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Exploring the Modulation of Motor Cortical Excitability by Duration of Transcranial Direct Current Stimulation

Master's Degree Programme in Digital Health and Life Sciences

Department of Computing, Faculty of Technology

Master of Science in Technology Thesis

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Abstract.

The motor cortex(MC) was important in human learning and memory, and the level of cortical excitability is a way of assessing its state. To non-invasively modulate cortical excitability, transcranial direct current stimulation (tDCS) is a widely employed technique. tDCS can be utilized to either enhance or inhibit motor MC excitability. However, in most studies, the optimal duration effect of tDCS on cortical excitability has been neglected. Additionally, there are no systematic analysis of tDCS and cortical excitability indexes. To bridge these gaps, we designed an experiment to systematically analyze the duration of tDCS and MC excitability it triggered.

We recruited 5 healthy, right-handed subjects. They don't have history of psychiatric disorders. Throughout the 30-minute tDCS stimulation at 1 mA, we continuously applied TMS to characterize the motor evoked potential.

Our experiment revealed the following: applying 1 mA tDCS to the participants for 30 minutes effectively increases cortical excitability, and this effect can last for at least 30 minutes after stimulation. We then analysed cortical excitability by measuring the motor-evoked potentials (MEPs) induced by TMS during the tDCS stimulation process. We found that the relationship between cortical excitability and tDCS duration does not appear to be linear. Instead, two peaks were observed at 2-5 minutes and 15-25 minutes, respectively. A decrease in cortical excitability was observed at 26-28 minutes. Finally, we analysed the response of different participants to tDCS and found that individuals vary in their response to tDCS. Additionally, the relationship between cortical excitability and tDCS duration is inconsistent across different individuals.

Keywords: Cortical excitability, Transcranial direct current stimulation, Stimulus duration, Neural mechanism.

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1 Introduction

1.1 Research Background

Cortical excitability is a crucial biomarker of the condition of the MC for the human brain. The biomarker measures the overall state of the synaptic population, which is mainly characterized by changes in neural circuits resulting from alterations in cortical plasticity. This phenomenon is the neurobiological basis of learning and memory. Neuromodulation techniques are often used to regulate brain connectivity or cortical excitability. [1]. Many neuromodulation techniques have been utilized to treat neurological disorders, including epilepsy, pain and other conditions [2]. Neuromodulation techniques are being increasingly utilized not only for the treatment of diseases but also for neurological disorders. Furthermore, ongoing research is actively exploring the potential of these techniques to modulate cortical excitability with the aim of enhancing motor training[1].

Transcranial magnetic stimulation (TMS) is a safe and environmentally friendly technique widely used for modulating brain function. It offers a unique avenue to explore the functionality of the cerebral cortex across various behaviours. By inducing changes in cortical plasticity, TMS allows us to explore the impact on cortical excitability. repetitive transcranial magnetic stimulation (rTMS) can change MC excitability and the modulatory outcomes of rTMS depend on the frequency used. Furthermore, the single-pulse TMS (SP-TMS) technique enables the measurement of changes in excitability within the MC. By stimulating specific regions of the MC that generate downward impulse potentials, TMS elicits MEP in the target muscles. MEP serve as a biomarker reflecting the MC excitability and are commonly employed by researchers to assess cortical excitability. Consequently, the utilization of TMS technology offers a valuable opportunity to study alterations in motor learning and MC excitability. [3].

A wealth of studies has firmly established the capability of non-invasive transcranial electrical brain stimulation (NTBS) techniques to effectively modulate the MC excitability. These techniques encompass various stimulation methods, including rTMS, tDCS, and transcranial alternating current stimulation (tACS)[4] . Among the extensively researched methods for brain modulation, tDCS stands out as a prominent non-invasive technique. It has found widespread application in modulating brain function and investigating its potential effects. At present, tDCS devices use the direct current through two electrodes (tDCS) or more electrodes (HD-tDCS), for a certain duration (about 20 minutes) to promote or inhibit spontaneous neuronal activity. In recent years, several articles have focused on tDCS-related

techniques and applications. Among these techniques, tDCS stands out as it utilizes anodic modulation to target the resting neurofunctional network connections, particularly focusing on the primary motor cortex for stimulation. This approach has shown good results to treat diverse neurological disorders and has been instrumental in significantly enhancing human motor performance[5]–[8].

While numerous studies have provided evidence of tDCS improving MC excitability and modulating the effects of exercise or learning in humans, there still exists considerable uncertainty regarding the medical evidence supporting its ability to enhance cortical excitability and the underlying mechanisms involved. The uncertainty surrounding regarding this matter stems from the limited availability of clinical studies and the absence of a standardized paradigm across these studies. Each study uses different stimulation parameters and experimental methods, leading to a high variability in the effects of tDCS stimulation. [9]. Hence, it is imperative to carry out systematic studies to test the impact of tDCS parameters for cortical excitability. There are currently several studies demonstrating the relationship between tDCS duration or tDCS intensity. For example, in early experiments Nitsche found that cortical excitability showed a tendency to increase first with increasing stimulus duration from 0-13 min, and then the effect became longer. They found tDCS had enhance the cortical excitability when stimulus durations were 22 and 24 minutes. Nevertheless, when the stimulation duration was 26, 28, and 30min, a paradoxical effect was observed where tDCS inhibited cortical excitability instead of enhancing it[10]. Although the available studies show approximate response curves for tDCS, we also found some contradictory findings, such as the finding in Fricke's article that a 5-min stimulus duration boosted excitability more than a 10-min stimulus [11]. Therefore, there is a need to search the mechanism of tDCS on cortical excitability using more refined experiments.

Additionally, most current studies investigate the after-effects of tDCS, which involve measuring MC excitability after the end of tDCS. Although this method can verify the effectiveness of tDCS on MC excitability, it overlooks the online influence of tDCS. It is crucial to learn the changes in mc excitability during tDCS to better comprehend the regulatory mechanisms of tDCS online. For instance, Lauro utilized a TMS-EEG-TDCS combination to investigate the difference of cortical excitability before, during, and after transcranial direct current stimulation in the PPC area [12] (as shown in Figure 1-1). This approach enables the exploration of changes in MC excitability when during and after tDCS, facilitating further investigation of tDCS mechanisms.

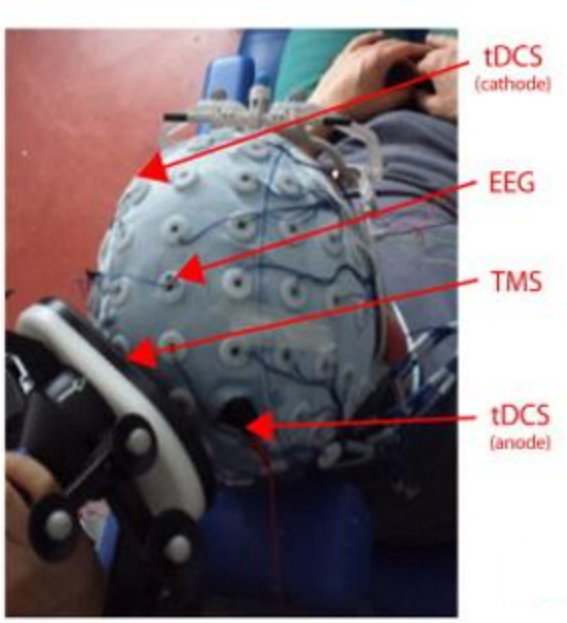

Figure 1-1: Image depicts the experimental arrangement employed to administer tDCS and TMS concurrently, with EEG recording conducted during tDCS. [12].

1.2 Research Purpose

Through an analysis of previous research, it has been determined that anodal tDCS is a reliable and effective method for augmenting MC excitability, but since there is no uniform standard between studies, there are some areas of improvement in the experimental design and contradictory findings have been found in different studies.

Based on previously investigated questions, we designed relevant experiments to further explore the stimulation effects of tDCS. Specifically, we aimed to investigate changes in motor cortex excitability under different tDCS durations by focusing on the MC and utilizing MEP as a measure of cortical excitability.

Although exist studies have used TMS-MEP to measure motor cortex excitability after tDCS, a more detailed study of tDCS duration and cortical excitability changes is lacking. Therefore, we aimed to search the relationship between tDCS duration and cortical excitability by measuring cortical excitability in real-time during tDCS stimulation. By doing so, we aimed to systematically find the relationship between tDCS duration and cortical excitability.

1.3 Research Significance

- For the first time, TMS, and tDCS, act simultaneously with human motor cortex. Based on the fact that few people have explored the impacts of tDCS during stimulation, we

used TMS to measure motor cortex excitability during tDCS, providing a guide to explore the online mechanisms during tDCS.

- A more complete protocol was designed to explore the link between tDCS duration and cortical excitability, refining the response curves between them in the same experiment.
- Whether or not the response to tDCS is specific to different subjects may be discovered during the study, which determines whether or not a fixed parameter should be used for subsequent treatment or modulation with tDCS.

1.4 Research Content and Overview

This study was undertaken to investigating the correlation between tDCS duration and cortical excitability, and to achieve several specific objectives. Firstly, the study sought to compare the correspondence between tDCS and cortical excitability at various stimulus durations. Additionally, the significance of the differences between different groups was observed using non-parametric analysis. Secondly, the study aimed to draw response curves for stimulus duration and MEP values by fitting curves to identify any uniform response pattern. Lastly, the response curves of different subjects were compared to determine whether the response curves of various individuals to tDCS were consistent.

Chapter 1 provides an introduction to the background and significance of our projects on cortical excitability regulation.

Chapter 2 provides an introduction to the background knowledge of the research content. It introduces the meaning of cortical excitability and the means of intrinsic and extrinsic modulation of cortical excitability. The chapter also describes the methods of measuring MC excitability, mainly by SP-TMS, PP-TMS, and EEG methods. It then describes the existing means of cortical excitability modulation, the various paradigms of TMS, TXCS, and their effects on cortical excitability under different parameters. We also discuss the data analysis methods used and present the results of the analysis. Finally, we examine the experimental results and potential mechanisms before highlighting the study's limitations and identifying future research directions.

Chapter 3 introduces the experiment. It first introduces the purpose of the study and the experimental paradigm. Then, it describes the exact procedure of the experiment, as well as the hardware and software used in the experiment. Finally, it discusses the data analysis methods and the results of the data analysis after the experiments.

Chapter 4 provides the summary and conclusion, which outlines the conclusions drawn from this project.

2 Fundamentals of Cortical Excitability Modulation

2.1 Cortical Excitability

The brain is composed of approximately 86 billion neurons, each serving a different function depending on the status of neural activity. Human brain operates on a closed-loop circuit, which primarily consists of two types of neurons: inhibitory and excitatory neurons. Excitatory neurons make up about 70-80% of cortical neurons, while cortical inhibitory interneurons make up only 20-30% of all cortical neurons [13] . The main manifestation of neuronal excitability is the modulation of synaptic plasticity. As humans and animals learn and remember, they adapt their behaviour to suit the changing environment. Synaptic plasticity refers to the capability of synapses in a cortical network to transmit information or the efficacy of neural connections, and is a critical aspect of information transfer between neurons[14] .

Changes in synaptic plasticity refer to alterations in the robustness of synaptic connections between pre- and post-synaptic neurons, and can either strengthen or weaken those connections. These alterations are fundamental for the optimal functioning of the nervous system. Short-term alterations in synaptic plasticity are linked to changes in behavioral states, while both short- and long-term modifications in synaptic strength can appear. Long-lasting modifications in synaptic plasticity are of paramount importance in the maturation of the developing nervous system as well as in the establishment of long-term memories. These persistent changes can be categorized into two distinct groups: Short-term synaptic plasticity [15] and Long-term synaptic plasticity, which includes both Long-term Depression (LTD) and Long-term Potentiation (LTP) [16].

Previous studies have consistently demonstrated that non-invasive brain stimulation techniques have the ability to modulate the excitability of the MC over an extended period. The characteristics of brain stimulation that lead to enduring changes in MC excitability align with the properties observed in LTP and LTD observed in animal models, specifically in recordings of single or multiple neurons in M1 slices. The sustained increase of cortical excitability inspired by brain stimulation techniques in human studies is referred to as LTP-like, while the sustained decrease of cortical excitability is referred to as LTD-like [17]. Therefore, in this study, we used changes in cortical excitability to reflect impacts of motor cortex plasticity.

2.1.1 Intrinsic Cortical Excitability Regulation

Cortical excitability is an important characteristic of brain activity that measures plasticity and neuronal excitability within the cerebral cortex. The functioning of higher functions in the cerebral cortex heavily relies on the neural systems consisting of intermediate excitatory and inhibitory neurons. Maintaining a delicate balance between enhancement and inhibition is vital for the optimal functioning of the cortex. Failure to balance excitability or inhibition can have a significant impact on the brain's homeostasis; for example, epilepsy is caused by cortical hyperexcitability [18] .

The brain circuits can be perturbed by external stimuli or by learning and memory processes, causing synaptic enhancement that can disrupt the stability between enhancement and inhibition in the brain. However, the brain is not significantly affected by the stimulus in the same way that it is affected by epilepsy. This is because the brain generates compensatory mechanisms to maintain the stability of excitability. Our brains are stimulated to produce changes in excitability, and compensatory mechanisms operate automatically to maintain balance. Research suggests that the brain employs a range of classical homeostatic negative feedback mechanisms to maintain homeostasis, such as global synaptic scaling and local synaptic scaling[19], [20] These homeostatic mechanisms allow neurons and cortical systems to monitor their level of activity and regulate their excitability to maintain this activity within a certain specific range. Collectively, these homeostatic mechanisms are known as homeostatic plasticity. In order to attain homeostatic plasticity, neurons must be capable of sensing specific aspects of "activity." When these measurements deviate from the desired target value, a compensatory force is triggered to regulate excitability and restore neuronal activity to a balanced state[21] .

