The Role of the Left Dorsolateral Prefrontal Cortex in Procedural Motor Learning

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Abstract

Procedural motor learning includes sequence learning and random learning. Neuroimaging studies have demonstrated that the dorsolateral prefrontal cortex (DLPFC) and primary motor cortex (M1) play important roles in procedural motor learning, yet the connectivity between DLPFC and M1 and its relationship with different types of procedural motor learning remain unclear. The present study utilized a serial reaction time task combined with transcranial magnetic stimulation (TMS) methods to investigate differences in connectivity from the left DLPFC to M1 across different procedural motor learning paradigms. Experiment 1 employed paired-pulse TMS to probe the optimal projection timing from DLPFC to M1; in Experiment 2, participants were divided into two groups to undergo sequence learning and random learning, respectively, with behavioral data as well as electrophysiological data including M1 motor evoked potentials and DLPFC-M1 connectivity collected before and after learning. Behavioral results revealed that the sequence learning group exhibited superior learning performance; electrophysiological results showed that M1 motor evoked potentials did not change before or after learning in either group. At the optimal projection time point and appropriate stimulation intensity, DLPFC-M1 connectivity was altered in the sequence learning group and correlated with learning performance, whereas no such change was observed in the random learning group. These findings suggest that enhanced connectivity from DLPFC to M1 may be an important factor underlying superior performance in sequence learning, providing crucial electrophysiological evidence for the role of DLPFC in motor learning.

Functional Role of the Left Dorsolateral Prefrontal Cortex in Procedural Motor Learning

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Abstract

Procedural motor learning encompasses both sequence learning and random learning. Neuroimaging studies have demonstrated that the dorsolateral prefrontal cortex (DLPFC) and primary motor cortex (M1) play crucial roles in procedural motor learning, yet the connectivity between DLPFC and M1 and its relationship with different forms of procedural motor learning remain unclear. This study employed the serial response time task combined with transcranial magnetic stimulation (TMS) to investigate differences in left DLPFC-to-M1 connectivity across distinct procedural motor learning contexts.

Experiment 1 utilized dual-site paired-pulse TMS to identify the optimal temporal window for DLPFC-to-M1 projections. In Experiment 2, participants were divided into two groups for sequence learning and random learning, respectively. Behavioral data, motor evoked potentials from M1, and electrophysiological measures of DLPFC-M1 connectivity were collected before and after learning. Behavioral results revealed superior learning outcomes in the sequence learning group. Electrophysiological findings showed no changes in M1 motor evoked potentials before versus after learning in either group. However, at the optimal projection time point and appropriate stimulation intensity, DLPFC-M1 connectivity changed in the sequence learning group and correlated with learning performance, whereas no such changes occurred in the random learning group.

These results suggest that enhanced connectivity from DLPFC to M1 may constitute an important mechanism underlying superior sequence learning performance, providing critical electrophysiological evidence for the role of DLPFC in motor learning from a connectivity perspective.

Keywords: dorsolateral prefrontal cortex; primary motor cortex; transcranial magnetic stimulation; procedural motor learning; sequence learning

1. Introduction

Procedural motor learning refers to the acquisition of new knowledge through repeated execution of motor tasks, involving skeletal muscles and corresponding neural reflexes. Based on the presentation order of learning materials, it can be divided into sequence learning and random learning. Sequence learning occurs through repeated presentation of fixed-length sequences (Yang, 2014), whereas random learning proceeds through unordered presentation (Pascual-Leone, Wassermann, Grafman, & Hallett, 1996). Procedural motor learning constitutes not only an essential component of daily human activities but also a critical mechanism for motor skill acquisition (Clegg, Digirolamo, & Keele, 1998). It provides a paradigm for investigating motor learning under complex conditions and reflects the adaptability and plasticity of the human brain' s motor-cognitive system across different states (Grafton, Woods, & Tyszka, 1994).

