal effect of tDCS stimulation before training on the rate of skill acquisition, for this reason, the before stimulation-training coupling was chosen for Experiment 2. Since no stimulation montage differences emerged, the Left-Anode, Right-Anode, sham montages were kept for the next experiment.

4. EXPERIMENT 2: METHODS

4.1 Participants

A total of 72 participants were recruited through the Motor Neuroscience pool in the Department of Health and Kinesiology at TAMU. The participants were randomly placed into six groups (Table 2). Participants were young adults ($M_{age} = 21 \pm 1.6$ yrs; 50 Female, 22 Male; 62 right-handed, 1 left-handed, 9 mixed-handed) and free of any neuromuscular disorders which inhibit upper limb movement or sensation. The experimental procedure, consent form, and all questionnaires were approved by the Human Subjects Interval Review Board at TAMU. All participants consented to participate in line with the Helsinki Declaration.

Table 3. Experimental groups: Stimulation Montage and Retest Delay.

		Stimulation Montage	
Retest Delay	Left-Anode	Right-Anode	Sham
2-hr Delay	N = 12	N = 12	N = 12
6-hr Delay	N = 12	N = 12	N = 12

4.2 Task and Experiment Setup

4.2.1 Bimanual task

Sitting in an upright and comfortable position, participants grasped two horizontal handles fixed on the desk (Figure 16). The task required flexion-extension motions of the index fingers in the vertical plane perpendicular to the tabletop. Augmented visual feedback was displayed via a Lissajous plot on a computer monitor in front of the participant (≈ 60 cm) at eye level. Vertical displacement of the right and left fingers was mapped to the *x*-axis and *y*-axis, respectively. For the familiarization trials, verbal instructions and visual feedback were provided by the Lissajous plot to perform 0° and 180° patterns (Figures 1 and 4). The training pattern was 90° relative phase also defined with the Lissajous plot (Figures 3 and 4). The handles the participants grasped were under a hood which removed vision of the participant's limbs (Figure 6A).

4.2.2 tDCS

This experiment investigated the training and consolidation process of the motor memory for a rhythmic bimanual coordination task over a 2-hr versus a 6hr retest interval. A 1x1 low-intensity transcranial electrical stimulator system (Soterix Medical, New York, NY) was used to deliver direct current stimulation to modulate cortical excitability before training the bimanual coordination pattern. The 2-mA current was transferred by an anodal and cathodal conductive rubber electrode of 5 × 5 cm resulting in a maximum current density of 0.08 mA/cm. The electrodes were placed in sponges soaked in 5 mL of a 0.9% saline solution. The center of the anodal and cathodal electrodes was placed over one of two target areas identified using the 10-20 EEG placement system: 1) C3 anode and C4 cathode (Left-Anode), or 2) C3 cathode and C4 anode (Right-Anode). The sham condition was pseudo-randomly chosen as one of these two combinations. In the active stimulation condition, the stimulation lasted for 20 minutes at an intensity of 2 mA with a ramping up and down over a 30-second interval. In the sham condition, the current ramped up and back down over a 60-second interval (to reach a peak of 2 mA) at the beginning and end of the same 20-minute period to produce the same initial and terminal sensation as commonly experienced with the active condition.

4.2.3 Experiment Procedure

The experiment required participants to partake in two separate sessions separated by either 2 or 6 hours. Session 1, the training session, lasted between 60 and 85 minutes while session 2, the retest session, lasted between 15 and 30 minutes. Each training and retest trial lasted 20 seconds

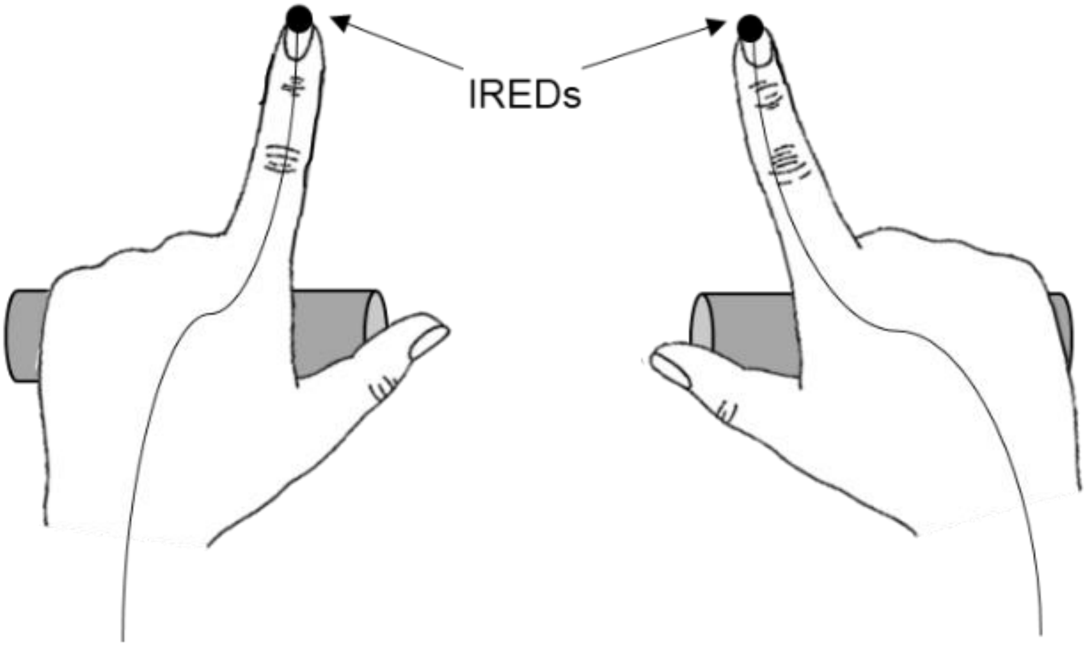

Figure 16. Dorsal view of participant grasping the horizontal handles fixed on the table. Two infrared light-emitting diodes (IREDs) were taped to the top of the index fingers. Participants were asked to flex and extend their index fingers at the metacarpophalangeal joint.

and was followed by a 20-second rest. In the first session, participants completed six familiarization trials using the Lissajous plot as feedback: three in-phase trials, three antiphase trials. After performing the familiarization trials, the tDCS electrodes were secured to the participant's scalp. Stimulation was applied before the 20 training trials (Figure 17). Participants waited in a relaxed position while abstaining from any unnecessary movements for the 20 min period. After stimulation, the 20 training trials began. The participants used the Lissajous plot and the provided template as a guide during the learning trials. After completing the training trials (T1-T20), the session ended, and participants completed the post-survey questionnaire as an opportunity to report any adverse side effects or sensations experienced due to stimulation.