Homeostatic plasticity consists mainly of synaptic homeostatic plasticity and intrinsic homeostatic plasticity. The synapse is the site of connection and transfer of information between neurons, and it is the structural basis for information transfer within the nervous system. Abnormalities in synaptic transmission are the most critical part of the brain, and Any abnormalities in the connection and transmission of neural signals can lead to modification in the functioning of the corresponding nervous system. The variability in the structure and function of synapses is recognized as synaptic plasticity. Synaptic plasticity is a fundamental process that holds great significance in various aspects of the nervous system, including its development, recovery following injury, and vital functions like learning and memory[22], [23] . Extensive research has highlighted the pivotal role of synaptic plasticity in cognition and the

various cognitive abilities exhibited by the brain. Therefore, the understanding of synaptic plasticity has become a current hot topic in neuroscience. The manifestations of synaptic plasticity in the nervous system vary, with Hebbian theory and homeostatic plasticity being the two most widely recognised. The two most prominent forms of Hebbian theory are LTP and LTD, which refer to the long-lasting functional potentiation and depression of synapses.

Studies have shown that LTP and the phenomenon of LTD have been recognised as the biological basis for learning and memory at the cellular level [24] [25]. The mechanism of regulation by Hebbian theory is that relevant pre-synaptic and post-synaptic communication will enhance the synaptic transmission efficacy, while irrelevant activity weakens them, a positive feedback process that causes a constant enhancement and inhibition of synaptic strength, which can lead to disruption of the nervous system. Therefore, in addition to Hebbian theory, we should also consider the homeostatic mechanism of the synapse, i.e. the homeostatic plasticity of the synapse. Unlike the positive feedback process of Hebbian theory, homeostatic plasticity involves the regulation of neural excitability and the maintenance of the stability of synaptic strength, which is necessary to maintain the stable of neural circuits and brain function.

Human brain undergoes numerous changes in its neural circuits when learning, remembering, or being disturbed. However, despite these changes, our brains remain in a stable state because they possess a homeostatic mechanism that regulates the production of ion channels or transmitters. Currently, there are various mechanisms of homeostatic regulation involving multiple molecular pathways. However, it is still difficult to clearly describe the process of homeostatic feedback [26] .

For instance, research has shown that during brain regulation, neurons have Ca ion-related receptors that detect changes in their firing rates. The neurons then modulate AMPA-type glutamate receptors to regulate neuronal excitability and inhibition[27] . Such like a neurofeedback loop that regulates synaptic strength. When neuronal activity wanes, somatic calcium levels decline, setting in motion the cascade of events known as synaptic scaling. This mechanism orchestrates the precise accumulation of neurotransmitters at the synapse, bolstering the strength of excitatory synaptic connections and subsequently elevating firing rates to reach their intended target levels. Similarly, when neuronal firing is enhanced, a rise in Ca ion concentration is induced. This activation leads to a downregulation of synaptic scaling, which regulates the accumulation of transmitters at the synapse. As a consequence of this reduction, excitatory synaptic strength diminishes while firing rates ascend to attain the desired target level. [28] .

Synaptic scaling stands out as one of the more comprehensible mechanisms underlying homeostatic plasticity, coming into play when a neural circuit undergoes disruption. This prompts a proportional adjustment, either reducing or augmenting the strength across all synapses by an appropriate magnitude, with the ultimate goal of reinstating firing rates to their baseline levels. The following diagram illustrates the neuromodulation mechanism for LTP: pre- and postsynaptic mutual firing, according to Hebbian theory, causes an enhancement of the synapse, and their association is increased. For unconstrained LTP, the enhancement of synaptic effects causes stronger firing, inducing a positive feedback synaptic firing process (as shown in Figure 2-1). The continued enhancement of strong synaptic firing eventually leads to abnormal firing. For the human brain, a homeostatic mechanism for synaptic plasticity changes exists. After detecting enhanced synaptic firing, the brain introduces synaptic scaling, which reduces the strength of the synaptic input by controlling the transmission of transmitters until the firing rate reaches a manageable level [19].

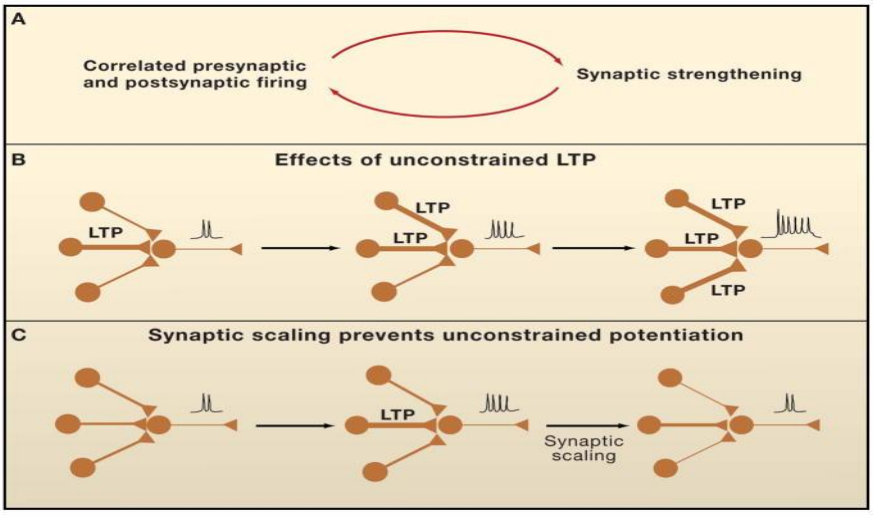

Figure 2-1: Mechanisms of Synaptic Plasticity [19].

2.1.2 Extrinsic Modulation of Cortical Excitability

Motor cortex excitability is a crucial biomarker of the condition for motor cortex of brain and characterizes overall state of the synaptic population [29]. Its primary manifestation stems from alterations within neural circuits, driven by fluctuations in cortical plasticity—a fundamental neurobiological process underpinning learning and memory. Currently, the study of functional plasticity in the motor cortex, such as the effects of sustained enhancement or reduction of MC excitability, has become one of the most interesting areas of cognitive neuroscience research [30].

Biomarkers of cortical excitability are now used in a variety of scenarios, including neurological disease monitoring and treatment, learning enhancement, and as important markers for exploring the brain. Hence, to foster human learning and facilitate the recovery of brain-injured patients, it is imperative to acquire a profound comprehension of motor cortex excitability and the intricate mechanisms governing plasticity. This comprehension lays the groundwork for the formulation of efficacious strategies and interventions. The field of cognitive neuroscience has witnessed rapid advancements in research techniques, leading to the emergence of numerous NIBS methods capable of effectively modulating cortical plasticity. These techniques include theta burst stimulation (TBS), tDCS, transcranial random noise stimulation (tRNS), tACS, rTMS, and paired associative stimulation (PAS) [31]. Primarily targeting glutamatergic and γ-aminobutyric acid-mediated (GABAergic) circuits, these techniques induce alterations in synaptic efficacy, either augmenting (LTP) or diminishing (LTD) synaptic connectivity and efficacy. Ultimately, they serve as powerful tools for brain modulation and manipulation. [32].

2.2 Measurement of Motor Cortical Excitability

Motor cortical excitability refers to the collective state of the synapses in the motor cortex. One way to induce neuronal firing is through an electrical field evoked by cranial magnetic stimulation. When the area of neuronal discharge is in the motor cortex, we can observe action potentials in peripheral muscles, which are known as MEP. To assess cortical excitability, various TMS paradigms have been developed, including MT, CSP, and SICI, in addition to MEP amplitude. Moreover, some studies have used EEG to explore cortical excitability and investigate EEG biomarkers of cortical excitability by correlating EEG features with TMS biomarkers like MEPs. Subsequently, we will delve into a more comprehensive description of these measures in the following section.

2.2.1 Introduction to TMS

The advent of TMS dates back to 1985, when Barker et al. pioneered the successful implementation of external magnetic stimulation on the cerebral cortex. TMS stimulation is a painless and contactless form of stimulation that uses a specific coil that is linked to the terminals of a big capacitor by a switch that, when closed, discharges the capacitor, causing a current of several thousand amperes to pass through the coil and provide TMS to the brain [33]

. Within the intricate framework of the human brain, the axons of nerve cells boast the highest concentration of ion channels, readily responsive to the influence of mild magnetic field stimulation. When one of these neuronal extensions is activated, an action potential propels along the core of the individual's axon, traversing until it reaches the presynaptic axon terminal. It is at this juncture that neurotransmitters are released, initiating the communication with the postsynaptic neuron. TMS pulses can activate a range of neurons, generating feedforward and feedback loops of excitation and inhibition. This property of TMS makes it particularly suitable for studies of cortical excitability for human brain. TMS can detect some neurological dysfunction caused by impaired cortical excitability or changes in the interaction between cortical and subcortical structures [34] . Currently, TMS is widely used in clinical neurophysiology, neuroscience, and psychiatry, mainly in applied research, but clinical applications are also increasingly being studied. In addition to its therapeutic use, low-frequency TMS techniques have been used to detect cortical excitability, which can be well activated, and cortical excitability can be manifested through the discernible presence of active electromyographic (EMG) signals.

2.2.2 Single-Pulse Transcranial Magnetic Stimulation (SP-TMS)

Within the realm of TMS, this method entails the administration of an electric current via an induction coil positioned on the scalp. This generates a magnetic field that permeates the skull, inducing eddy currents parallel to the coil within the corresponding cortex. This generates magnetic field of force that pass through the skull and produce eddy currents parallel to the coil in the corresponding cortex. For traditional measures of cortical excitability, we typically use the MEP value produced by a single pulse of TMS. This produces various biomarkers of cortical nerve function, such as the MEP amplitude, motor threshold (MT), and cortical silent period (CSP) [33], as shown in the Figure 2-2.

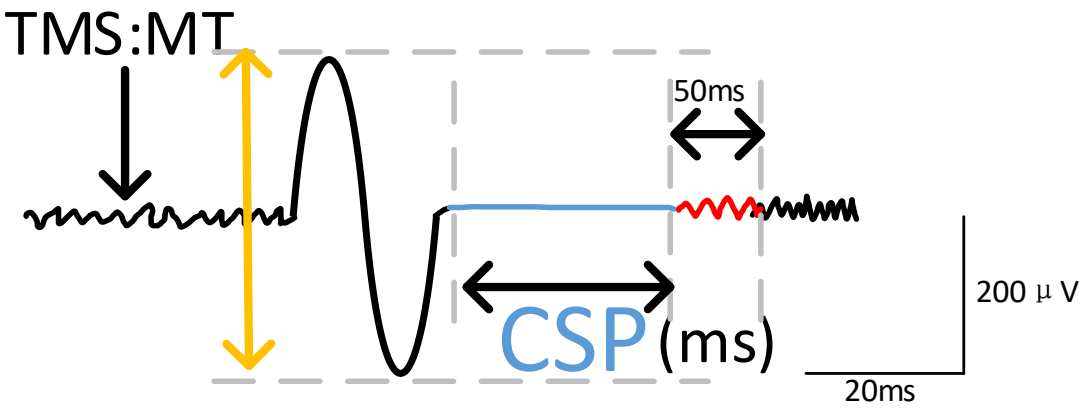

Figure 2-2: Typical profile of the biomarker for the single-pulse TMS stimulation.

The Figure 2-2 illustrates how applying TMS to the motor cortex can produce action potentials in peripheral muscles. For instance, when TMS is implemented to the MC responsible for hand movements, we can measure the electromyography (EMG) response evoked in the first dorsal interosseous (FDI) muscle. This particular measurement is commonly known as a MEP. By analyzing the recorded MEP, several standard indicators can be derived, including MEP amplitude, MEP latency, MT, and CSP. The areas of the hand generally used for analysis include the abductor digiti minimi (ADM), the adductor pollicis brevis (APB), and the FDI, as shown in the Figure 2-3. Eliciting MEPs in leg muscles presents a greater challenge compared to hand muscles, requiring relatively higher stimulation intensity. This primarily stems from the M1-LEG's deeper location within the brain and the elongated pathway that the stimulation must traverse from the brain to the leg, as compared to the hand. The anterior tibialis muscle is typically the preferred site for stimulation when targeting the leg. [35].

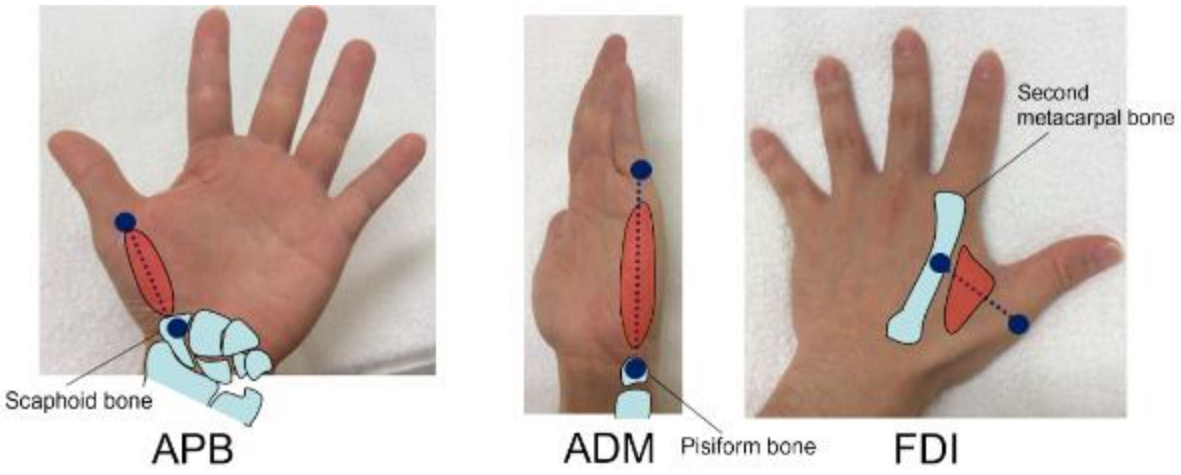

Figure 2-3: Location of the muscles at each target [36].