Research indicates that procedural motor learning involves coordinated activity across multiple brain regions, including the primary motor cortex (M1), supplementary motor area (SMA), premotor cortex, and dorsolateral prefrontal cortex (Leonora, Teo, Ignacio, Rothwell, & Marjan, 2010; Poldrack et al., 2005; Schendan, Searl, Melrose, & Stern, 2003; Seidler et al., 2005). The connectivity and projections between motor functional areas (represented by M1) and cognitive functional areas (represented by DLPFC) serve as important bases for explaining differential motor learning performance (Cao et al., 2018; Lam et al., 2015).

Compared with the right prefrontal cortex, the left dorsolateral prefrontal cortex, particularly Brodmann Area 46 (Kielan, Peter, & Krakauer, 2009), represents a critical region integrating motor learning and motor control functions (Miller, 2000). Although DLPFC participates in motor-cognitive processes related to motor learning, the specific mechanisms of its involvement remain unresolved. Neuroimaging studies have demonstrated sustained DLPFC activation during sequence learning tasks (Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Sakai et al., 1951; Toni, Krams, Turner, & Passingham, 1998). Shadmehr and Holcomb (1997) reported activation of both prefrontal and motor cortices during sequence and random learning tasks, with concurrent improvements in sequence learning performance. According to Hikosaka' s theory, DLPFC participates in spatial sequence acquisition, processing initial sensory input and mapping spatial sequences before ultimately projecting to motor cortex (Hikosaka, Nakamura, Sakai, & Nakahara, 2002). Conversely, when tasks lack sequence learning components, prefrontal cortex activation does not occur (Willingham, 1998). These findings indicate that DLPFC activation varies with task context. Disruption of DLPFC excitability through non-invasive brain stimulation increases response times and impairs learning in spatial information processing tasks (Robertson, Tormos, Maeda, & Pascual-Leone, 2001), reflecting the impact of DLPFC excitability on motor learning outcomes. Pathological studies have shown that patients with cerebellar dysfunction or Parkinson' s disease exhibit impaired procedural learning (Pascual-Leone et al., 1996), whereas patients with temporal lobe and dorsomedial thalamic damage (Gordon, 1988; Squire, 1992) or Alzheimer' s disease retain procedural learning abilities (Grafman et al., 1990). These results demonstrate that procedural learning depends on the integrity of pathways connected to the dorsolateral prefrontal cortex, particularly when frontal lobe damage occurs (Fuster & Alexander, 1971).

Previous research has primarily focused on activation patterns of DLPFC as a single region, rather than examining connectivity between DLPFC as a crucial node in the cognitive functional network and M1 as the final common pathway for motor output. Although DLPFC plays an important role in motor learning, neuroimaging studies cannot reveal changes in DLPFC-M1 connectivity and their functional significance across different motor learning contexts (Friston, 2011). Effective neural functional connectivity is prerequisite for rapid information processing, making the investigation of DLPFC-M1 connectivity an important approach to understanding the mechanisms of motor learning.

This study employed dual-site paired-pulse TMS, an electrophysiological technique that can directly demonstrate effective connectivity or direct influence from one neural system to another, thereby precisely characterizing plasticity changes (Lafleur, Tremblay, Whittingstall, & Lepage, 2016). In paired-pulse stimulation, the first pulse serves as conditioning stimulation (CS) applied to DLPFC, while the second pulse serves as test stimulation (TS) applied to primary motor cortex. By varying the interstimulus interval and test intensity, this method can probe excitability of neural pathways connecting multiple target brain regions, particularly characterizing neural circuits beyond M1 (Ni & Chen, 2012; Ni, Florian, Chen, & Ziemann, 2011; Ni et al., 2009). Its advantage lies in linking different brain regions to investigate their functional connectivity and excitatory or inhibitory circuits. Changes in motor evoked potential (MEP) amplitude evoked by TS reflect functional pathways at the cortical level from the CS-stimulated region to the TS-stimulated region, i.e., inter-regional connectivity (Lazzaro et al., 1999; Rothwell, 2011). With advances in paired-pulse TMS technology, this method is now widely used to assess interhemispheric and intrahemispheric connections from non-homologous regions and cerebellar-cortical connections in scientific research (Ziemann et al., 2015), as well as in clinical diagnosis of stroke, movement disorders, and amyotrophic lateral sclerosis (Ridding & Rothwell, 2007), demonstrating high reliability and validity (Marco, Carlo, & Elena, 2011).