After the retest delay period, either 2 or 6-hrs, participants returned to the lab to complete eight more trials. In the retest session, participants were asked to perform the 90° coordination pattern from memory without any feedback (Lissajous plot) for four 20 sec trials (R1-R4). Then participants were given the Lissajous plot to complete the last four 20 sec trials of the session (R5-R8).

4.3 Data Collection and Analysis

The data collection and analysis procedures were the same as experiment 1. The Optotrak Certus 3D camera system (Northern Digital, Inc.) was used to record the motion of two IREDs attached to the end of the index fingers. The camera was positioned approximately two meters from the participants' hands and approximately one meter parallel to the floor (Figure 6A). The markers were sampled at 100 Hz with 2000 data points collected per trial. All dependent measures were calculated using Matlab 2014b (The Mathworks, Inc.) and the *y*-axis time series of each marker were dual-pass filtered (Butterworth) with a cutoff frequency of 10 Hz.

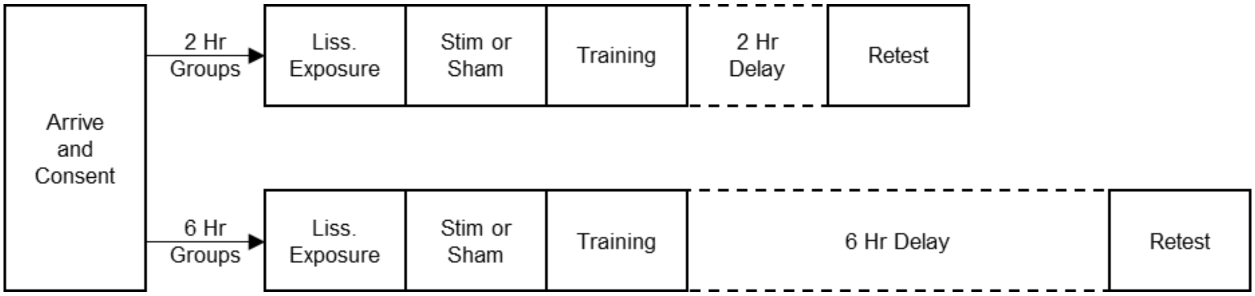

Figure 17. Experimental timeline. Participants were separated into either 2-hr (top) or 6-hr (bottom) Retest Delay Groups: Lissajous familiarization, stimulation or sham procedure, training begins. Following the session was either a 2-hr or 6-hr delay before the retest trials.

4.4 Performance Measures

The vertical displacement motion of the index fingers is represented by the *y*-axis in the *XYZ* plane of the Optotrak system. The same performance measures used in experiment 1 were computed for this experiment, RMSE, distributions of relative phase ($\boldsymbol{\phi}_{C}$), relative phase variability ($\boldsymbol{\phi}_{SD}$), movement frequency (Freq), and movement amplitude (Amp).

4.5 Statistics

The data means from the four performance measures ($\boldsymbol{\phi}_{SD}$, RMSE, Freq, and Amp) from the initial training session were analyzed using Stimulation Montage (Left-Anode, Right-Anode, Sham) × Retest Delay (2-hr, 6-hr) × Trial (T1-20) ANOVAs with repeated measures on Block. To further analyze the training trials, the $\boldsymbol{\phi}_{C}$ distributions for T1, T4, and T20 were analyzed using a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (T1, T4, T20) × Bin (B1, B5, B9) ANOVA with repeated measures on Trial and Bin design. These three trials were chosen to represent the beginning of training (T1), the point in training where participants plateaued or maintained performance (T4), and the end of training (T20).

The data means ($\boldsymbol{\phi}_{SD}$, RMSE, Freq, and Amp) from the retest trials were analyzed using Stimulation Montage (Left-Anode, Right-Anode, Sham) × Retest Delay (2-hr, 6-hr) × Trial (T20, R1-8) ANOVAs with repeated measures of Block. Post hoc comparisons were performed using a Bonferroni test ($\alpha = 0.05$) when appropriate. To further analyze the retest session, the distributions of the $\boldsymbol{\phi}_{C}$ time series were analyzed three ways. First, a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (T20, R1-4) × Bin (B1, B5, B9) ANOVA with repeated measures on Trial and Bin was performed. This analysis compared the end of training (T20) to the four no feedback retest trials (R1-4). Next, a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (T20, R5-8) × Bin (B1, B5, B9) ANOVA with repeated measures on Trial and Bin was performed. This ANOVA compared the end of training to the four retest trials where visual feedback was re-introduced. Finally, a Stimulation Montage (Left-Anode, Right-Anode, Sham) × Stimulation Timing (Before, During) × Trial (R1-8) × Bin (B1, B5, B9) ANOVA with a repeated measure of Trial and Bin was performed. Here, all eight retest trials were compared to determine if there were any differences in retention based on the availability of visual feedback.

4.6 Results

To provide a qualitative measure of trial performance, a visual inspection was conducted of the ϕ_C time series to categorize each trial as either: in-phase, antiphase, 90°, or wrapping (Table 4). For each trial, the count for each category is displayed as well as the percent of occurrence. Displayed are training trials T1 and T20 and retest trials R1-8 all of which are separated by Retest Delay. Participants improved from T1, where 50% of the trials were classified as 90°, to T20, where 97% of the trials were classified as 90°. A difference emerged between the Retest Delay groups during trials R1-4. Participants tested after a 2-hr delay was able to maintain 90° (82% of trials) more often than the groups retested after the 6-hr delay (65%). When visual feedback was reintroduced, R5-R8, participants consistently performed 90°.

Table 4. Categorization of trails from visual inspection.