2.2.2.1 MEP Amplitude

When the cerebral cortex is stimulated with a magnetic pulse, it generates a magnetic field that elicits direct (D) waves in the spinal cord, which are subsequently succeeded by a sequence of indirect (I) waves. These waves represent the response of cortex and spinal cord to TMS pulse. The cumulative effect of these direct (D) and indirect (I) waves manifests as the MEP observed in our target muscle during measurement[37], [38], as shown in the Figure 2-4. The amplitude of the MEP was the most direct measure reflecting the response elicited by the stimulation. The MEP amplitude wave exhibits a relationship with the stimulus intensity that follows a sigmoidal pattern. This pattern is representative of the dose-response curve, which describes the correlation between the intensity of the stimulus and the corresponding amplitude of the MEP wave[39], [40]. Therefore, MEP amplitude is largely related to MC and spinal cord excitability during TMS time. Many clinical studies have used MEP amplitude as the gold standard for cortical excitability measurement.

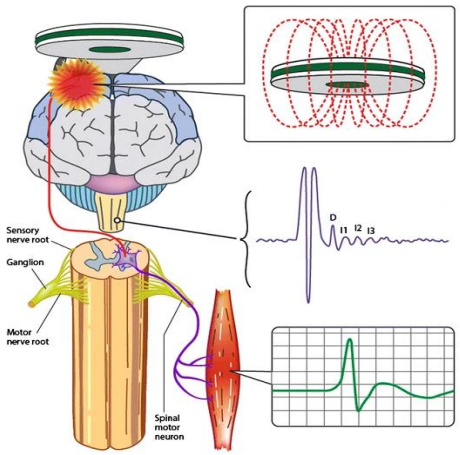

Figure 2-4: Showing the D-wave and I-wave produced on the corticospinal tract, as well as the MEP produced in the muscle [37].

2.2.2.2 Cortical Silent Period (CSP)

The CSP is a brief period of reduced electromyographic activity that occurs during an isometric contraction of the muscle. This quiet phase is induced by TMS pulses applied to the M1 on the opposite side. The duration of the CSP is influenced by the intensity of the stimulation, and this relationship can be effectively illustrated by a sigmoidal curve[41]. The duration of the CSP serves as an informative reflection of cortical inhibition and can be readily discerned through a single pulse of TMS. Therefore, the CSP serves as a valuable biomarker for evaluating neural activity in the M1 of the brain. It possesses the capacity to monitor alterations in cortical neural activity, making it particularly useful for assessing individuals with

brain disorders. For instance, in patients with epilepsy, the excitability of the cerebral cortex can serve as an additional diagnostic indicator and aid in evaluating the effectiveness of epilepsy treatments. SP-TMS parameters show a trend of reduced resting motor threshold (rMT) and prolonged duration of the CSP in individuals with generalized epilepsy. These findings suggest alterations in cortical excitability and inhibitory processes in the MC of patients with this condition[42] .

2.2.2.3 Motor Threshold(MT)

The MT provides insights into the excitability of corticomotoneurons and is suggested by the International Federation of Clinical Neurophysiology as a means of evaluation. The rMT is defined as the minimal stimulus intensity necessary to evoke a small MEP in the targeted muscle. Typically, the amplitude of the MEP is around 50 μV when the individual is at rest and increases to approximately 200 μV during muscle contraction. The rMT is determined by observing the response in the target muscle during 50% of the trial attempts[43] . If the measured stimulus intensity is lower than MT, it indicates that cortical neurons can induce the corresponding MEP value by using a weaker stimulus. This, in turn, suggests that the cortical neurons are in an inherently more excitable state. Additionally, MT may also serve as an biomarker of cortical excitability [34] .

MT is typically divided into two types: rMT and aMT (active Motor Threshold). The rMT is typically measured when the muscle is in a state of rest and is commonly employed to gauge cortical excitability. However, for populations affected by movement disorders, where eliciting a MEP from the muscle may be challenging or unattainable, a more sensitive alternative known as the aMT is introduced. The aMT is specifically designed to determine the optimal intensity of TMS output for evaluating and treating a range of movement disorders.

2.2.3 Paired-Pulse Transcranial Magnetic Stimulation (PP-TMS)

The MC, located within the brain, assumes a crucial role in controlling and coordinating motor functions by intricately interacting with other neural networks throughout the brain. The delicate balance and interplay within the cortical circuits ultimately dictate the ultimate outcome and expression of the motor cortex's function[44]. PP-TMS serves as a valuable tool for monitoring the status of cortical loops. PP-TMS, depending on the particular experimental setup, enables the evaluation of intra-cortical inhibition and facilitation. This technique involves the application of a conditional stimulus (CS) followed by a test stimulus (TS) to examine the

state of cortical circuits. By comparing the amplitudes of the MEP generated by the TS and CS with those elicited by the TS alone, can deepen understanding of cortical circuit function. Various paradigms of paired-pulse TMS have been explored to examine different intracortical circuits, including short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), short-interval intracortical facilitation (SICF), intracortical facilitation (ICF), short-latency interhemispheric inhibition (SIHI), long-latency interhemispheric inhibition (LIHI), short-latency afferent inhibition (SAI), and long-latency afferent inhibition (LAI)[33], as shown in the Figure 2-5. In Rossini's and Paulus's review, the pharmacologic effects and general principles of these biomarkers have been discussed [33], [45].

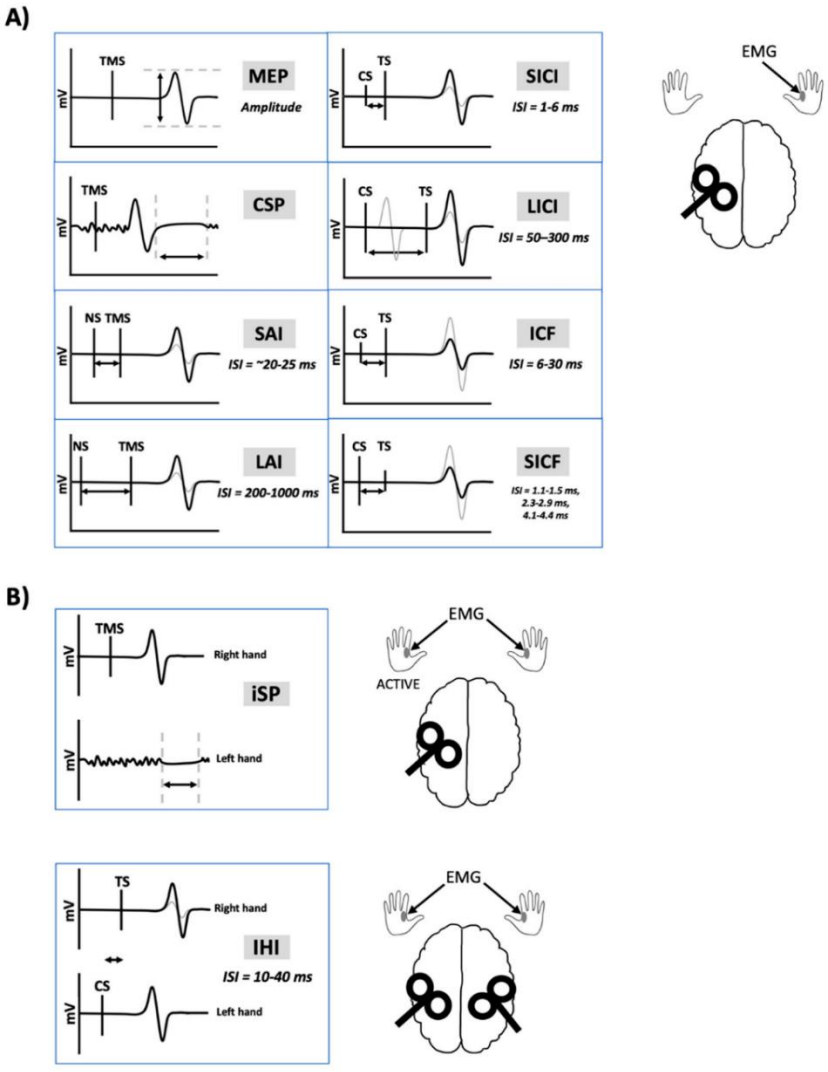

Figure 2-5: Showing EMG responses to the TMS stimulation protocols. [46].

The EMG signal resulting from a single pulse of TMS is represented by the black line. In contrast, the grey line depicts the EMG signal obtained when the neural stimulus (NS) and TMS are combined or when the CS and the TS have a cumulative effect.

Both PP-TMS stimuli exert their effects on the motor cortex. The phenomenon known as SAI occurs when electrical peripheral nerves are stimulated with intervals (ISI) of 20-25 ms prior to the implement of the TMS pulse. Similarly, LAI occurs when the peripheral nerves are stimulated with intervals of 200-1000 ms before the TMS pulse.

When the conditioned stimulus is set as a subthreshold stimulus and the test stimulus is a suprathreshold stimulus, an intriguing phenomenon known as SICI emerges. This phenomenon occurs when the time interval between the two stimuli was of 1-6 ms, leading to the inhibition of the TS. On the other hand, when the time interval between the two stimuli extends to 6-30 ms, a contrasting phenomenon called intracortical facilitation (ICF) occurs, leading to an enhancement of the test stimulus effect. SICI, known as short-interval intracortical inhibition, represents one of the predominant neural circuits within the motor cortex. Despite not eliciting MEPs in the targeted muscle, the conditioned stimulus effectively activates inhibitory interneurons within the motor cortex. This activation is facilitated by the unique characteristics of inhibitory interneurons, such as their low threshold, short latency, and brief delay. Initially, a subthreshold stimulus is given which is small enough not to cause corticospinal neurons to fire and so no MEP occurs, however this stimulus activates inhibitory interneurons in M1. The neural mechanism of intracortical facilitation of ICF is not known, and some studies suggest that it may be mediated by glutamatergic transmitters[47] .

When the conditioned stimulus exceeds the threshold level and the time interval between the CS and the TS falls within the range of 50-300 ms, the action-evoked potentials elicited by the test stimulus undergo suppression, resulting in a phenomenon known as LICI. LICI primarily operates by activating the cortical inhibitory circuit, which subsequently diminishes the magnitude of the MEP amplitude.

SICF is observed when the suprathreshold conditioned stimulus is delivered approximately prior to the subthreshold test stimulus, the interval is 1.1-1.5 ms, 2.3-2.9 ms, or 4.1-4.4 ms. This temporal configuration leads to an enhance effect in MEP amplitude during SICF, potentially attributed to the cumulative effect of various I wave, leading to an overall larger MEP value.

Additionally, when TMS is administered to the left MC while the corresponding muscle on the left side is engaged in isometric contractions, it triggers a transient cessation of voluntary contraction, referred to as the iSP. Notably, when both hemispheres of the brain are simultaneously stimulated, novel cortical circuits come into play. Hemispheric inhibition (IHI) is assessed by giving a suprathreshold TS to the left MC prior to a suprathreshold CS sent to the right MC, resulting in MEP inhibition. SIHI manifests at an approximate interval of 10 ms

between the stimuli, whereas LIHI is observed around 40 ms between the stimuli. These time intervals represent the interstimulus intervals (ISI) at which the inhibitory effects on MEP occur. This inhibition may be produced by the intercerebral excitatory pathway that reaches local inhibitory circuits in target M1 via the corpus callosum and synapses, resulting in MEP values being suppressed when TMS is used contralaterally[48] .

2.2.4 EEG Biomarker

EEG is a non-invasive technique used to measure the state of the brain. By recording the potentials generated by currents in and around neurons through electrodes applied to the scalp, EEG provides a comprehensive measure of the activity of the brain's nervous system. EEG can be traced back so that the brain can be analysed at the site of the lesion, greatly reducing the influence of subjective factors on the diseases being studied.

EEG plays a crucial clinical role in diagnosing a wide range of conditions, including epilepsy, sleep disorders, assessing the depth of anesthesia, coma, encephalopathy, and even determining brain death. Additionally, EEG provides a tool for studying brain activity in the field of experimental psychology and is also widely used as a neuroimaging method in computational neuroscience. Therefore, searching for EEG biomarkers of cortical excitability is crucial for the diagnosis of neurological disorders.

EEG metrics can be divided into two types: one measures MEP values before and after TMS stimulation, and EEG values in the resting state of the brain after TMS stimulation. By performing correlation analysis of EEG and MEP, we can determine which EEG metrics correlate with cortical excitability [29], [49]–[56] . The EEG metric can be the average power or phase of a band. If we find a strong correlation between an EEG metric and cortical excitability, then that metric may be an important biomarker.

Sauseng utilized this approach and made a fascinating finding. Among healthy individuals, a strong negative correlation was identified between the power of alpha frequency and the phases of alpha frequency that preceded TMS stimulation[51]. Many researchers have also used open-loop validation methods to find new EEG metrics and verify their reliability. This is done by using EEG band features to induce TMS stimulation, selecting the EEG features of interest through previous exploration, and then triggering TMS stimulation when the EEG features reach a specific threshold to detect cortical excitability. The features associated with cortical excitability were explored by analyzing the consistency of TMS-induced cortical excitability at the same features and thresholds [57]–[63] .