This study aimed to investigate differences in left DLPFC-to-M1 connectivity between two types of procedural motor learning. The serial response time task (SRTT) was employed (Leonora et al., 2010), where target stimuli appear in one of four possible locations per trial, requiring participants to press corresponding keys as quickly as possible. Stimulus sequences can be presented in either sequential or random order depending on experimental goals. The "sequence operability" (Willingham, Salidis, & Gabrieli, 2002) of SRTT makes it particularly advantageous compared to tasks such as virtual mechanical manipulation (Krebs, Hogan, Hening, Adamovich, & Poizner, 2001) or pursuit rotor tasks (Noguchi, Demura, Nagasawa, & Uchiyama, 2009), and it has become widely used in procedural learning research as an important behavioral paradigm for comparing learning under sequential versus random conditions (Mayor-Dubois, Zesiger, Van der Linden, & Roulet-Perez, 2016). Experiment 1 investigated the temporal course of DLPFC-to-M1 projections by varying interstimulus intervals. Experiment 2 combined SRTT with TMS to explore how different motor learning types affect DLPFC-M1 input-output curves.

Based on previous literature and theory (Hikosaka et al., 2002; Robertson et al., 2001; Toni et al., 1998; Willingham, 1998), DLPFC participates in spatial sequence acquisition and shows sustained activation during sequence learning but not during implicit learning, while DLPFC inhibition impairs accuracy and reaction time in button-press learning tasks. Therefore, we hypothesized that sequence learning would enhance DLPFC-M1 connectivity correlated with improved learning performance, whereas random learning would produce no such changes.

2. Methods

2.1 Participants

Forty-four participants took part in one or more experiments in this study. Twenty-one participants (7 female) with a mean age of 21.10 ± 1.97 years participated in Experiment 1; forty participants (15 female) with a mean age of 21.75 ± 1.74 years participated in Experiment 2. The two experiments were separated by at least two weeks to prevent carryover effects (Ni, Gunraj, Kailey, Cash, & Chen, 2014). All participants were right-handed (Oldfield, 1971) and had normal or corrected-to-normal vision. All provided written informed consent according to the Declaration of Helsinki. The TMS experiments were approved by the local ethics committee.

2.2 Experiment 1: Temporal Course of Left DLPFC-to-M1 Projections

This experiment employed a single-factor repeated-measures design, with the independent variable being the interstimulus interval (ISI) between CS and TS in dual-site paired-pulse TMS, and the dependent variable being paired-pulse MEP amplitude. Continuous paired-pulse stimulation of DLPFC and M1 was used to investigate changes in corticospinal output excitability.

First, CS and TS intensities were determined. Following previous literature, TS intensity (M1) was set to the stimulator output intensity required to evoke a 1 mV MEP at rest using the same TMS coil, establishing this as the baseline unit for subsequent comparison of paired-pulse TMS-evoked MEP amplitudes (Hallett, 2000). CS intensity (DLPFC) was set to 110% of resting motor threshold (RMT). Suprathreshold stimulation better facilitates conduction between frontal and primary motor cortices, though higher intensities may cause discomfort (Jacinta, Catherine, Boorman, Heidi, & Rushworth, 2010; Koch et al., 2007). Prior to the formal experiment, RMT was determined for each participant's right first dorsal interosseous muscle, defined as the minimum stimulation intensity that evoked MEPs > 50 V in at least 5 of 10 consecutive trials with the target muscle at rest.

Based on differences in projection fiber length from various brain regions to M1 (Oh et al., 2014; Wedeen et al., 2012) and neurotransmitter properties (Ziemann, 2004), ten consecutive ISIs were selected (2, 4, 6, 8, 10, 12, 15, 20, 25, 30 ms) to determine the temporal course of projections from the stimulated brain region to M1 under specific CS intensities, thereby characterizing the nature (excitatory or inhibitory) of the resulting neural circuits and the ISI required to achieve peak effects (Figure 1 [Figure 1: see original paper]A,B). Each participant completed 10 trials at each ISI, with a 5-second inter-trial interval. The 10 ISIs were presented in random order across 100 total trials. Based on previous reports, a 5-second inter-trial interval prevents interactions between trials (Hallett, 2000).