Retest Delay	Relative Phase	T1	T20	R1	R2	R3	R4	R5	R6	R7	R8
2 Hr	In-Phase	**2**	**0**	**3**	**1**	**2**	**2**	**0**	**0**	**0**	**0**
N = 36		6%	0%	8%	3%	6%	6%	0%	0%	0%	0%
	Antiphase	**1**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
		3%	0%	3%	0%	0%	0%	0%	0%	0%	0%
	90 deg	**19**	**34**	**29**	**29**	**30**	**30**	**33**	**35**	**35**	**35**
		53%	94%	81%	81%	83%	83%	92%	97%	97%	97%
	Roaming	**14**	**2**	**3**	**6**	**4**	**4**	**3**	**1**	**1**	**1**
		39%	6%	8%	17%	11%	11%	8%	3%	3%	3%
6 Hr	In-Phase	**2**	**0**	**4**	**4**	**4**	**4**	**0**	**0**	**0**	**0**
N = 36		6%	0%	11%	11%	11%	11%	0%	0%	0%	0%
	Antiphase	**1**	**0**	**1**	**1**	**1**	**2**	**0**	**0**	**0**	**0**
		3%	0%	3%	3%	3%	6%	0%	0%	0%	0%
	90 deg	**17**	**36**	**23**	**24**	**23**	**23**	**31**	**34**	**35**	**34**
		47%	100%	64%	67%	64%	64%	86%	94%	97%	94%
	Roaming	**16**	**0**	**8**	**7**	**8**	**7**	**5**	**2**	**1**	**2**
		44%	0%	22%	19%	22%	19%	14%	6%	3%	6%

4.6.1 Training session: Initial exposure to 90º relative phase

The ANOVA of RMSE found a main effect of Trial ($F_{(19,1254)} = 6.452$, $p < 0.001$, $\eta_p^2 = 0.089$). The post hoc analysis found that performance during training trials T1 and T2 were characterized by larger error than all other training trials (*ps* ≤ 0.025) (Figure 18B). The analysis of $\boldsymbol{\phi}_{SD}$ revealed a significant performance improvement over the training session with a main effect of Trial ($F_{(19,1254)} = 6.452$, $p < 0.001$, $\eta_p^2 = 0.089$). Bonferroni post hoc tests revealed a significant decrease for each of the first three training trials (*ps* ≤ 0.016). Trials T4 through T20 were not significantly different (Figure 18A).

The ANOVA of the Freq data revealed a main effect of Trial ($F_{(19,1254)} = 8.473$, $p < 0.001$, $\eta_p^2 = 0.114$) (Figure 18C). Movement frequency was significantly slower during trials T1 and T2 compared to trials T6-20 (*ps* ≤ 0.046). The analysis of Amp data also found a main effect of Trial ($F_{(19,1254)} = 8.880$, $p < 0.001$, $\eta_p^2 = 0.119$). Participants moved with smaller amplitude during training trials T1 and T2 compared to trials T3-20 (*ps* ≤ 0.042) (Figure 18D).

To further explore performance during the training session, the distribution of the continuous relative phase was analyzed across three trials: T1, T4, and T20. The distribution bins analyzed were Bin1, Bin5, and Bin9 which contain in-phase, 90°, and antiphase, respectively. The ANOVA found main effects of Trial ($F_{(2,132)} = 8.694$, $p < 0.001$, $\eta_p^2 = 0.116$), Bin ($F_{(2,132)} = 136.616$, $p < 0.001$, $\eta_p^2 = 0.679$), and an interaction of Trial × Bin ($F_{(4,264)} = 19.643$, $p < 0.001$, $\eta_p^2 = 0.229$) (Figure 19). For training trials T1, T4, and T20, all participants spent more time in Bin5 than Bin1 and Bin9 (*ps* ≤ 0.003). The post hoc analysis also revealed differences within the bins. Participants spent more time in Bin1 and Bin9 during T1 compared to T4 and T20 (*ps* ≤ 0.003). Participants also spent more time in Bin1 during T4 compared to T20 ($p = 0.002$). All participants spent more time performing the 90° relative phase during T4 than T1 (*ps* ≤ 0.001), but they spent the most time in B5 during T20 (*ps* ≤ 0.002).

4.6.2 Retest and consolidation of the 90° pattern

In this section, the performance measures $\boldsymbol{\phi}_{SD}$, RMSE, Freq, and Amp were compared across training trial T20 and retest trials R1-8. Then the distribution of the continuous relative phase was analyzed using Bins 1, 5, and 9 in three tests: T20 and R1-4, T20 and R5-8, then R1-8. For RMSE, the ANOVA revealed a min effect of Trial ($F_{(8,528)} = 13.532$, $p < 0.001$, $\eta_p{}^2 = 0.170$). The post hoc analysis found that participants performed with less error during training trials T20 and retest trials R5-8 compared to retest trials R1-4 (*ps* ≤ 0.028), indicating that participants' error increased during the retest session when visual feedback was removed (Figure 20A). No other main effects or interactions were found for the RMSE measure. The ANOVA of $\boldsymbol{\phi}_{SD}$ (Mean = 32.169°, SE = 0.425) found no main effect or significant interactions.

Analysis of the movement frequency (Mean = 0.62 Hz, SE = 0.014) revealed no main effects or interactions. However, the ANOVA for movement amplitude found main effects of Stimulation Montage ($F_{(2,66)} = 3.481$, $p = 0.037$, $\eta_p{}^2 = 0.095$) and Trial ($F_{(8,528)} = 10.134$, $p < 0.001$, $\eta_p{}^2 = 0.133$). The Bonferroni post hoc test for groups that received Right-Anode tDCS made smaller movement amplitudes compared to the groups which received Sham tDCS ($p = 0.039$). Post hoc analysis of the main effect of Trial found several differences (Figure 20B). Participants produced larger movement amplitudes during retest trials R3 and R4 compared to training trial T20 and retest trials R1, R2, and R5 (*ps* ≤ 0.014). Additionally, movement amplitude in retest trial R4 was significantly greater than trials R6-8 (*ps* ≤ 0.005).