Prior research has established a potential connection between cortical excitability and the power of the alpha band. To further examine this association, some studies employ a real-time EEG-triggered TMS system. In a similar vein, Madsen conducted a study employing this methodology to explore the impact of the mu band on corticospinal excitability. Their findings indicated a modest reduction in MEP wave amplitude with higher mu power, whereas longer ISI intervals yielded increase in MEP amplitude[57] .

Another EEG indicator is TMS-EEG (TEP), which is similar to MEP values. TEP, also known as Transcranial Evoked Potentials, refers to the alteration in EEG potential resulting from the application of TMS to the brain. This phenomenon generates a complex waveform that aligns with the timing of the TMS stimulation pulse. It is more clearly characterized relative to resting EEG and is composed of waves with different peaks and troughs of latency, typically lasting 300ms or more. TEPs serve as a valuable measure to capture alterations in cortical circuit excitability and inhibition consequent to stimulation. They offer a quantifiable physiological marker to assess changes in cortical excitability. For example, negative and positive potentials are generated during specific latencies: N15 (Approximately 15 ms after stimulation, a negative deflection in the EEG waveform can be observed at the vertex), P30, N45, P55, N100, P180, and N280 [64] , as shown in the Figure 2-6. In addition to potential metrics in the time domain, metrics currently studied in TMS-EEG include frequency band power, time-frequency domain metrics, and phase [65], [66] .

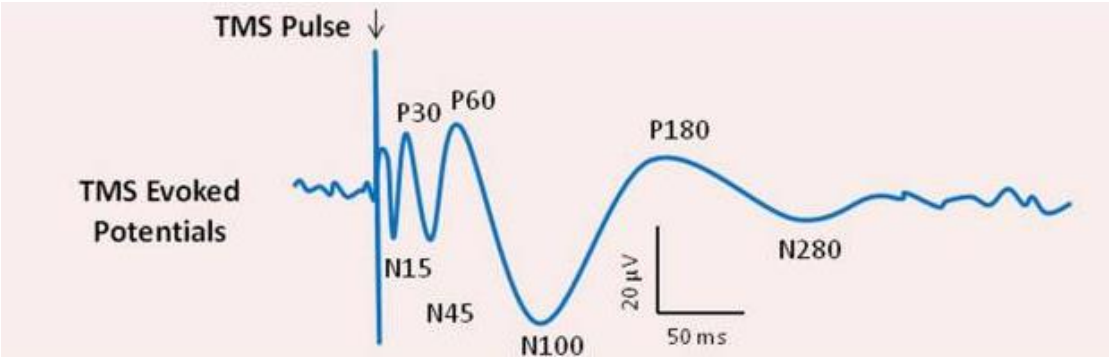

Figure 2-6: Showing the TEP waveform [64].

2.3 SP-TMS Parameter Setting

The gold standard for measuring cortical excitability is MEP data evoked by SP-TMS. However, there is no standardized parameter setting for SP-TMS, so we need to choose the appropriate parameters. The parameters for SP-TMS mainly include the TMS stimulation interval and the TMS stimulation intensity.

2.3.1 TMS Stimulus Interval Inter-Pulse Intervals

For TMS stimulation, the stimulation frequency is an important parameter that modulates cortical excitability differently. For instance, rTMS at 5 Hz (120% rMT) increases cortical excitability, likely due to a reduction in γ-aminobutyric acid (GABA)-mediated inhibition of intracortical inhibitory circuits that leads to a facilitatory effect [67]. In contrast, low-frequency rTMS (115% rMT) at around 1 Hz inhibits cortical excitability, possibly due to a facilitation of GABA-B transmission that increases GABA mediators, leading to an inhibitory effect [68], [69]. On the other hand, rTMS stimulation at less than 1 Hz (120% rMT) has no effect on the magnitude of MEP values [70]. Chen's experiments demonstrated that one hour of TMS stimulation with 105% rMT at 0.1 Hz did not cause changes in cortical excitability [71]. However, Aya Sato found an increase in MEP values after six minutes of TMS stimulation at 120% rMT and 0.1 Hz [72]. This increase in MEP size may be associated with the duration and intensity of stimulation. Another study similarly demonstrated a positive relationship between stimulation interval and MEP amplitude. Specifically, TMS stimulation with an inter-pulse interval (IPI) of 10s resulted in larger MEP compared to TMS stimulation with an IPI of 4 seconds (at 120% rMT). This effect may be attributed to the recovery time required by neurons to regain normal levels of haemoglobin following stimulation-induced reductions[73]. Therefore, during the experiment, to experimentally exclude TMS modulation of the motor cortex, we also need to extend the IPI length as much as possible to reduce the effect of IPI on TMS stimulation and to keep the IPI consistent from experiment to experiment.

2.3.2 TMS Stimulation Intensity

Stimulus intensity is a major factor influencing the effect of TMS stimulation, and the correlation between MEP and TMS stimulus intensity can be modeled by a sigmoid curve. MEP amplitude increases more slowly at first as TMS stimulus intensity increases, then increases linearly at 120%-140%rMT stimulus intensity, and eventually saturates. In many studies, experiments assess MEP magnitude at only a single intensity. In such instances, it is customary to calibrate the intensity of TMS to 120% rMT of the individual. This setting ensures that the experiment targets the ascending linear phase of stimulus-response curve, leading to a more dependable MEP magnitude[74]. Moreover, research in the literature has shown that the stimulus intensity that is used more often is 120% of the rMT stimulus intensity for TMS stimulation [75].

2.4 Methods for Modulating Motor Cortical Excitability

Transcranial magnetic stimulation (TMS) and transcranial current stimulation (TCS) are indispensable techniques in the fields of neuroscience. These methods offer valuable understanding into the importance of distinct brain structures and patterns of neuronal activity in relation to a wide range of brain functions. There are already several neuromodulation paradigms based on TMS and TCS that can affect brain activity in various methods, such like increasing or decreasing cortical excitability. The Table 2-1 illustrates the different paradigms and their effects. In this section, we will outline the principles of common neuromodulation and how the various parameters of each paradigm can change cortical excitability.

Table 2-1: Summarizing the Methods of NTBS.

Method	Excitatory mode	Inhibitory Mode
tDCS[76]	Anodal	cathodal
tACS[77]	20 Hz,140 Hz	15 Hz
tRNS[78]	100-640Hz	unknow
TI[79]	20Hz,70Hz	unknow
rTMS[80]	High frequency,>5hz	Low frequency, 0.5-1.0 Hz
PAS[81]	ISI=10ms	ISI=25ms
TBS[82]	Intermittent	continuous

2.4.1 Transcranial Direct Current Stimulation(tDCS)

tDCS is a widely used non-invasive, painless, and well-tolerated method in clinical practice. Neuromodulation techniques, such as tDCS, have gained widespread use in regulating neural activity within the motor cortex. This electrical stimulation is administered using two electrodes that penetrate the layers of skin, skull, meninges, and cerebrospinal fluid, ultimately reaching the cortex and subcortical tissues. As a result, the membrane's permeability to ions and larger molecules undergoes alterations[76]. In addition to conventional tDCS, a new technique called High-Definition Transcranial Direct Current Stimulation (HD-tDCS) is now available. HD-tDCS uses a 4x1 ring electrode as the return electrode. Compared to tDCS, HD-tDCS provides a more sustained focus and stimulation effect. The enhanced stimulation effect may be related to the location of the current focus, as the large electric field of HD-tDCS is concentrated below the stimulating electrode, allowing for more focused current to induce changes in cortical plasticity [83], as shown in the Figure 2-7.

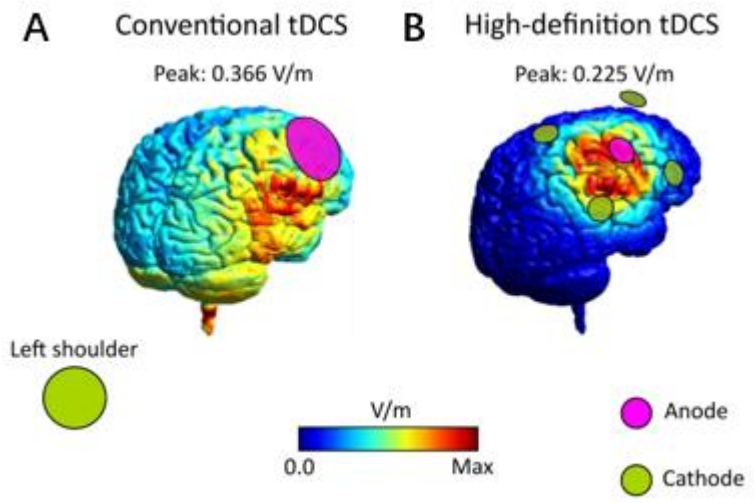

Figure 2-7: The image A shows the electric field of conventional tDCS. The image B shows the electric field of HD-tDCS. As depicted, HD-tDCS produces a significantly more focused electric field compared to conventional tDCS, where the current tends to disperse more widely. [84]

2.4.2 Transcranial Alternating Current Stimulation(tACS)

tACS is a brain stimulation method that uses an oscillating current to modulate excitability and activity. Transcranial alternating current stimulation (tACS) has the capacity to either enhance or inhibit cortical excitability, with its effectiveness contingent upon the frequency and amplitude of the stimulation signal[77]. Feurra's experiments showed that cortical excitability was enhanced only during 20 Hz tACS (75% increase in the mean amplitude of the MEP) after using tACS at frequencies of 5, 10, 20, and 40 Hz. In Moliadze's experiments, 140 Hz tACS stimulation (0.2mA-1mA) was used, and the results showed that only 0.4mA stimulus intensity had a more pronounced inhibitory effect, 1mA tACS had a facilitative effect on cortical excitability, while no significant changes were found for 0.2mA, 0.6mA, and 0.8mA stimulus intensities [85].

In addition to conventional tACS, which uses sine waves of different frequencies, there are now other tACS paradigms such as oscillatory tDCS (otDCS) formed by combining tDCS and tACS, as shown in the Figure 2-8. The AC signal does not necessarily need to be sinusoidal and can also be square, as shown in the following diagram [77], [86].

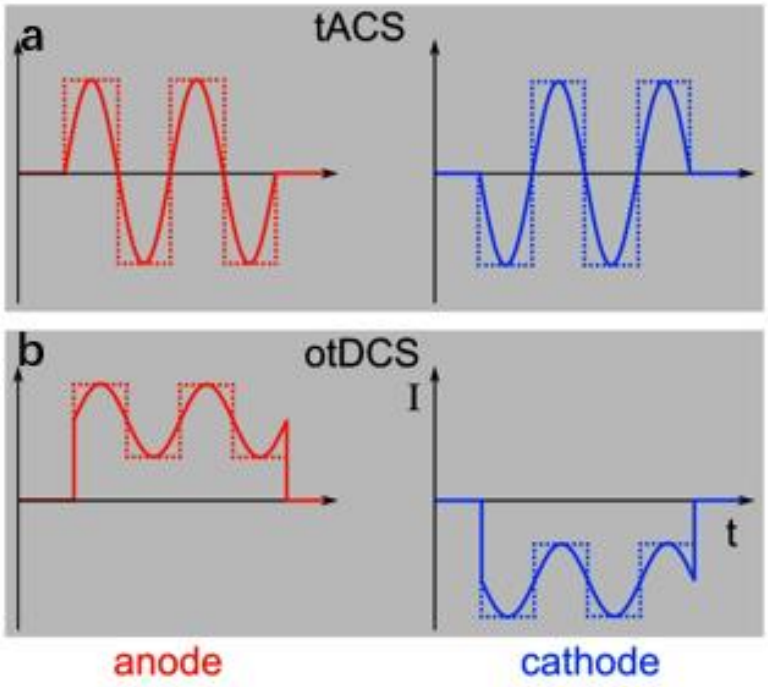

Figure 2-8: Showing the various stimulation profile for tACS. (a) Stimulation profile for conventional tACS. (b) oscillatory tDCS (otDCS), in which AC is superimposed on DC. The AC can also be sinusoidal or rectangular [77].

2.4.3 Transcranial Random Noise Stimulation (tRNS)

tRNS is a new technique of transcranial electrical stimulation which was first experimented on human subjects in 2008[78]. It entails the administration of low-intensity alternating current (AC) at randomized frequencies via a serial of electrodes placed on the scalp, and has demonstrated effectiveness in augmenting cortical excitability[78], [87] .

In conventional tRNS (transcranial random noise stimulation), the signal spectrum spans from 0 to 640 Hz, with each sample generating a random current level. The random values adhere to a normal distribution, characterized by a bell-shaped density function. The stimulus intensity is commonly set at 1 mA, resulting in approximately 99% of the amplitude values falling within the range of +500 μA and -500 μA, as shown in Figure 2-9.