2.3 Experiment 2: Effects of Different Motor Learning Types on DLPFC-M1 Connectivity

Experiment 2 employed learning type (sequence vs. random) as a between-subjects factor to examine effects on DLPFC-M1 connectivity input-output curves at two optimal ISIs (10 and 25 ms, determined from Experiment 1 results) (Figure 2 [Figure 2: see original paper]A). For the learning task, a 2 (group: sequence learning, random learning) × 2 (block: block 1, block 10) mixed design was used with block as the within-subject variable, and mean block reaction time as the dependent variable. For physiological measures, a 2 (group) × 2 (time: pre-test, post-test) × 5 (stimulation intensity) mixed design was employed with stimulation intensity and time as within-subject factors, and MEP amplitude as the dependent variable.

Stimulation intensity levels for paired-pulse TMS included CS intensities of 50% RMT, 70% RMT, 90% RMT, 110% RMT, and 130% RMT (CS applied to DLPFC, with high sensitivity to both sub- and suprathreshold intensities, thus using RMT as baseline) (Lam et al., 2015). Single-pulse TMS intensities were set at 70%, 90%, 100%, 110%, and 120% of 1 mV (requiring comparison with the 1 mV standard single-pulse MEP, thus using 1 mV intensity as baseline) to complete input-output curve measurements. Each participant received 10 trials at each intensity level, with intensities randomly presented every 5 seconds. The experimental sequence was pre-test → learning task → post-test (Figure 2A). Following the learning task, sequence learning group participants were asked whether they had detected the sequence and to recall it.

2.4 TMS and EMG Procedures

Two small figure-of-eight coils (5 cm diameter) connected to two single-pulse monophasic TMS stimulators (Magstim200, Whitland, Dyfeld, UK) were used to deliver transcranial magnetic stimulation to left DLPFC and left M1. Both stimulation sites (dorsolateral prefrontal cortex and primary motor cortex) were in the left hemisphere to reduce interhemispheric measurement bias and focus on the dominant hemisphere. To investigate left hemisphere DLPFC-M1 connectivity, CS was applied to left DLPFC, and the degree of excitability change in ipsilateral M1 output was measured to quantify excitatory or inhibitory effects of DLPFC on M1.

The TS coil position was the "motor hotspot" corresponding to the optimal activation site for the right first dorsal interosseous muscle in M1. The TS coil handle was oriented nearly perpendicular to the central sulcus and at 30°–45° to the cerebral mid-sagittal line, with current flowing in a posterior-to-anterior direction. To determine the precise location of left DLPFC, a neuronavigation system connected to the TMS device (eemagine visor2) modeled each participant' s brain MRI scan to identify the CS location based on Talairach coordinates for left BA 46 (x, y, z: -40, 28, 30) (Rowe, Stephan, Friston, Frackowiak, & Passingham, 2005).

MRI scans were performed using a Siemens 3.0T whole-body scanner (Siemens Magnetom Trio 3.0T) for structural data acquisition. A 12-channel array coil and echo-planar imaging (EPI) sequence were used to collect T1* structural images with the following parameters: TR 2000 ms, TE 30 ms, slice gap 1 mm, flip angle 90°, slice thickness 3 mm, matrix 64×64, field of view 240×240 mm^2, voxel size (3.75×3.75×5.0) mm^3, 33 slices, interleaved scanning (Zhang L. L. et al., 2017).

2.5 EMG Recording

Nine-millimeter bowl-shaped Ag-AgCl surface electrodes recorded surface electromyography from participants' right first dorsal interosseous muscle. The active electrode was placed over the muscle belly and the reference electrode over the metacarpophalangeal joint. Signals were collected via a neurodischarge signal acquisition system (CED Micor1401), filtered (bandpass 20–25,000 Hz) and amplified 1000-fold using an electrophysiological signal conditioning amplifier (Model 2024F). Filtered and amplified signals were digitized at 1 kHz and analyzed offline using Signal 6.0 software.