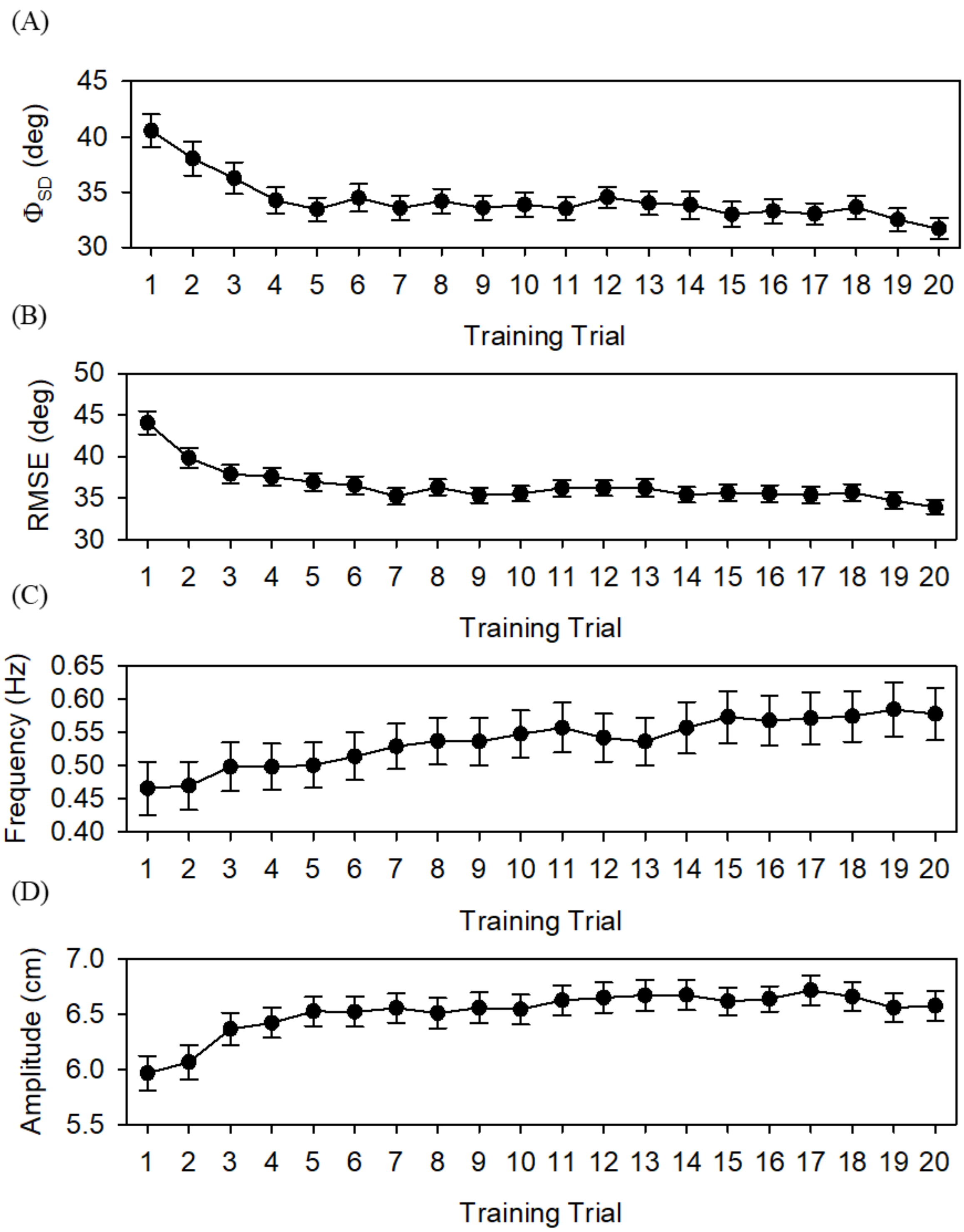

Figure 18. (A) The standard deviation of the continuous relative phase is plotted across trials. The root mean square error (B), movement frequency (C), and movement amplitude (D) are plotted across all training trials. The *error bar* represents one standard error of the mean.

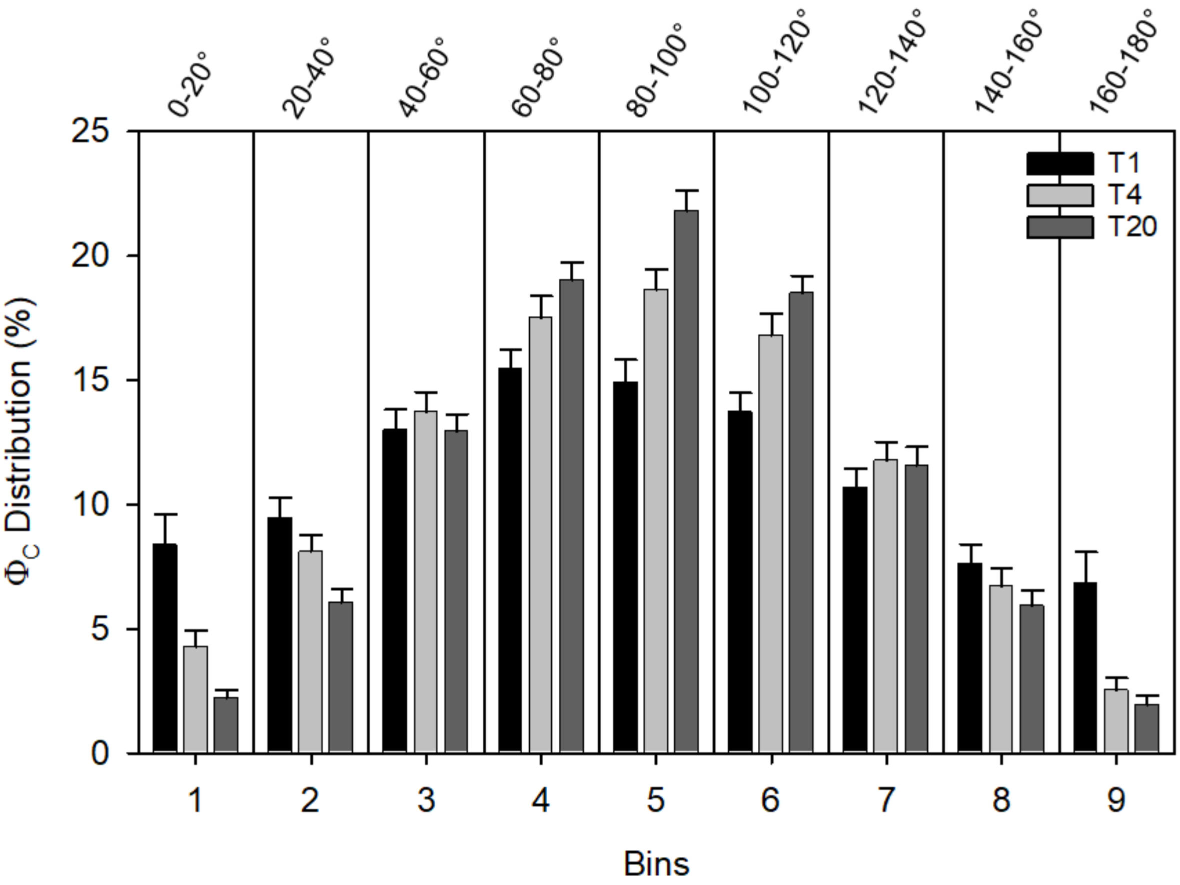

Figure 19. Plotted is the distribution of continuous relative phase for training trials T1, T4, and T20. Each bin represents a range of 20° from 0° to 180°. The three training trials plotted were chosen to represent the beginning of training (T1), the point in training when most participants began to plateau their performance (T4), and the end of training (T20). Each *error bar* represents one standard error of the mean.