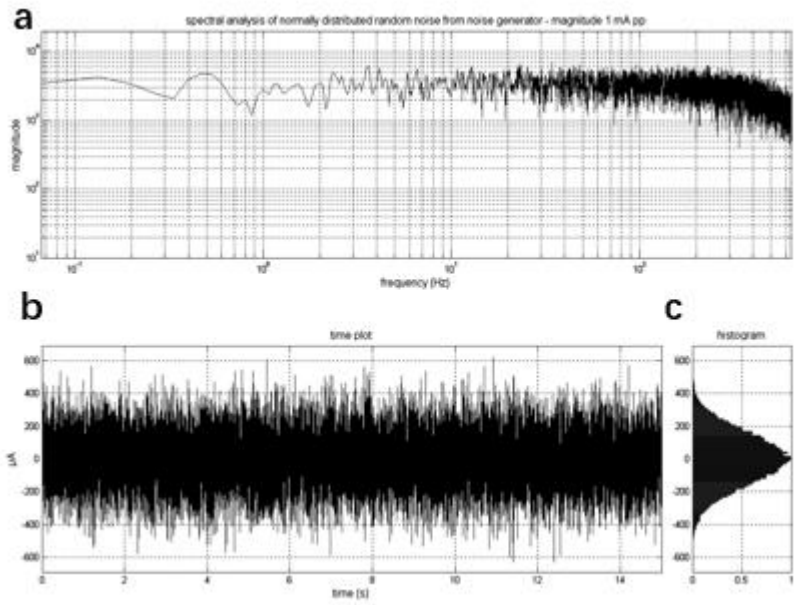

Figure 2-9:Various representations of tRNS signal. (a) Frequency distribution, (b) time plot, and (c) histogram of the tRNS signal [78].

The increase in motor evoked potential (MEP) due to tRNS was found to be more pronounced than for tDCS, despite the same current intensity [88]. Additionally, in terms of subject perception, it was found in Terney's experiment that 78 out of 80 subjects did not notice the use of tRNS compared to the mild painful skin irritation of tDCS, suggesting that tRNS appears to have better potential for blinding applications [78].

2.4.4 Repetitive Transcranial Magnetic Stimulation(rTMS)

The TMS technique involves utilizing a coil to produce a strong magnetic field, which is then directed towards the head. As the magnetic field through the scalp, it induces an electric current within the brain cortex, effectively stimulating the brain. Unlike the single TMS stimulation used for testing, which usually lasts only a few dozen or a few hundred TMS stimulations with a very low frequency (0.1Hz - 0.3Hz), more than 600 TMS pulses are typically delivered continuously for rTMS (as shown in the Figure 2-10), inducing LTP and LTD and change the excitability of brain[51], [89]. In general, applying low-frequency rTMS stimulation (below 1Hz) tends to decrease cortical excitability, while high-frequency rTMS stimulation (5Hz to 20Hz) typically enhances cortical excitability[33].

2.4.5 Theta Burst Stimulation(TBS)

TBS is a novel technique that utilizes rTMS to modulate cortical excitability through different combinations of cerebral cortex stimulation. The core of TBS involves continuous TMS pulse stimulation using pulse packs, each spaced at 5 Hz, with each pulse containing three 50 Hz TMS pulses. The presence or absence of interruptions classifies TBS as either intermittent (iTBS) or continuous (cTBS). Early TBS experiments it was shown that iTBS (the TBS sequence, consisting block of 2 seconds of stimulation, and the block repeated every 10 seconds, is performed for 20 cycles, resulting in a cumulative delivery of 600 pulses) increased cortical excitability for at least 20 minutes; cTBS (a continuous TBS sequence of 40 s and 600 pulses) reduced cortical excitability with 60 min[82] .The potential advantages of TBS over rTMS are the following - shorter stimulation duration (40 seconds for 600 cTBS pulses compared to 10 minutes for 600 1 Hz rTMS pulses).

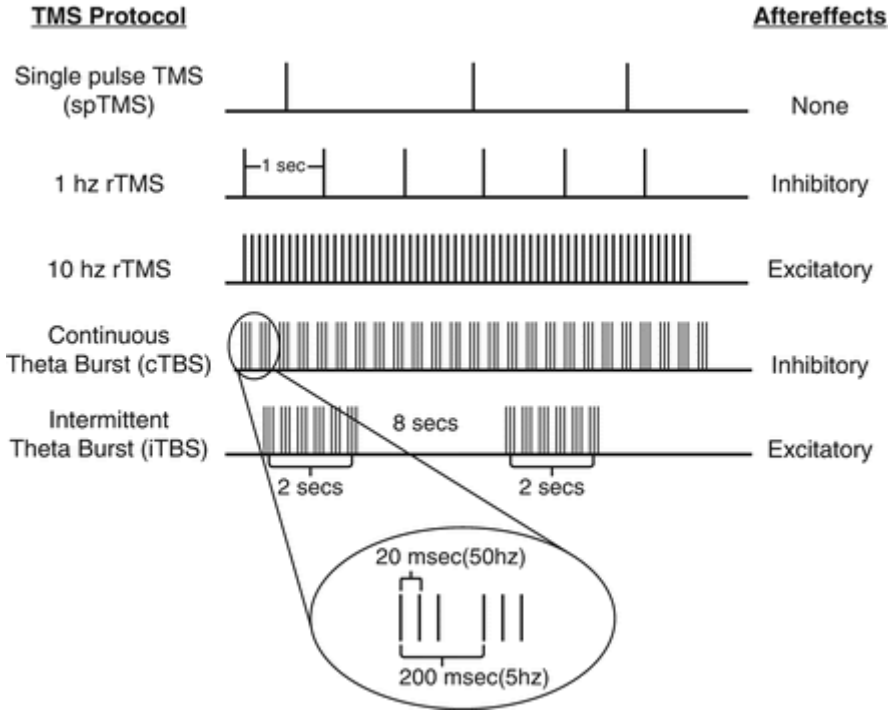

Figure 2-10: Paradigms of TBS and rTMS [82].

2.4.6 Paired Associative Stimulation (PAS)

PAS is a stimulation paradigm that combines electrical stimulation of the nerve in the hand with TMS of the corresponding hand motor area in the opposite motor cortex. The synergistic interplay of this distinctive combination of stimuli results in the induction of plastic changes within the motor cortex of the human brain. These changes result in significant functional

alterations and adaptations within the motor system, facilitating enhanced motor performance and neuroplasticity, as shown in Figure 2-11 [90] . The time interval between peripheral and central stimulation determines the outcome of motor cortical plasticity changes. Weise's study found that a similar cortical excitability-promoting effect to PAS25 was found when the inter-stimulus interval was 21.5 ms (PAS21.5) [91].

In Schabrun's experiments, the effects of moderate inter-stimulus intervals (50 ms, 65 ms, 80 ms) and long negative inter-stimulus intervals (-100 ms, -200 ms, -250 ms, -300 ms, -350 ms, -450 ms) on PAS effects were specifically investigated. The results showed that only long negative intervals (-250-450 ms) produced an inhibitory effect on cortical excitability when PAS was used [92].

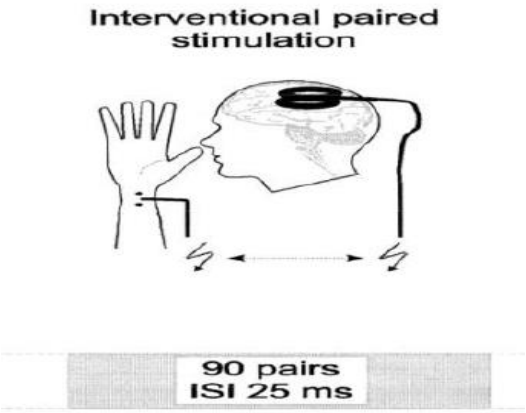

Figure 2-11: Paradigms of TBS [90].

2.4.7 Temporally Interfering Stimulation

Temporal Interference (TI) stimulation, pioneered by Grossman in 2017, is a technique that transmits two high-frequency electric fields of slightly different frequencies in the kHz range to the brain[93]. The two oscillating electric fields interact to create an amplitude-modulated electric field, as shown in Figure 2-12. TI, or transcranial electrical stimulation, offers several advantages over other methods, such as targeted spatial stimulation and non-invasiveness. Unlike the broad areas covered by tDCS, TI stimulation can provide more precise and focused stimulation, reaching deeper brain positons. Numerous studies have demonstrated the efficacy of TI stimulation in humans. In a notable study conducted by Ma, the left M1 in healthy individuals was targeted for TI stimulation. Two tasks were designed to evaluate motor function. In the random reaction time task (RTT), 70 Hz TI stimulation resulted in improved reaction times (RT) for the participants. Similarly, in the sequential reaction time task (SRTT),

20 Hz TI stimulation enhance motor learning. These findings result the potential of TI electrical stimulation in enhancing motor activity[79].

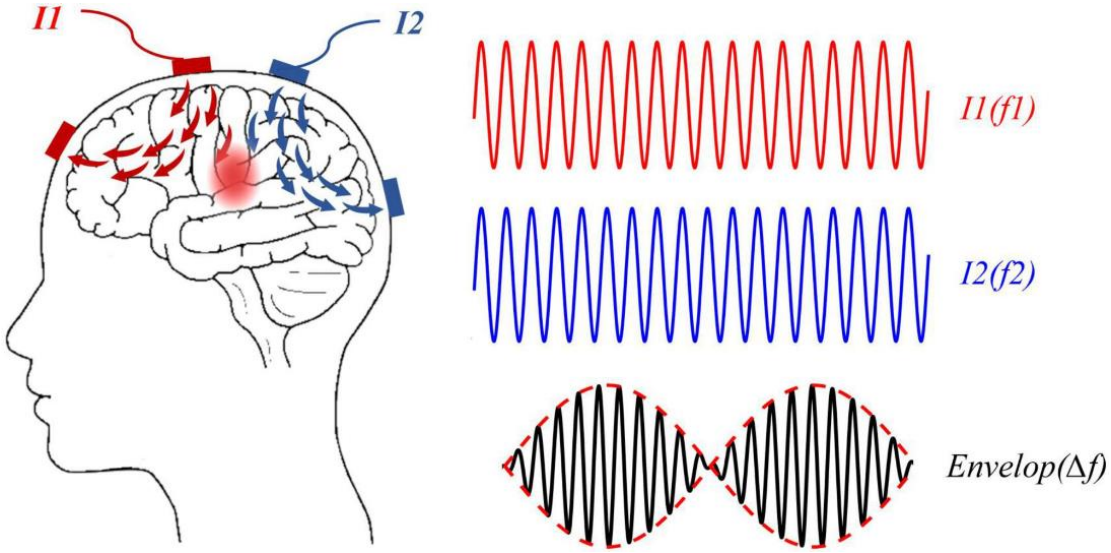

Figure 2-12: TI stimulation applying two high-frequency sinusoidal currents *I1* and *I2* to the human brain. Stimulation is administered at frequencies *f1* and *f2*, the difference between their frequencies is *Δf* – which is chosen to be small. The combined stimulation produces a modulated waveform and the envelope of the modulated waveform.

2.5 Effect of Stimulation Parameters on Cortical Excitability

tDCS is currently the most widely used form of neuromodulation, offering promising opportunities for altering neurological, cognitive, and behavioral functions. One of its primary effects is to increase motor cortex excitability, with the extent of this increase dependent on factors such as stimulus polarity, duration, and intensity. While anodal tDCS is thought to increase cortical excitability and cathodal tDCS to suppress it, the precise modulatory effects of stimulus duration, intensity, and location on motor cortex excitability are still not fully comprehended[94].

2.5.1 Stimulation Intensity

The impact of tDCS intensity on MC excitability lacks a clear consensus in the literature. Initial studies by Nitsche indicated that affect anodal tDCS became more pronounced as the intensity increased within the range of 0.2 to 1 mA, while keeping the stimulation duration constant at 5 minutes[76]. However, the Jamil system studied affects anodal stimulation at current intensities of four intensity with a stimulation duration of 15 minutes, and they did not observe significant changes in excitability at different intensities, with the same promotion of cortical excitability observed at each intensity level [9].

2.5.2 Stimulation Duration

Stimulation duration plays a crucial role in tDCS, as it determines the extent of cortical excitability enhancement or inhibition. Nevertheless, our understanding of how stimulation duration influences cortical excitability remains limited, as there is no systematic study that has investigated this factor alone. Thus, it becomes necessary to examine the findings from various studies to evaluate the correlation between the duration of stimulation and cortical excitability.

Through review of the literature shows that stimulation duration has a proportional effect on cortical excitability enhancement at durations ranging from 0 to 11 minutes. For instance, Nitsche found that longer durations (up to 5 minutes) of tDCS at 1 mA led to a stronger effect on cortical excitability [76]. In a subsequent study, Nitsche explored the effect of cortical excitability and tDCS duration and found that durations between 5 to 13 minutes resulted in similar cortical excitability enhancement, but longer durations led to more sustained enhancement [95] .

In contrast, Monte-Silva's experiments found that tDCS had a boost in cortical excitability at 13 minutes but an inhibitory effect on cortical excitability at 26 minutes [96]. Therefore, the relationship between stimulation duration and cortical excitability begins to be such that, as the duration of stimulation increases, cortical excitability first increases before reaching a plateau and eventually leading to inhibition. However, some studies have reported contradictory results, such as Fricke's study, which found that a 5-minute stimulation duration led to more cortical excitability enhancement than a 10-minute duration [11]. Thus, the relationship between stimulation duration and cortical excitability enhancement remains to be thoroughly investigated.

2.5.3 Stimulation Position

The placement of electrodes plays a critical role in tDCS, as it directly influences the electrical current in the brain. Pioneering research conducted by Nitsche revealed that altering electrode positions could impact the magnitude of MEP induced by TMS using identical tDCS parameters[76]. Subsequent modelling studies have shown that different electrode positions cause the electric field of tDCS to exhibit significant differences in the brain, and even a small movement of the electrodes can cause significant changes in the electric field of tDCS [84], [97]. Therefore, ensuring consistent electrode placement is crucial in tDCS. Traditional tDCS devices typically involve positioning one electrode on the desired stimulation area and the other on a separate location on the head or neck. This configuration allows the electrical current to

pass through and modulate the neuronal activity in the targeted stimulation area. Nevertheless, accurately pinpointing the exact target area for stimulation based on the selected electrode site can present challenges because of variations in head size and shape among subjects. To address this issue, several methods have been developed, including the international 10-20 electrode placement system, physiology-based placement techniques such as TMS-MEP, or neuronavigation systems utilizing MRI guidance. These approaches help improve the accuracy and reliability of electrode placement in tDCS procedures[98].