2.6 Behavioral Paradigm

The behavioral task employed the serial response time task (SRTT). Experimental programs were created using E-prime software and presented on a laptop screen (HP Pavilion, 15.6 inches, 1980×1020 resolution, 75 Hz refresh rate). Participants responded using a standard keyboard while seated in a quiet laboratory approximately 80 cm from the screen. A black arrow appeared in the center of a white screen in one of four possible directions (9, 11, 1, and 3 o' clock positions), each requiring a button press with a different finger (index, middle, ring, little finger, respectively) (Figure 2B). Stimulus duration was 800 ms with a 300 ms inter-stimulus interval. The task comprised 10 blocks, each containing 10 sequences of 12 arrow stimuli. Participants rested between blocks. Prior to the experiment, participants completed 10 practice trials to familiarize themselves with the procedure.

For each 12-stimulus sequence, two different orders were used. In the sequence learning group, black arrows appeared in a fixed order: 1-9-3-1-11-3-11-9-1-3-9-11 o' clock positions, repeating throughout. In the random learning group, the 120 trials per block were presented in pseudorandom order following these constraints: equal probability at each position, no direct repetitions (e.g., 1111), and exclusion of runs (e.g., 1234) or partial repetitions (e.g., 1212). The 40 participants in Experiment 2 were randomly assigned to two groups with no demographic differences (Table 1).

2.7 Data Analysis

Peak-to-peak MEP amplitude was recorded for each trial. Paired-pulse (CS-TS) MEP amplitudes were expressed as a percentage of single-pulse TS amplitude (Figure 3 [Figure 3: see original paper]). Data are reported as mean ± standard error.

In Experiment 1, one-way repeated-measures ANOVA was conducted on MEP amplitudes across different DLPFC-to-M1 projection intervals to examine inhibitory and facilitatory peaks. In Experiment 2, two-way repeated-measures ANOVA (2 group × 2 block) was performed on behavioral task results to examine learning differences between groups. For physiological data, three-way repeated-measures ANOVA (2 group × 2 time × 5 intensity) was conducted to examine differences in input-output curves for single-pulse and paired-pulse TMS across conditions. Statistical analyses were performed using SPSS 17.0 with significance set at $p < 0.05$.

3. Results

3.1 Experiment 1: Temporal Course of DLPFC-to-M1 Projections

One-way repeated-measures ANOVA revealed significant differences in paired-pulse MEP amplitude relative to single-pulse TS amplitude across different ISIs ($F(10, 200) = 8.88$, $p < 0.001$, $p^2 = 0.31$, Figure 4 [Figure 4: see original paper]). Bonferroni-corrected multiple comparisons between MEP amplitude at each ISI and single-pulse 1 mV amplitude showed significant MEP reduction at 10 ms ($p = 0.019$) and 20 ms ($p = 0.021$), and significant MEP facilitation at 25 ms ($p = 0.047$). Other ISIs showed no significant effects. To identify time points of maximal inhibition and facilitation, the 10 ms (peak inhibition) and 25 ms (peak facilitation) intervals were selected for Experiment 2.

3.2 Experiment 2: Behavioral Task Performance

Figure 5 [Figure 5: see original paper] shows mean reaction times for the first and last blocks in both learning groups. Two-way repeated-measures ANOVA on reaction times revealed significant main effects of group ($F(1,38) = 8.33$, $p = 0.006$, $p^2 = 0.18$) and block ($F(1,38) = 138.62$, $p < 0.001$, $p^2 = 0.79$), and a significant group × block interaction (F(1,38) = 33.49, $p < 0.001$, p^2 = 0.47). Simple effects analysis showed significant reaction time decreases in both sequence learning ($p < 0.001$) and random learning groups (p = 0.018), indicating practice effects. However, sequence learning group reaction times in block 10 were significantly faster than those of the random learning group ($p < 0.001$).