To analyze the distribution of the continuous relative phase for the retest trials without visual feedback, T20 was compared to R1-4 for Bin1, 5, and 9. The ANOVA revealed main effects of Bin ($F_{(2,132)} = 58.658$, $p < 0.001$, $\eta_p^2 = 0.471$) and Trial ($F_{(4,264)} = 7.357$, $p < 0.001$, $\eta_p^2 = 0.100$). The analysis also found a significant interaction for Bin × Trial ($F_{(8,528)} = 5.424$, $p < 0.001$, $\eta_p^2 = 0.076$). Overall, everyone spent more time performing 90° relative phase than 0° or 180° ($ps \leq 0.001$) (Figure 21A). Additionally, participants spent more time in Bin1 during retest trials R1 and

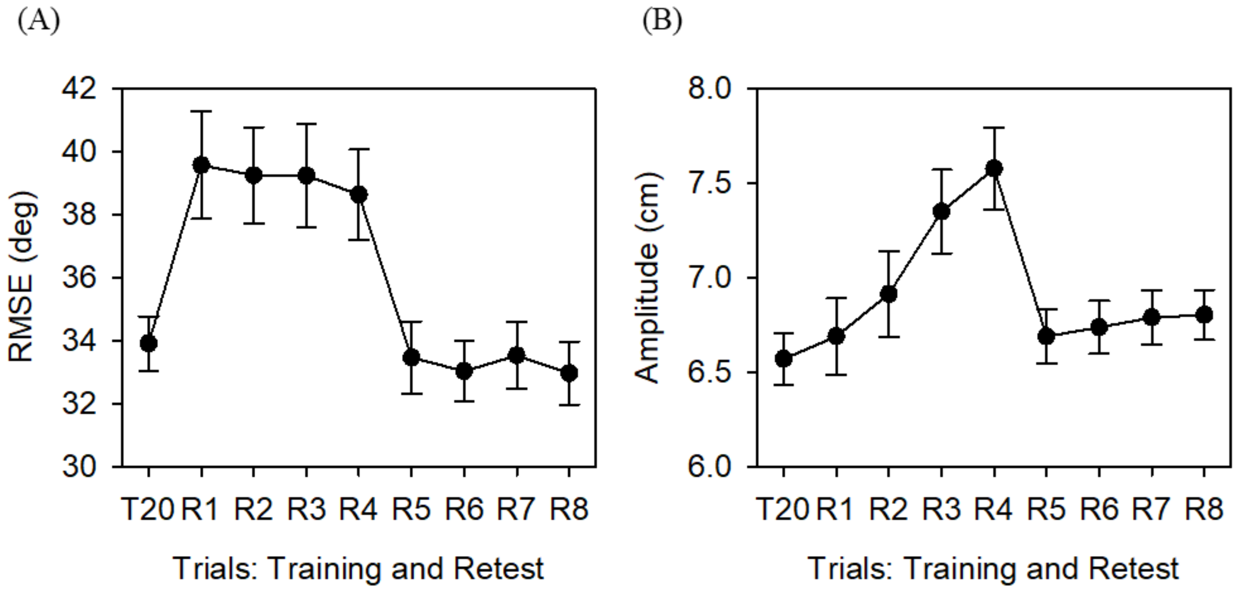

Figure 20. Plotted are the root mean square error (A) and movement amplitude (B) for training trials T20 and retest trials R1-8. Each *error bars* represent one standard error of the mean.

R2 compared to the end of the training, T20 (*ps* ≤ 0.012). Time spent in Bin5 was significantly greater for participants during the end of the training, T20, compared to retest trial R2 ($p = 0.017$). Participants also spent more time performing antiphase during retest trial R3 than at the end of the training, T20 ($p = 0.036$). Next, we analyzed the retest trials after visual feedback was reintroduced. An ANOVA was performed with Trial (T20, R5-8) and Bin (1, 5, 9) as repeated measures. A main effect of Bin ($F_{(2,132)} = 325.761$, $p < 0.001$, $\eta_p^2 = 0.832$) and a three-way interaction of Bin × Trial × Delay ($F_{(8,528)} = 2.557$, $p = 0.027$, $\eta_p^2 = 0.037$) was found. Post hoc analysis of the interaction revealed no differences other than between bins. A Bonferroni adjustment was performed for the main effect of Bin. All participants spent more time performing 90° than 0° or 180° (*ps* ≤ 0.001) (Figure 21A).