2.6 Stimulus Parameter Setting and Safety of tDCS

2.6.1 Electrode Position

Conventional tDCS involves two different electrodes and battery-powered devices, along with control software to set the output of the stimulation type. The electrodes are divided into cathode and anode, and commonly used stimulation electrodes have an area of 20-35 cm^2. In this study, since we aim to stimulate the motor cortex, various current modeling approaches can be used in two paradigms. If electrical stimulation is performed using sponge electrodes, the motor cortex can be stimulated by placing the electrodes mainly at C3 above M1 and in the contralateral orbit, as demonstrated in Nitsche's study [76] . For more precise and targeted stimulation, the stimulating electrode can be positioned directly in the MC region responsible for the right hand's movements, as determined by TMS. This approach ensures that the desired muscle group is specifically targeted for stimulation, allowing for a more accurate and effective intervention[9]. If one wishes to stimulate the motor cortex using HD-tDCS, there are also a number of optimal sites for stimulation, as shown by several studies [84], [99]–[101]. For convenience, a 4x1 circular montage based on the conventional 10-20 system can be used, with the anode electrode putted on C3 and the cathode electrode on FC1, FC5, CP1, CP5.

2.6.2 Current Intensity

The current study mostly used tDCS current strengths in the range of 1-2 mA. Ho tested tDCS stimulation impact for MC excitability using electrodes of 1.0 mA or 2.0 mA and an area of 16 cm^2 or 35 cm^2, respectively. However, they did not find significant differences in cortical excitability when comparing the effects of 1.0 mA and 2.0 mA currents[102]. Batsikadze et al. explore impact of tDCS on cortical excitability(2.0 mA). They administered tDCS stimulation with 2.0 mA intensity, using anodal and cathodal stimulation on the left M1 area of healthy participants. Two additional control groups received 1.0 mA cathodal stimulation and sham

tDCS. The study findings unveiled that the application of cathodal 1.0 mA stimulation resulted in a decrease of cortical excitability[5]. Thus, the relationship between tDCS current strength and effect remains unclear and requires further exploration. To avoid too low a stimulus intensity resulting in no modulation of the brain and also to ensure the safety of the stimulus, we propose using a tDCS stimulus of 1 mA.

2.6.3 Stimulation Duration

Currently, there is no strict limit to the duration of tDCS stimulation, but 30 min is generally considered the maximum stimulation time, and the stimulation current applied to humans should be less than 3 mA[103]. Additionally, the current intensity should be slowly increased and decreased during tDCS treatment to avoid causing discomfort to the patient, and a slow increase and decrease time of 15s is typically used. The objective of this study was to examine the impact of stimulation duration on MC excitability, requiring the implementation of extended periods of tDCS stimulation. We aimed to examine how different durations of tDCS stimulation would influence cortical excitability. Therefore, we propose using 30 min of tDCS stimulation to investigate the complete response curve.

2.6.4 Safety and Side Effects

Current research consistently demonstrates that tDCS is a safe and dependable way of brain stimulation, characterized by minimal side effects. Over the course of nearly 60 years of clinical and human studies, conventional tDCS protocols (lasting no more than 40 minutes, using currents of no more than 4 mA, and delivering no more than 7.2 coulombs) have been used on over 1,000 subjects in more than 33,200 stimulation sessions, with no reports of serious or irreversible damage [104] .

In this study, we aim to implement a tDCS stimulation protocol that strictly adheres to established safety guidelines. Our proposed approach involves the use of a 1 mA current for tDCS stimulation, administered over a duration of 30 minutes. Furthermore, we will be targeting the traditional motor cortex with the contralateral orbit as the stimulation site, ensuring an additional layer of safety for our participants.

2.7 Summary

In this chapter we discuss the existing research on motor excitability modulation. The meaning of cortical excitability, methods of measuring cortical excitability and common means

of modulating cortical excitability are discussed. The tDCS affect cortical excitability are then explored for different parameters. Although tDCS stimulus duration affect cortical excitability has been studied by many people, it has been divided into two, which allows us to explore the relationship between tDCS on cortical excitability at different durations through several different experiments, but not to find a more consistent pattern, which may be the result of inconsistent experimental conditions. So, the best-case would be to examine all the ways in which tDCS duration affects cortical excitability states under the same experiment, so that the modulatory effect of tDCS on MC excitability at different stimulus durations can be more easily compared. Finally, the safety of tDCS was explored. From the current studies, it appears that tDCS is very safe if experimented within a safe range, and no studies have found irreversible damage to the brain caused by tDCS.

3 Assessing the Effects of tDCS on Cortical Excitability

3.1 Goals

For our study, the target area for randomized interventions using tDCS and sham stimulation was the M1-FDI on the left side of the brain. TMS-MEP data were collected before and after the stimulation, each lasting 3 minutes and conducted at an intensity of 120% of rMT. In addition, TMS-MEP data were collected during tDCS.

The main purpose of the study was to alterations in MC excitability during tDCS stimulation. Through the analysis of MEP data, the researchers aimed to provide insights into the effect of tDCS on MC excitability within the M1 region.

3.2 Participants

Five healthy subjects from Fudan University (all male, age 26 ± 2.83) were recruited for this study(One subject withdrew from the experiment for his own reasons), and the inclusion criteria for the subjects are presented in the table below. Before the commencement of the experiment, all participants were provided with detailed information about the study's objectives and procedures. They were given the opportunity to clarify any concerns before voluntarily providing their informed consent by signing a consent form. The whole experiment has been approved by the Ethics Committee of Fudan University.

To ensure reliable data, subjects were instructed to avoid strenuous exercise, sleep deprivation, and consumption of substances such as coffee, alcohol, and tea that can induce neurological changes for 24 hours before each test. Additionally, they were instructed not to consume any such substances for at least 4 hours prior to testing.

Subject inclusion criteria:

1. Healthy school students or social workers.
2. Able to sign an informed consent form on their own.
3. Age 18-40 years old.

Subject exclusion criteria:

1. Have epilepsy or have had convulsions or seizures.
2. Has had severe (i.e. unconscious) head trauma.
3. Those who are pregnant or may become pregnant.
4. Metal (except titanium) in the brain/skull.
5. has a cochlear implant.

6. Those with implantable neurostimulators (e.g. DBS, epidural/subdural, VNS)
7. Anyone with a pacemaker or intracardiac catheter or metal in their body.
8. Those with drug infusion devices.
9. Those who have undergone surgical procedures on the spinal cord.
10. Any other brain injury or mental illness related to the brain, etc.

3.3 Experimental Procedure

The experimental procedure is illustrated in its entirety in Figure 3-1 and is summarized as follows:

- Search for hotspots: The corresponding brain regions is searched for hotspots of the TMS-FDI area, and the resting action threshold rMT values.
- Baseline acquisition: subjects were given 30 SP-TMS stimuli and MEP data were collected at the corresponding moment, with an interval of 5s between each TMS and the stimulus intensity was 120% rMT.
- Intervention: after the baseline MEP data were collected, subjects were randomised be given tDCS or sham stimulation for 30 min, and SP-TMS was also performed continuously while stimulating and recording the corresponding EMG data MEP (TMS intensity: 120% rMT; TMS ISI: 5s).
- Post-effect acquisition: 30 times TMS at 0 min, 10 min, 20min and 30 min after stimulation, and measure the corresponding MEP values.

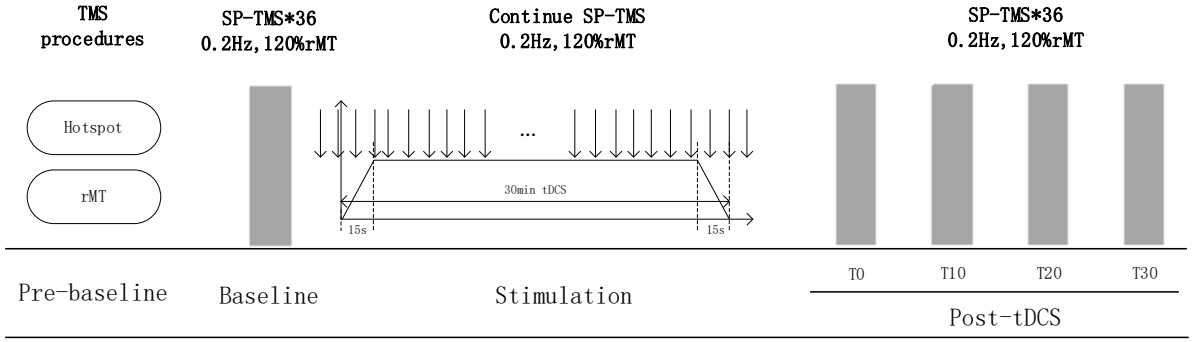

Figure 3-1: Experiment process for investigate the relationship between the duration of tDCS stimulation and cortical excitability

3.4 Experimental Protocol for Real and Sham Stimulation

3.4.1 Real tDCS Stimulation

In this experimental tDCS stimulation protocol, we employed conventional tDCS stimulation. The active electrode was positioned over the MC representational field of the right FDI, determined through a TMS mapping experiment. The reference electrode was putted on the right frontopolar cortex, situated above the eyebrow. The tDCS intensity was 1 mA, and the stimulation duration lasted for 30 minutes.

3.4.2 Sham Stimulation

The sham stimulation protocol closely resembled the actual tDCS stimulation protocol, with a few notable differences. The duration of the sham stimulation was set to 1 minute, and the timing of the stimulation differed as well. Specifically, the sham stimulation occurred half a minute before the initiation of the actual stimulation and one minute before its conclusion. Additionally, the sham stimulation incorporated a fade-in period of 15 seconds and a fade-out period of 15 seconds.

3.5 Experimental Hardware and Software

3.5.1 Wireless EMG acquisition system

The equipment used for EMG (electromyography) acquisition in this study was the wireless surface EMG test system MiniWave, manufactured by Cometa Systems in Italy. The hardware and software included in this system consisted of the EMG sensor miniX, as well as EMG and MotionTools_8.7.6 software, as shown in Figure 3-2. The system offers two options for data storage: local storage or wireless transfer to the software. The EMG sampling rate was 2000 Hz, and system endurance was up to 10 hours.

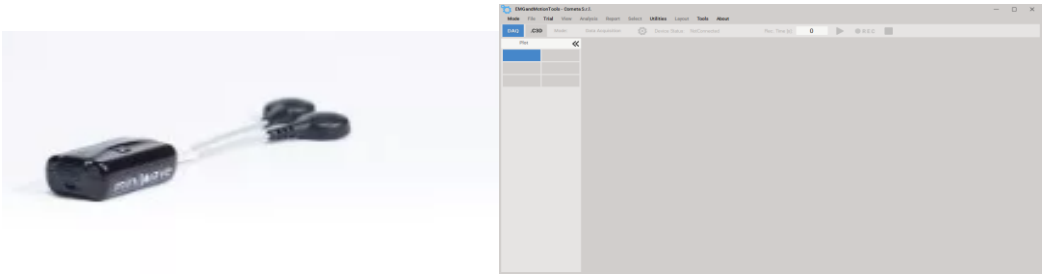

Figure 3-2: Sensor and acquisition interface. (a) The recording electrodes and (b) The software acquisition interface.

3.5.2 Transcranial magnetic stimulator

The TMS device uses the Magstim Rapid2 system from Magstim UK. The Magstim Rapid2 system is a magnetic stimulator that uses short pulses of electromagnetic energy to introduce small currents into the neuromuscular tissues, thereby providing non-invasive stimulation of the neuromuscular tissues. The experimental apparatus consisted of the Rapid^2 UI device, the main unit and the power supply unit, as shown in Figure 3-3. The stimulation coil is connected to the coil output socket of the mainframe before the experiment and the stimulation parameters are set by adjusting the Rapid^2 UI device. The mainframe outputs the signal to the stimulation coil according to the parameters set by the Rapid^2 UI. The Magstim Rapid2 stimulator has a stimulation frequency range of 0-100 Hz with a resolution of 0.1 Hz.

Figure 3-3: The TMS system (Magstim-Rapid2).

3.5.3 Transcranial electrical stimulator

Using Neuracle's wireless electrical neurostimulation system Neustim from China, the stimulation mode is multiple stimulation modes including tDCS, tACS and tRNS, sham stimulation mode, as shown in Figure 3-4. And other parameters are as follows:

- DC current intensity range: 0±5000μA;
- AC intensity range: 0-10mA (peak-to-peak);

- Stimulation frequency range 1-1000HZ, stimulation frequency resolution 1hz;
- Number of leads: 2/4/8, up to 32 channels electrode type:
- High precision focusing electrode, sponge electrode;
- Control command transmission method: wireless transmission.

Figure 3-4: The TES system (Neustim).

3.6 Experimental Procedure

3.6.1 Subjects Check

Confirm that the subject has not stayed up late or exercised strenuously for 24 hours prior to the test, and has not consumed or taken nerve-forming beverages or drugs such as coffee or alcohol for 6 hours prior to the test, and complete the corresponding informed consent form.