3.3 Experiment 2: Single-Pulse MEP Input-Output Curves

Figure 6 [Figure 6: see original paper] shows MEP changes in the first dorsal interosseous muscle before and after different procedural motor learning types. Three-way repeated-measures ANOVA on MEP data revealed a significant main effect of stimulation intensity (F(4,152) = 140.34, $p < 0.001$, p^2 = 0.79), but no significant main effect of time (F(1,38) = 0.17, p = 0.681, p^2 = 0.00) or three-way interaction (F(4,152) = 0.11, p = 0.105, p^2 = 0.00).

3.4 Experiment 2: Paired-Pulse MEP Input-Output Curves

Based on Experiment 1 results, two ISIs showing peak effects (10 and 25 ms) were selected. At 10 ms ISI, three-way repeated-measures ANOVA showed no significant main effects of time (F(1,38) = 0.53, p = 0.472, p^2 = 0.01) or intensity (F(4,152) = 1.00, p = 0.411, p^2 = 0.03), but a significant three-way interaction (F(4,152) = 2.61, p = 0.038, p^2 = 0.06). Subsequent two-way ANOVA in the sequence learning group revealed no main effects of intensity (F(4,76) = 0.98, p = 0.426, p^2 = 0.05) or time (F(1,19) = 1.73, p = 0.204, p^2 = 0.08), but a significant time × intensity interaction (F(4,76) = 4.24, p = 0.011, p^2 = 0.18, Figure 7 [Figure 7: see original paper]A). Post-hoc tests showed significantly higher MEP amplitude at 110% RMT after sequence learning compared to baseline (p = 0.002). In the random learning group, no significant effects were found for intensity (F(4,76) = 0.57, p = 0.683, p^2 = 0.03), time (F(1,19) = 0.12, p = 0.736, p^2 = 0.01), or their interaction (F(4,76) = 0.77, p = 0.551, p^2 = 0.04) (Figure 7B).

At 25 ms ISI, three-way ANOVA revealed no significant main effect of time (F(1,38) = 1.08, p = 0.304, p^2 = 0.03), a significant main effect of intensity (F(4,152) = 3.86, p = 0.005, p^2 = 0.09), and a significant three-way interaction (F(4,152) = 2.48, p = 0.046, p^2 = 0.06). Two-way ANOVA in the sequence learning group showed no main effects of intensity (F(4,76) = 1.07, p = 0.376, p^2 = 0.05) or time (F(1,19) = 3.00, p = 0.099, p^2 = 0.14), but a significant time × intensity interaction (F(4,76) = 3.29, p = 0.015, p^2 = 0.15, Figure 7C). Post-hoc tests revealed significantly lower MEP amplitude at 110% RMT after sequence learning compared to baseline ($p < 0.001$). In the random learning group, ANOVA showed a significant main effect of intensity (F(4,76) = 3.23, p = 0.017, p^2 = 0.15), with post-hoc comparisons revealing significant differences between 50% RMT and 90% RMT (p = 0.041), 50% RMT and 110% RMT (p = 0.011), and 110% RMT and 130% RMT (p = 0.033). However, no significant effects were found for time (F(1,19) = 0.09, p = 0.772, $p^2 = 0.00$) or time × intensity interaction (F(4,76) = 0.65, p = 0.626, $p^2 = 0.03$) (Figure 7D).

3.5 Correlation Between Behavioral and Physiological Data

Figure 8 [Figure 8: see original paper] shows correlations between the difference in mean reaction times (block 1 vs. block 10) and physiological MEP differences in both learning groups. Positive MEP differences indicate decreased motor cortex excitability after learning, while negative values indicate increased excitability. Correlation analysis revealed that in the sequence learning group, greater increases in motor cortex excitability at 10 ms ISI correlated with larger reaction time reductions (r = -0.448, p = 0.048, Figure 8A). At 25 ms ISI, greater decreases in motor cortex excitability correlated with larger reaction time reductions (r = 0.467, p = 0.038, Figure 8C). The random learning group showed no significant correlations at either ISI (10 ms: p = 0.121, Figure 8B; 25 ms: p = 0.373, Figure 8D).