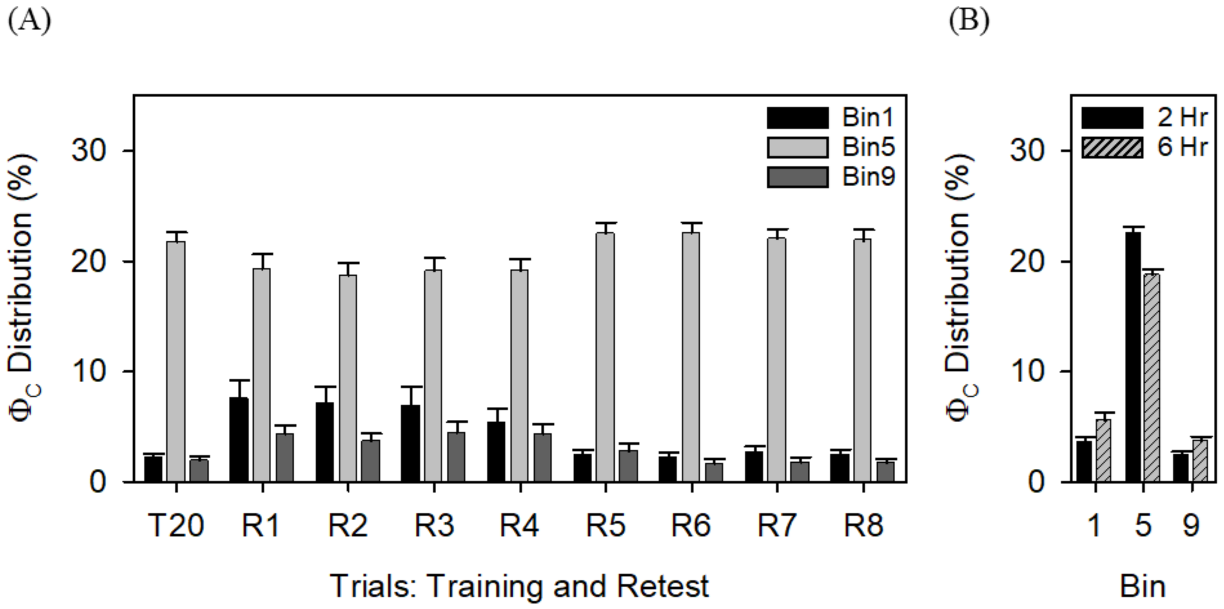

Figure 21. (A) Plotted are Bin1 (0°), 5 (90°), and 9 (180°) for training trials T20 and retest trials R1-8. T20 is the performance at the end of training. R1-4 is the retest when visual feedback was removed while R5-8 is the retest when visual feedback was reintroduced. Each error bar represents one standard error of the mean. (B) Plotted is the distribution of the continuous relative phase data for the Retest Delay × Bin interaction. Each *error bar* represents one standard error of the mean.

Finally, a comparison was performed between the retest trials without visual feedback (R1-4) and the retest trials where visual feedback was reintroduced (R5-8). To analyze the distribution of the continuous relative phase, Bin1, 5, and 9 were compared across trials. Main effects of Bin ($F_{(2,132)} = 122.398$, $p < 0.001$, $\eta_p^2 = 0.650$) and Trial ($F_{(7,462)} = 6.308$, $p < 0.001$, $\eta_p^2 = 0.087$) were found. The ANOVA also revealed interactions for Bin × Delay ($F_{(2,132} = 3.346$, $p = 0.049$, $\eta_p^2 = 0.048$) and Bin × Trial ($F_{(14,924)} = 7.463$, $p < 0.001$, $\eta_p^2 = 0.102$). For the Bin × Delay interaction (Figure 21B), the Bonferroni post hoc analysis found that participants performing at the 2-hr retest performed 90° more often than the participants who performed at the 6-hr retest ($p = 0.032$). There was no difference in time spent performing in-phase or antiphase between the 2-hr and 6-hr delay groups. The post hoc analysis for the Bin × Trial interaction found differences between trials and across bins (Figure 21A). For all trials, participants spent more time performing the 90° relative phase than 0° and 180° (*ps* ≤ 0.001). The 0° pattern was more frequently performed during trials R1 and R2 than during trials R5, R6, and R8 (*ps* ≤ 0.044). The 180° pattern was more frequently performed during trial R2 than during R8 ($p = 0.037$). For the 90° relative phase pattern, participants spent more time in Bin5 during retest trials R5 and R6 than during R1, R2, and R3 (*ps* ≤ 0.045).

5. DISCUSSION

The purpose of experiment 2 was to explore the time-dependent nature of the consolidation process for rhythmic bimanual coordination tasks. To probe this consolidation process, the number of mechanical DoF was reduced and the experiment manipulated the delay for the retesting period. For the training session, all participants improved performance of the 90° relative phase as evident by the decrease in $\boldsymbol{\phi}_{SD}$ and RMSE which plateaued in the first few trials (Kovacs et al., 2009a; McCulloch et al., 2020). Participants increased movement frequency and amplitude before establishing a comfortable movement speed and amplitude. The distribution of the continuous relative phase revealed the frequency of in-phase and antiphase was reduced from the start to the end of training. As training went on, participants were able to maintain the 90° relative phase more often as a result. Stimulation did not increase the rate of acquisition of the 90° pattern compared to the sham condition.

The first four retest trials were performed without visual feedback. Without visual feedback, performance variability was similar to the end of practice while performance error increased. This is suggestive that the motor memory is somewhat stable yet fragile at the same time. The reintroduction of visual feedback allowed participants to immediately improve their performance to or near the same level as the end of training in the first trials, again suggesting that consolidation occurred to some extent. An effect of movement amplitude did emerge without the aid of visual feedback. Amplitude slowly increased across the four trials when participants were not able to rely on external information on their movement amplitude. The distribution of the continuous relative phase revealed that participants were able to efficiently perform the target relative phase at the end of the training, while visual feedback was removed, and after the reintroduction of visual feedback. However, most participants were able to perform the target relative phase at the same level as the end of the training, an increase in in-phase performance emerged when participants were not provided visual feedback. This suggests that participants who were roaming without feedback were instead drawn to a more stable state (0°). There appears to be a slight retest delay difference. Participants retested after a 2-hr delay was able to perform 90° relative phase at a higher percentage than the groups retested after a 6-hr delay. Overall, participants were able to maintain the same percentage of time spent performing the target relative phase from the end of the training, to the retest trials without visual feedback, and during the retest with reintroduced visual feedback. This would suggest that a stable memory had formed and was maintained across the retest delay periods (2-hr or 6-hr). No effect of stimulation montage was evident during the training or retest session.

6. GENERAL DISCUSSION

Two experiments were conducted to explore the time-dependent nature of the consolidation process for rhythmic bimanual coordination tasks. To explore this consolidation process, the experiments manipulated the mechanical DoF, stimulation-training coupling, and time delay of the retesting period. Determining the time scale of consolidation is important because it can reveal the strengths and weaknesses of different types of training protocols. The underlying processes that support new memory formation are initially fragile or unstable and then consolidate over a period of time. An important aspect of the training protocol was the use of the Lissajous plot for concurrent visual feedback. Similar training protocols have produced large improvements over short practice bouts compared to the use of visual metronomes for various rhythmic bimanual tasks (Kovacs et al., 2009a; Kovacs, Buchanan, & Shea, 2009b; Kovacs et al., 2010a). For these experiments, we sought to determine if the rapid improvements which occur over the short training period can initiate the process of attractor formation (consolidation from a dynamical systems perspective).

In Experiment 1, subjects experienced tDCS either before or during training on the 90° relative phase pattern and retested at 6-hr. To perform the rhythmic bimanual task, participants manipulated two handles by flexing/extending their whole arms, requiring six mechanical DoF: shoulders, elbows, and wrists. The results detected a significant improvement in performing the target relative phase, 90°, across the training trials. It is important to establish that participants did improve performance as a result of training. As a whole, participants all began with similar performance and ended the training session with similar performance values. This indicates that neither the timing of stimulation nor the stimulation montage affected how participants performed when initially exposed to the 90° pattern or the final level of performance by the end of training.