3.6.2 Data Collection Process

3.6.2.1 Skin Treatment

Before the experiment, the skin needs to be prepared. First, apply an appropriate amount of scrub onto the surface of the muscle skin in the areas of APB, FDI, and ADM. Rub the scrub onto the skin (resulting in a slight redness). After the treatment is completed, wipe off the scrub using a tissue, and cleanse the skin with an alcohol-soaked cotton ball.

3.6.2.2 FDI Position Determination and Electrode Fixation

The subject placed the hand palm upwards, allowing the hand muscles to relax on a table or chair, and then installed the positive and negative EMG electrodes at the corresponding points and then secured them with medical tape, as shown in Figure 3-5.

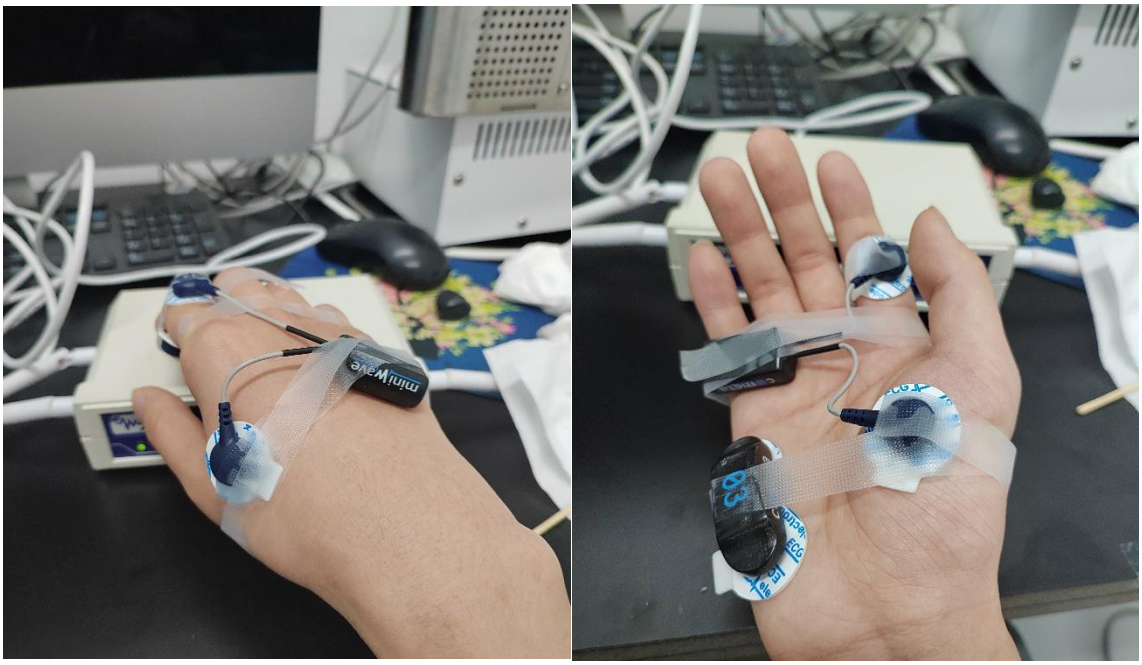

Figure 3-5 Diagram of the connection of the hand and electrodes

3.6.2.3 Electromyographic Acquisition Testing

After the electrodes have been fitted, the EMG acquisition software is opened and the EMG signal is observed on the software without force. Have the subject relax the muscle and confirm that the baseline EMG amplitude is less than 20μV. If the baseline signal is consistently greater than 20μV, reprocess the EMG block acquisition surface and the subject's skin until the relax EMG amplitude is less than 20μV.

3.6.2.4 Formal Experimental Data Collection

After starting the experiment, TMS stimulation will be administered to the subject at different experimental stages and the corresponding EMG data will be recorded. During the acquisition, the subject should attentively monitor the EMG values of the muscle., keeping the muscle in a relaxed state, i.e., EMG amplitude less than 20uV, as shown in the Figure 3-6.

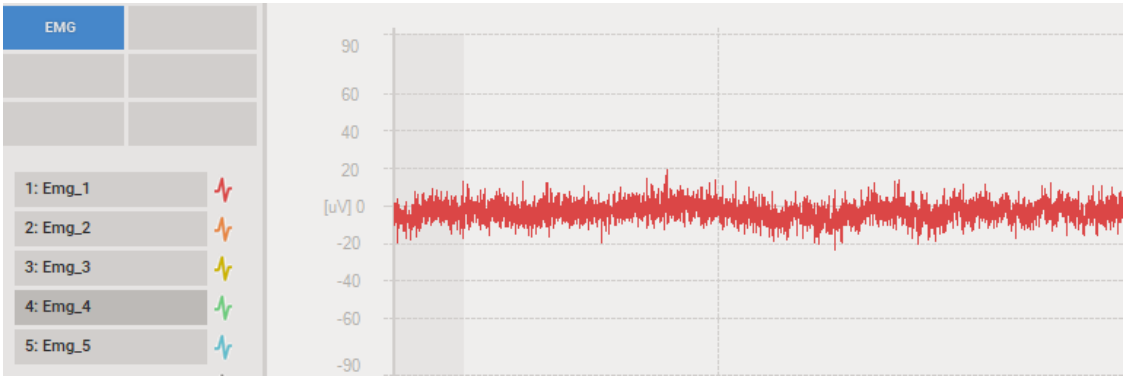

Figure 3-6: A sample of EMG signal in relaxed state.

3.6.3 Electrical Stimulation Process

3.6.3.1 Determine the Location of the "hot spot"

Once the EMG electrodes were attached, the subject took a seated position in a chair, placing their arm on the table with the palm facing upward. The stimulator's "8" coil was connected and positioned on the left M1-FDI area. The coil was placed tangentially on the skull, at a 45° angle to the midsagittal line. The stimulation process began at an intensity of 40% of the maximum stimulator output (MSO) and an interstimulus interval (ISI) of 0.2 Hz. The coil was then moved in different directions to locate the optimal point that elicited the largest MEP response. Once identified, the stimulate parameter was reduced until 5 out of 10 pulses produced a MEP amplitude greater than 50μV. This specific stimulus intensity was then recorded as the rMT, and the corresponding scalp location was marked as the "hot spot" using a pen.

3.6.3.2 Record the "hot spot" Coordinates

To measure the "hot spot," mark it with a marker and place the coordinates of the soft ruler at point CZ. The horizontal axis should be the line connecting the ear screens, and the vertical axis should be the line connecting the brow and the occipital ridge. Record the coordinates of the "hot spot" based on the vertical and horizontal axes.

3.6.3.3 Electrode Settings for Electrical Stimulation

Once the "hot spot" of the M1-FDI was found, preparations for electrical stimulation were initiated. The tDCS was administered using a pair of sponge electrodes. The anode electrode, saturated with saline solution, was positioned over the target M1-FDI, while the cathode electrode was putted on the right supraorbital region, specifically Fp2[105] . The impedance is

then checked using TXCS software and if the impedance is higher than 10kΩ, the headband holding the electrodes in place is adjusted to allow more contact with the head or the sponge electrodes are filled with saline.

3.6.3.4 Start Stimulating

To control the stimulation process, the TXCS software is used. After configuring the stimulation protocol, tDCS is initiated by clicking on the "Start Stimulation" button. A constant current of 1mA is then implemented for 30 minutes, with a fade in/fade out period of 15 seconds, as shown in Figure 3-7. For sham tDCS, the electrode position and stimulation parameters are same. However, in this case, the stimulator is switched off after 15 seconds.

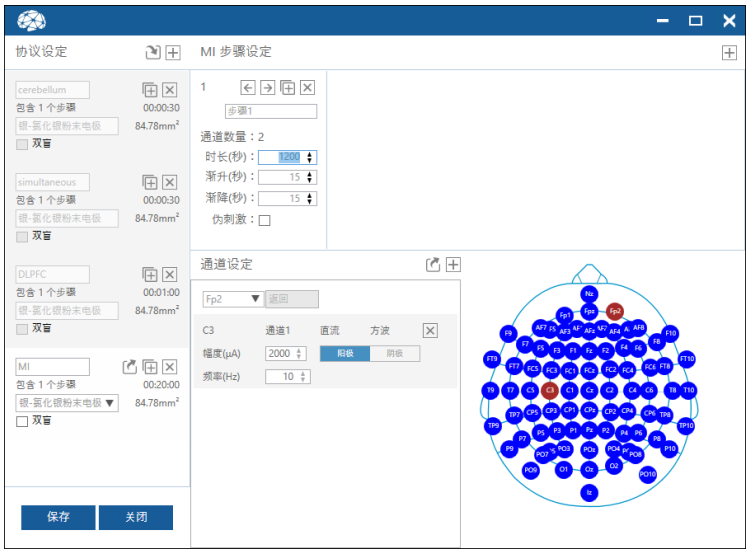

Figure 3-7: Settings for stimulation protocol.

3.6.3.5 Experimental Data Recording

In addition to the recording of EMG signals, information on the subject's discomfort, experimental interruptions, and average impedance are also recorded.

3.7 Data Processing

The data were imported from Comate software, and algorithms were used to extract the MEP values from the signals, as shown in Figure 3-8. The biological indicators used in this study were the peak-to-peak values of MEP. Our TMS-MEP signal appeared once every 5 seconds, and the amplitude of TMS-MEP was much larger than the resting state EMG values.

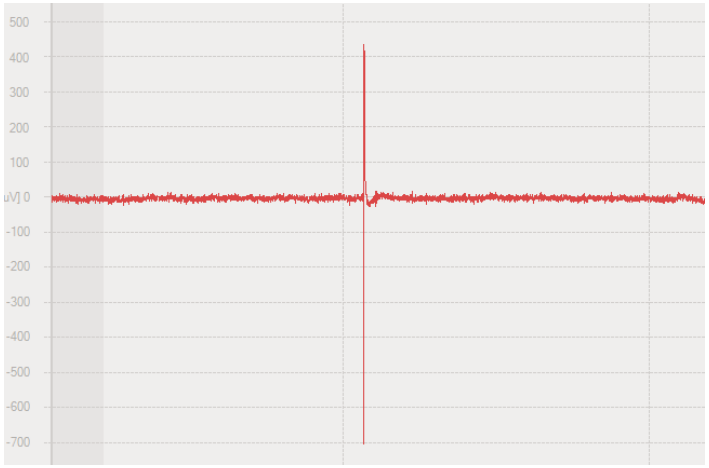

Figure 3-8: Raw MEP signal.

To analyze the MEP signal, we imported the EMG signal into MATLAB R2022b. Utilizing the properties identified in the preceding analysis, we initially segmented the data into 5-second intervals. Subsequently, we identified the highest and lowest values within each 5-second segment from the EMG signal. By computing the disparity between these highest and lowest values, we obtained the MEP amplitude (peak-to-peak of signal), as shown in the Figure 3-9.

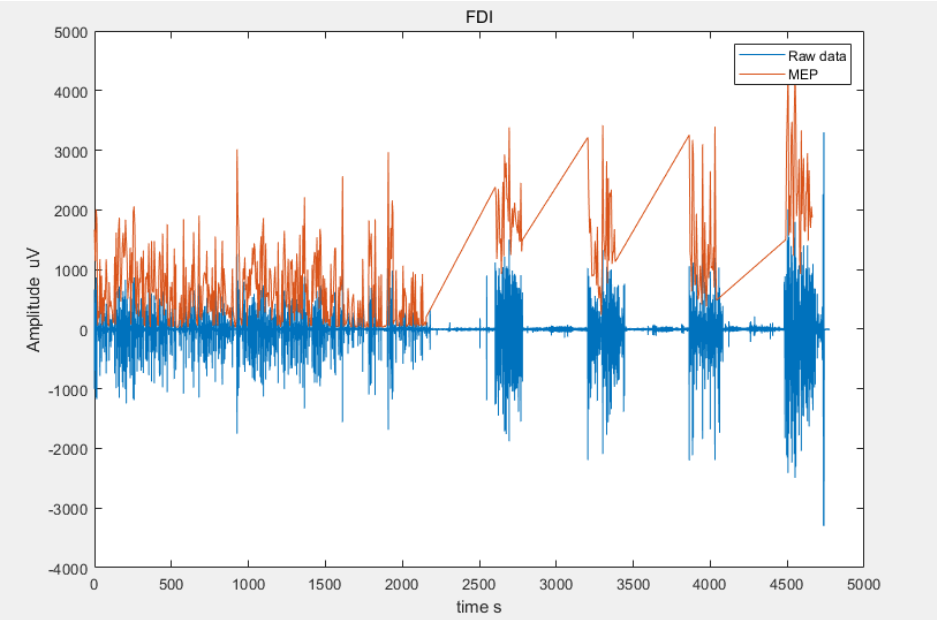

Figure 3-9: Plot of MEP amplitude and raw signal of FDI muscle. The blue line is the raw EMG signal, and the orange line is the MEP amplitude.

Upon acquiring the amplitudes of the MEP, we encountered the task of comparing these MEP values among different subjects, which presented a significant challenge. Since the baselines of each individual were different, a direct comparison was not feasible. To overcome this issue, we introduced the concept of MEP normalized amplitude. Specifically, we used the

mean resting MEP values before the experiment as the baseline, and then calculated the MEP normalized magnitude by dividing all the MEP magnitudes with the baseline.

The formula for MEP normalized magnitude can be expressed as follows:

$$\text{MEP normalized magnitude} = \frac{\text{MEP magnitude}}{\text{Baseline}}$$

By using this metric, we could effectively compare and contrast the MEP values of different subjects. A MEP normalized amplitude less than 1 indicated weaker cortical excitability in stage A as compared to the resting condition, implying that cortical excitability was suppressed. On the other hand, a MEP normalized amplitude greater than 1 indicated facilitated cortical excitability.