4. Discussion

This study used SRTT and TMS to investigate whether left DLPFC-to-M1 connectivity differs between two types of procedural motor learning. Behavioral and electrophysiological data revealed that: (1) the sequence learning group showed superior learning outcomes with significant reaction time reductions; (2) left DLPFC-to-M1 projections exhibited significant inhibitory (10 ms) and facilitatory (25 ms) peaks at two time points; (3) single-pulse TMS-evoked MEPs remained unchanged in both learning conditions, whereas paired-pulse MEPs showed altered DLPFC-M1 connectivity in the sequence learning group at 110% RMT that correlated with behavioral data, with no changes in the random learning group.

4.1 Temporal Characteristics of DLPFC-to-M1 Projections

The results revealed early inhibitory and late facilitatory peaks from DLPFC to M1 (Figure 4), indicating that the first CS either inhibited or facilitated primary motor cortex responsiveness to the second test pulse through prefrontal cortex activation. These two projection time points likely result from differences in fiber pathway lengths from specific brain regions to M1 and variations in neuronal thresholds (Wedeen et al., 2012). The early 10 ms inhibitory peak may represent the optimal time for direct synaptic transmission from DLPFC to M1, depending on direct cortical projections via white matter tracts such as the superior longitudinal fasciculus (Koch et al., 2007). Additionally, inhibiting DLPFC excitability increases M1 cortical excitability, while facilitating DLPFC excitability decreases M1 excitability (Cao et al., 2018). This bidirectional inverse relationship suggests the existence of inhibitory projection pathways between DLPFC and M1, consistent with our findings. The later 25 ms facilitatory peak may reflect signal transmission not directly from DLPFC to M1 but via intermediate "relay stations" such as dorsal premotor cortex, forming polysynaptic circuits. Most cortical projections from DLPFC travel through premotor cortex before reaching primary motor cortex, resulting in facilitatory effects at the 25 ms time point (Koch & Rothwell, 2009).

In addition to the inhibitory and facilitatory peaks at 10 ms and 25 ms, MEPs at ISIs of 2, 4, 6, 8, 15, 20, and 30 ms also showed inhibitory projection states. Facilitatory effects appear only at limited ISIs and require specific stimulation intensities, whereas inhibitory projections dominate across most ISIs and intensities, consistent with previous findings (Koch et al., 2007). The predominance of inhibitory projections remains unclear. One possible explanation is that facilitatory connections represent projections within a limited number of pathways, while inhibitory projections are more widespread. As a focal stimulation method, TMS can only target specific foci within regions, making it more likely to detect widely distributed inhibitory connections (Hanajima et al., 2001).

4.2 Differential Role of DLPFC in Different Motor Sequence Learning

The study found differential changes in DLPFC-M1 pathway excitability between sequence and random learning groups, particularly strongest at 110% RMT CS intensity (Figure 7A,C). Based on previous research, suprathreshold stimulation better elicits functional interactions between frontal cortex and M1 compared to subthreshold stimulation (Jacinta et al., 2010; Koch et al., 2007), while excessively high suprathreshold intensities (130% RMT) may suppress synaptic transmission and inhibit TS-evoked MEPs (Uehara, Morishita, Kubota, & Funase, 2013).

Experiment 2 results demonstrate that sequence learning can alter DLPFC-M1 excitability at two optimal time points, indicating differences between sequence and random learning in DLPFC-M1 projections. In sequence learning, visual signals rapidly reach and are processed by prefrontal regions. With sufficient training trials, DLPFC becomes further activated and sends facilitatory signals to motor cortex, resulting in significantly enhanced DLPFC-M1 connectivity at the 10 ms interval post-learning (Figure 7A). At the 25 ms interval, paired-pulse MEPs significantly decreased after sequence learning (Figure 7C), showing opposite changes from baseline similar to those at 10 ms. This synchronous reversal demonstrates learning-induced effects on inter-regional connectivity, where memory traces produce unique, lasting representations that act on brain networks, generating plastic changes that subsequently alter reaction times (Zhang, Tang, Cha, Huang, & Liu, 2016). Previous fMRI studies have also reported enhanced resting-state functional connectivity between DLPFC and M1 after sequence learning (Steel et al., 2016).