However, a difference between Stimulation Timing did emerge for the $\boldsymbol{\phi}_{SD}$ measure early in training. The groups which experienced stimulation before training reduced performance variability at a quicker rate than the groups which experienced stimulation during training. This means they stabilized the 90° pattern at a faster rate. Although this was a significant interaction between Stimulation Timing and training Trials, there was no significant difference between the stimulation montages: Left-Anode, Right-Anode, and Sham. The Before groups reduced their performance variability at a quicker rater regardless of receiving active or sham stimulation. This difference leveled off around training trial T9. It is not evident with the current experiment whether tDCS before training provides a slight advantage to the rate of improvement compared to tDCS during training, or if tDCS during training somehow hinders or slows the rate of improvement compared to tDCS before training. In a different experiment using a uni-manual tack, a simple repetitive abduction-adduction right-hand thumb motion was performed and was paired with tDCS either before, during, or after training. The anode electrode was placed over left M1 and the cathodal reference electrode was placed over the contralateral supraorbital area (1 mA for 13 min). Only the before training tDCS produced a significant increase in cortical excitability (Cabral et al., 2015). Although this study used a different stimulation montage than Experiment 1, there appears to be some congruence in the benefits of stimulation before training.

Participants did increase their movement frequency and movement amplitude early in training. This is not surprising as part of the task required a specific target movement amplitude to trace the provided template on the Lissajous plot. As participants became familiar with using the feedback and while learning the target relative phase, they then began to hone in on the target movement amplitude. An increase in movement frequency is also not surprising since participants were instructed to increase the frequency until they felt like they were at a comfortable rate.

A key feature of the learning process is identifying how the stability of a pattern changes across practice and the extent that consolidation occurs as measured through the accuracy and stability of a recalled pattern. One problem with averaging the continuous relative phase within each trial is that unstable or roaming performance can mask good performance that occurs intermittently. The idea of intermittent attraction to the trained relative phase is consistent with the idea that the newly developing motor-memory is fragile. If a participant can maintain the 90° pattern for several cycles during a trial but then transitions to either in-phase or antiphase, the mean continuous relative phase would not properly represent the behavior during this trial. This transition from an unstable pattern to a more stable pattern has been highly documented (Carter et al., 2015; H. Haken, J. A. Kelso, & H. Bunz, 1985a; J. A. Kelso, 1984; Schoner, Haken, & Kelso, 1986; P. G. Zanone & J. A. Kelso, 1992). Analyzing the distribution of the relative phase for each trial revealed how participants were performing as a percentage of each trial. Using this analysis, it was found that participants spent more time performing in-phase and antiphase at the start of the training session with the time spent performing the 90° pattern increasing across the training trials. This suggests that everyone was beginning to stabilize a new attractor state around the 90° relative phase and were less likely to transition to either in-phase or antiphase.

For the retest session, participants were unable to maintain the same level of performance as the end of training. Performance error and variability increased when visual feedback was removed at the 6-hr retest. This would suggest that memory stabilization did not occur for the rhythmic bimanual skill given the initial retesting conditions without visual feedback. This sort of behavior was seen in previous experiments when the Lissajous plot was removed at a retest 5-15 minutes after training (Kovacs et al., 2009a, 2010a). The relative phase classification scheme and distribution analysis suggest that performance was stable on some trials, with intermittent performance on other trials. This mixing of stable and unstable performance across trials and within trials reveals the fragility of the motor memory, yet also shows that consolidation is occurring. With the reintroduction of the Lissajous plot for the last four trials, participants were able to rapidly hone back in on the target relative phase. Because the performance was able to quickly return to levels comparable to the end of the training, some form of memory consolidation had likely occurred for the motor memory, but this memory was dependent, to some degree, on the visual information supplied via the Lissajous plot. Over time, a newly practiced bimanual pattern consolidates (stabilizes) into a memory, a stable representation of that pattern that can be recalled or reactivated (McCulloch et al., 2020).

All participants were able to effectively perform the target relative phase when the Lissajous plot was available, both at the end of the training session and during the retest session. However, some participants were able to perform the target relative phase without visual feedback at the 6-hr retest while others were not. The ability to recall and perform the correct relative phase without visual feedback appears to be participant-specific, and not due to any experimental conditions. The ability to accurately recall the correct pattern varied between and within each participant. For example, 13 of the 96 participants were able to perform the 90° relative phase on all 4 trials without feedback. Others performed in-phase or antiphase during trial R1 then performed the target relative phase on trials R2-4. Some participants performed the 90° pattern well in the first few trials then switched to a roaming behavior for the remaining trials. Interestingly, 14 participants performed only in-phase or only antiphase during trials R1-4. The other 69 participants, which didn’t perform only in-phase, 90°, or antiphase across the four trials, had performances that were categorized with at least one trial where they performed either the target pattern or were roaming between patterns. This sort of inconsistent behavior between and even within participants suggests that most participants had at least a weak or fragile memory (attractor) at the time of retest. Without the visual feedback, this weak or fragile (unstable attractor) memory was unable to produce the same level of performance as the end of training.

As a whole, performance without visual feedback was not comparable to the end of the training, so we cannot conclude that consolidation in the form of memory stabilization occurred for all participants. Performance during the retest without visual feedback was quite worse than hypothesized, one reason for this may have been the number of mechanical DoF required to perform the motor task. From the perspective of dynamical systems, as the number of DoF increases, the system must account for more interacting components (joints and muscles) to achieve the task. In previous work by McCulloch et al. (2020), participants were able to perform the 90° pattern after a 6-hr delay, even after practicing an interference task. However, the task required participants to flex/extend their index fingers (two mechanical DoF). Therefore, it is hypothesized that the greater number of DoF contributed to the poor retention of the target pattern when visual feedback was removed.

Overall, there appears to be no significant difference between the main effect of Simulation Montage, however, there were a few interactions with Stimulation Timing. For the Right-Anode groups, experiencing tDCS during training, compared to before training, resulted in increased $\boldsymbol{\phi}_{SD}$ during the training session. Studies finding little to no results from tDCS exposure is not uncommon (Chen et al., 2020; Furuya et al., 2013; McCambridge et al., 2016; Vancleef et al., 2016).

The dynamic pattern approach to motor control focuses on rhythmic coordination tasks and is centered on the concept of self-organization which proposes that behavior is not a result of an internal model or motor program controlling movements. Rather, the system's DoF (limbs, muscles, nerve cells) self-organizes into stable patterns of coordination. For Experiment 2, we reduced the number of mechanical DoF by switching to index finger flexion/extension at the metacarpophalangeal joint to produce the rhythmic bimanual task, and subjects experienced tDCS before training. To further explore the consolidation process and to test the strength of the attractor formation process at different time intervals, the time delay for the retest period was manipulated. Participants performed their retest after either a 2-hr or 6-hr delay. The results of Experiment 2 revealed a significant improvement in performing the target relative phase, 90°, across the training trials. Performance measures at the beginning of the training session were similar for all participants. The level of performance improvement by the end of the training session did not differ between groups. No differences between Stimulation Montage and Retest Delay emerged during the training session.

Participants did increase their movement frequency and movement amplitude early in training. Part of the task was to perform the pattern at a specific movement amplitude to trace the template provided on the Lissajous plot. As a result of this requirement, participants needed to increase the amplitude to match the external feedback display. As participants became more familiar with using the visual feedback, they increased their movement frequency. Part of the task instructions mentioned increasing movement frequency until the participants felt they were moving at a comfortable rate, so this increase is not surprising.

Using the distribution of the relative phase, we found that participants spent more time performing in-phase and antiphase at the start of the training session. Both of these patterns are intrinsically stable patterns and performance seemed to be attracted to these patterns early in training. This attraction decreased over the training session as participants spent significantly less time performing in-phase and antiphase at the end of training. The time spent performing the 90°

pattern increased across the training trials suggesting everyone was beginning to establish a new attractor state around the 90° relative phase and were less likely to transition to either in-phase or antiphase. These results are comparable to Experiment 1.

When visual feedback was removed during the retest, performance error increased whereas coordination variability was maintained. This suggests that participants were drifting from the target relative phase but were maintaining similar within-trial coordination variability. This is different from Experiment 1 where both performance error and variability increased without visual feedback. With the reintroduction of visual feedback, all participants were able to rapidly improve their performance error to or near the same level as the end of training. Because performance error was able to quickly return to levels comparable to the end of the training, some form of memory consolidation had likely occurred for the motor memory. It is probably that at least part of this memory was dependent on the visual information supplied via the Lissajous plot. Participants were able to effectively perform and maintain the target relative phase at the end of training and during the retest with and without visual feedback. This is dissimilar to Experiment 1 where the performance of the 90° target relative phase in retest trials R1-4 was more intermittent (fragile). This would suggest that a stable memory had formed and was maintained across the retest delay periods (2-hr or 6-hr). When visual feedback was removed, an increase in in-phase emerged suggesting that participants who were roaming when using visual feedback (performance outside of Bins 1, 5, or 9) were instead drawn to the more stable 0° or in-phase pattern. In contrast, the individuals in Experiment 1 who were unable to recall the correct movement pattern without visual feedback during any of the four retest trials tended to favor antiphase over in-phase.

It was hypothesized for Experiment 2 that performance would be maintained at the 6-hr retest and performance would be unstable at the 2-hr retest. This hypothesis was supported by previous work which found similar results (McCulloch et al., 2020). A difference in Retest Delay did emerge when performing after a 2-hr delay resulted in a higher percentage of 90° relative phase performance than performing after a 6-hr delay. This was not anticipated as a result of the retest delay period. Both the 2-hr and 6-hr delay groups performed the target relative phase more often than in-phase and antiphase across all retest trials, but participants who performed at the 2-hr delay retest performed the target pattern more often. The previous study by McCulloch et al. (2020) does not predict these results. The previous study by McCulloch et al. used an interference task to test the stability or strength of the attractor formation process at different time intervals. They concluded the difference between the 2-hr and 6-hr retest delays suggested the initial formation of persistent memory in the form of an attractor was occurring around a 4-hr window post-practice. Their results support the conclusion that a 6-hr delay was required for the consolidation of an attractor at 90° to emerge, whereas a 2-hr delay resulted in interference at the retest. However, there are some key differences between the McCulloch et al. (2020) experiment and Experiments 1 and 2. During the retest session of the McCulloch et al. study, participants trained on a 45° pattern (the interference pattern) for ten trials using a Lissajous plot and template as visual feedback to guide them during training. This could be described as the reintroduction of the visual feedback after the initial exposure to the 90° pattern. During the present two experiments, participants returned after the delay period and immediately began performing without a reintroduction to the feedback. For participants in Experiment 1 and 2, their reintroduction to the visual feedback was during retest trial R5, after the first four retest trials. It is possible that using the visual feedback to train on the interference task immediately before retesting the 90° pattern allowed participants to reactivate the attractor formation process, but the interference task was able to disrupt the recall of the pattern at 2-hr but not 6-hr. The poor performance in R1-4 for Experiment 1 could be contributed to the increase in degrees of freedom required for moving the whole arms to perform the task. Unfortunately, the only retest delay period for Experiment 1 was at 6-hr, so we cannot compare results to a shorter delay period.

In Experiment 2, an interesting effect of movement amplitude emerged when visual feedback was removed. Amplitude increased across the four retest trials when participants were unable to rely on external information. Once the visual feedback was reintroduced, participants immediately adjusted their movement amplitude back to the target as defined by the template on the Lissajous plot. Because most participants were able to maintain the 90° pattern with and without visual feedback, the difference in amplitude would suggest the motor memory might not contain the specific amplitude of the movements that were trained but may be more focused on the timing and spatial accuracy of the flexion/extension for the index fingers to achieve the 90° relative phase.

7. CONCLUSION

The underlying motor-memory (perception-action) representation developed during training is initially fragile or unstable and requires a time interval to consolidate or become stable. In the present two studies, we attempted to explore the time-dependent nature of the consolidation process for rhythmic bimanual tasks. To explore this consolidation process, the experiments manipulated the mechanical DoF, stimulation-training coupling, and time delay for the retesting period. The results indicate that rapid performance improvement occurred as a result of training with concurrent augmented feedback in the form of a Lissajous plot. However, the degrees of freedom required to complete the task seem to impact the dependency on visual feedback to detect and correct errors in performance after only a short bout of training. A novel finding of Experiment 2 is that participants were able to maintain the target relative phase at similar levels of performance at both the 2-hr and 6-hr delayed retest. Regarding the effects of tDCS on the training or the consolidation process of a novel rhythmic bimanual task, minimal to no effects were found as a result of both experiments. Previous research has presented conflicting findings regarding the validity of this form of non-invasive brain stimulation, and the present studies do not present any robust findings to help clarify any of these blurry lines.

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