Sequence learning is considered to occur through accumulated experience (Mayor-Dubois et al., 2016). Its primary distinction from random learning involves activating DLPFC to deploy spatial attention with conscious pattern detection to master regularities and complete memory and decision processes

(Yang & Wang, 2018). Neuroimaging studies confirm left DLPFC activation during motor learning (Toni et al., 1998). For sequence learning tasks, previous studies show that TMS pulses to DLPFC significantly enhance M1 excitability in button-press learning tasks, consistent with our 10 ms paired-pulse TMS results in the sequence learning group (Hasan et al., 2013; Lam et al., 2015). Willingham et al. (2002) proposed that DLPFC may transmit a function parallel to conscious attention direction—conscious selective responding. This conscious selection before target appearance reduces reaction times in sequence learning conditions.

Notably, although participants were not informed about the sequence beforehand, all sequence learning group participants successfully recalled the entire 12-item sequence after the motor learning task. Thus, left DLPFC activation relates to sequence search processes through observation of cue occurrences. DLPFC participates in sequence search as an implicit process within goal-directed pattern search, activating dorsolateral prefrontal regions (Fatma, Thorsten, Christof, & John-Dylan, 2012). Correlation analyses between physiological and behavioral data showed significant relationships in the sequence learning group at both 10 ms and 25 ms intervals between reaction time differences (block 1 vs. block 10) and paired-pulse TMS differences (Figure 8A,C), indicating close relationships between DLPFC-M1 connectivity and learning improvement. This further demonstrates that left DLPFC activation levels relate to sequence learning ability.

4.3 Importance of Motor-Cognitive Networks in Motor Learning

We found no changes in single-pulse MEP input-output curves after approximately 20 minutes of either motor learning task (Figure 6), whereas paired-pulse MEP and input-output curves changed significantly in the sequence learning group (Figure 7A,C), indicating that sequence learning specifically affects DLPFC-M1 pathway excitability. Jueptner et al. (1997) reported similar findings in a neuroimaging study where participants trained on a similar sequence learning task using eight fingers. Comparison of motor excitability before and after training revealed no significant short-term, within-session plasticity changes during early training stages (Jueptner et al., 1997), a result supported by other studies (Grafton, Hazeltine, & Ivry, 1995; Stefan et al., 2006). These findings suggest that short-term motor learning activates relevant brain networks without specifically and significantly activating or increasing excitability in primary motor cortex.

Many studies have investigated inhibitory and facilitatory effects in primary motor cortex, with test stimulation applied to primary motor cortex and conditioning stimulation from ipsilateral cerebellum (Ugawa et al., 1991) or prefrontal regions (Cao et al., 2018; Civardi, Cantello, Asselman, & Rothwell, 2001). These pathways and inter-regional connectivities demonstrate that cortical information transmission and organization are global, requiring long-term working platforms across the entire brain rather than being confined to single functional regions. Specific neuronal populations with different physiological properties are distributed across each node of the cortical network, forming extremely complex connections with other neurons near and far (Oh et al., 2014). Previous studies primarily examined DLPFC-M1 connections and projections at rest without analyzing how motor learning affects connectivity between these two functional regions. By incorporating procedural motor learning tasks, our study further demonstrates that DLPFC-M1 connectivity significantly contributes to improved motor learning performance, highlighting the importance and causality of the DLPFC-M1 pathway in sequence motor learning. Understanding the differential roles of DLPFC-to-M1 projections in sequence versus random learning provides greater insight into the differentiation and cooperation of DLPFC functions. Investigating cooperative and network-based working modes among human brain functional regions remains an important topic for revealing everyday psychological activities.

5. Conclusion

This study combined the serial response time task with transcranial magnetic stimulation to investigate the role of left dorsolateral prefrontal cortex in procedural motor learning. Behavioral results demonstrated superior learning outcomes in the sequence learning group. Electrophysiological results showed no changes in M1 motor evoked potentials before versus after learning in either group. However, at optimal projection time points and appropriate stimulation intensities, DLPFC-M1 connectivity changed in the sequence learning group and correlated with learning performance, whereas no changes occurred in the random learning group. These findings indicate that enhanced connectivity between DLPFC and M1 may be an important mechanism underlying superior sequence learning performance, providing critical electrophysiological evidence for the role of DLPFC in motor learning.

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