3.8 Data Analysis Methods

The mean MEP amplitudes were computed for each time, encompassing both the baseline and post-stimulation. The post-stimulation MEP were normalized within each individual, representing baseline ratios. All findings are reported as the mean accompanied by the standard error of the mean (SEM).

3.9 Result

3.9.1 Aftereffects of Transcranial Electrical Stimulation on Cortical Excitability

To assess the post-stimulation effects on cortical excitability, we analysed the mean and standard deviation of the normalized MEP values. Following 30 minutes of anodal tDCS stimulation, we observed a general augmentation in cortical excitability across all muscles. Immediately after the stimulation ended (at the 0-minute mark), there was a mild inhibition of cortical excitability. However, between 10 and 30 minutes after the stimulation, we observed a increase in cortical excitability, reaching its peak at the 10-minute mark. Subsequently, cortical excitability gradually declined at both the 20 and 30-minute marks, as shown in the Figure 3-10.

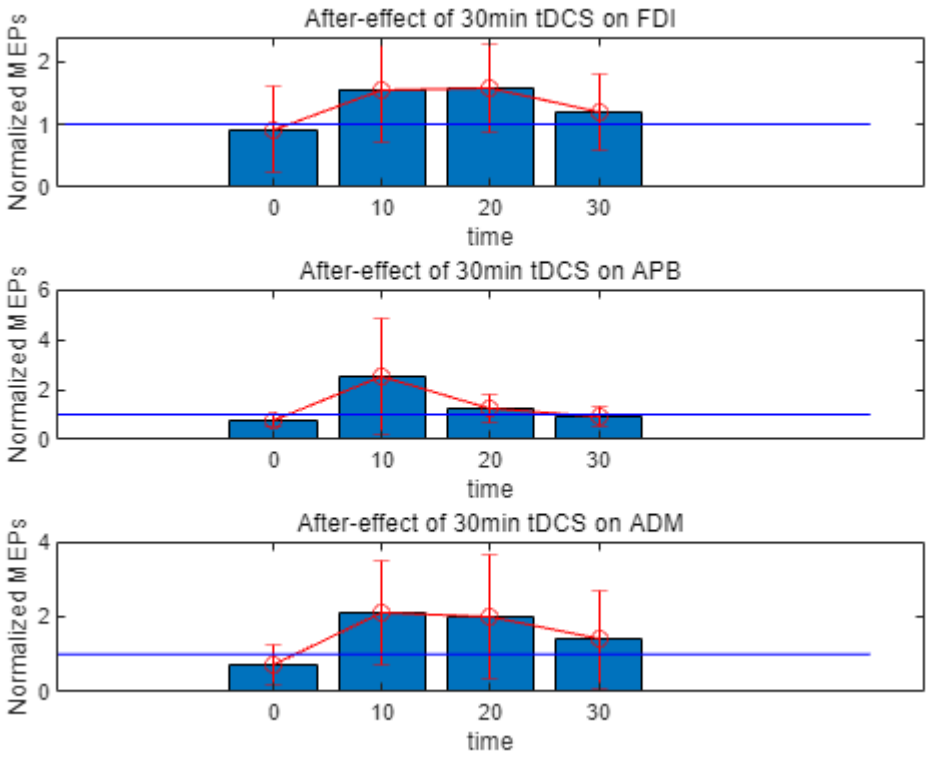

Figure 3-10: Aftereffect of 30min tDCS for subjects.

3.9.2 Online Effect of Transcranial Electrical Stimulation on Cortical Excitability

To analyse the impact of tDCS on MC excitability over time, our algorithm divided the 30-minute stimulation session into ten equal segments, with each segment spanning a duration of 3 minutes. We then calculated the response curves of MEP values for all target muscles. The results showed that cortical excitability increased rapidly within the first 3 minutes, reaching its highest state. Subsequently, it gradually decreased and reached the lowest point at 6-9 minutes, before rising again and staying constant until around the 24th minute, when it started to decline once more, as shown in Figure 3-11. These results suggest that tDCS has a time-dependent effect on MC excitability, with the strongest effect observed within the first few minutes of stimulation.

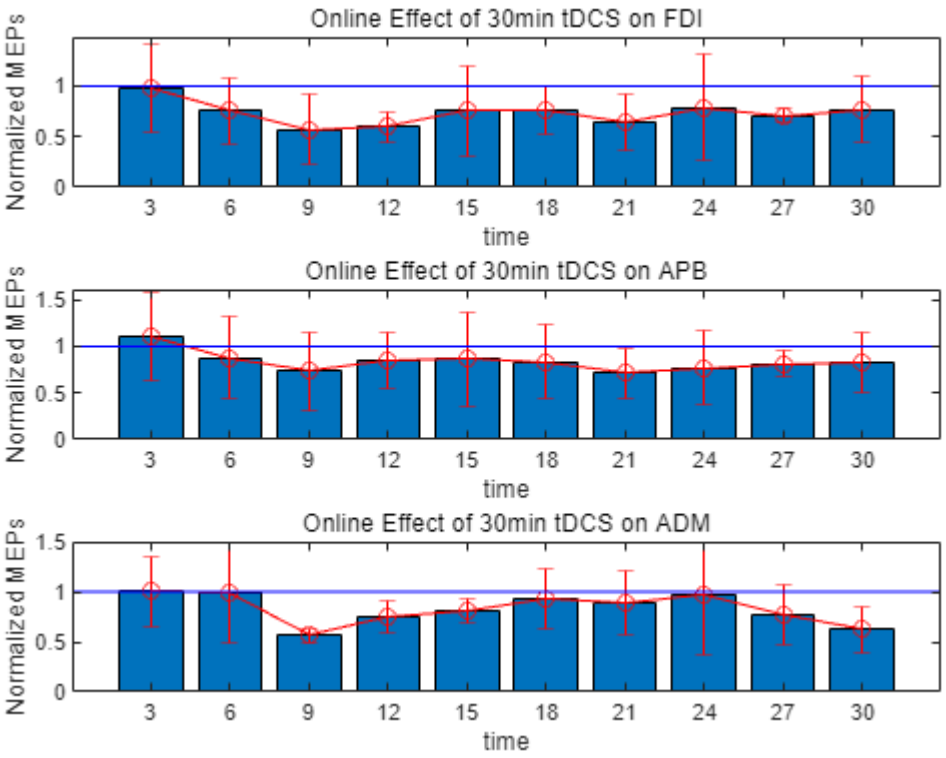

Figure 3-11: Online effect of 30min tDCS.

To obtain a detailed response curve, we divided the data into 30 segments, with each segment representing one minute of the stimulation session. We then fit the data to a curve, which is presented in Figure 3-12. The curve shows an initial rise in cortical excitability, reaching its first peak between 1-3 minutes. Subsequently, cortical excitability starts to decline before reaching a second peak at around 20 minutes. Finally, the lowest value of cortical excitability is observed between 26-30 minutes. These results suggest that the impact of the stimulation on MC excitability varies over the course of the session, exhibiting multiple peaks and troughs at different time intervals.

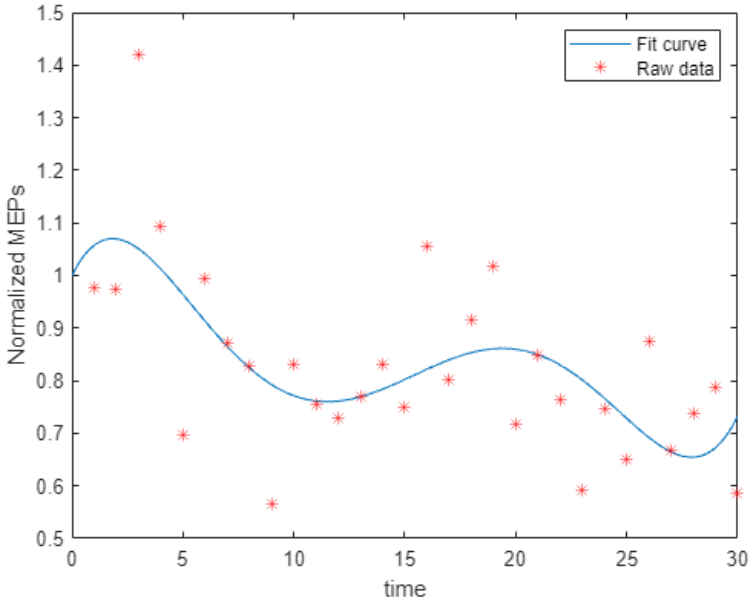

Figure 3-12: Curve of cortical excitability for various stimulus duration.

3.9.3 Analysis of stimulus response curves between different subjects

Although the response to tDCS varied between subjects, all participants showed a consistent pattern of changes in cortical excitability. Specifically, two moments of maximal cortical excitability were observed during the tDCS stimulation. However, the timing of these peaks varied between subjects. The first peak occurred between 0-10 minutes, while the second peak occurred between 15-25 minutes, as shown in Figure 3-13. These findings indicate that although individual responses to tDCS may vary, there is an overall pattern of cortical excitability changes that remains consistent across participants.

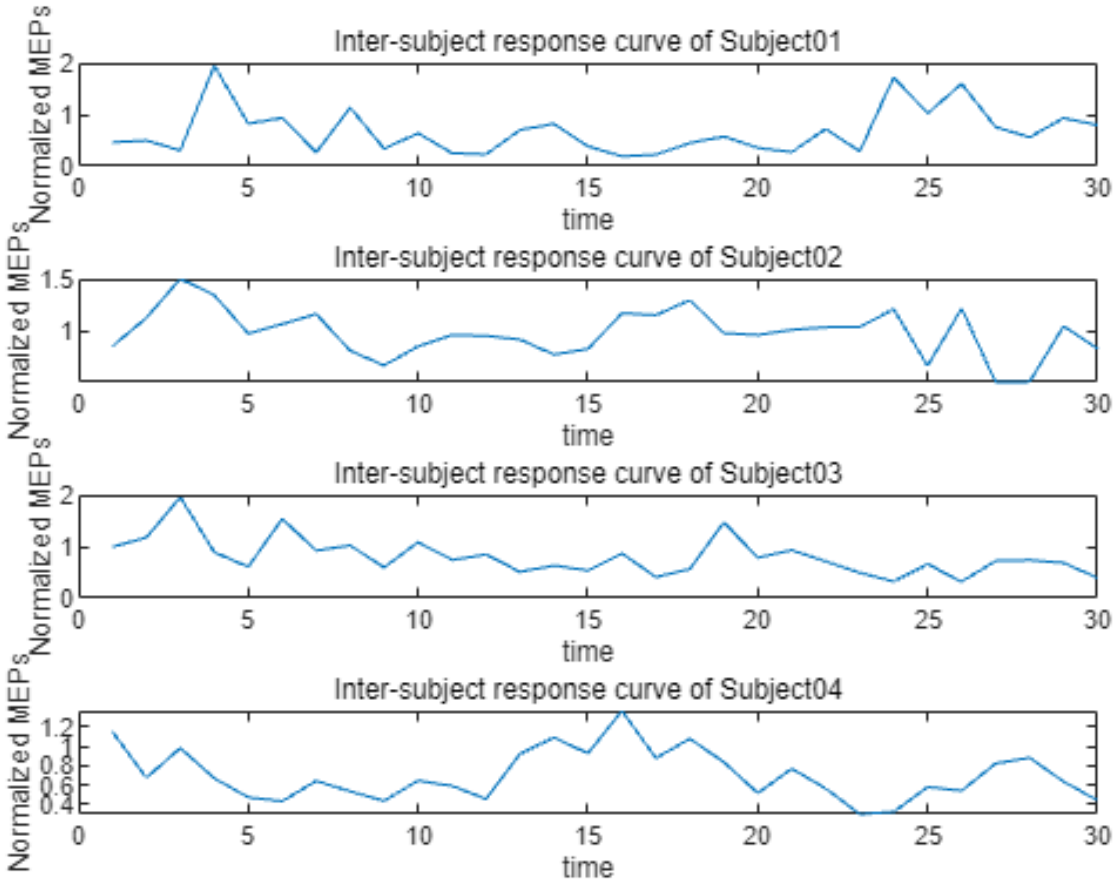

Figure 3-13: Inter-subject response curve.

3.10 Discussion

3.10.1 Assessment of tDCS After-effects on cortical excitability

This study aimed to examine the impact of a 30-minute session of anodal tDCS, set at 1 mA intensity, on MC excitability in a sample of four participants. The results revealed a notable improve of MC excitability in all subjects. Furthermore, an analysis of the after-effects demonstrated a sustained increase in MC excitability for at least 30 minutes following the conclusion of stimulation, with the most substantial effect observed 10 minutes after the session.

The present findings align with prior studies conducted by Farnad and Fujiyama, which similarly observed an elevation in MC excitability following a 1mA-30 min session of anodal tDCS. Notably, the effect was found to persist until the following morning, highlighting the long-lasting impact of the stimulation[103], [106]. However, the observed inhibition of cortical excitability measured at 0 min at the end of stimulation may be attributed to a combined TMS-

tDCS effect, as 33 min of TMS can almost be identified as low-frequency rTMS, which can cause inhibition of cortical excitability [33].

Our experiment also supports previous studies that reported anodal tDCS impact on MC excitability, with a duration of 5 min [11], [95], [107], [108], 10 min [109], [110], 20 min [111], and an intensity of 2 mA with duration of 10 min [88] or 20 min [112], as well as with 1.5 mA and durations of 10, 20, and 30 min [103].

3.10.2 Online effect of transcranial electrical stimulation on cortical excitability

The results demonstrated that by applying TMS and tDCS simultaneously, two peaks of cortical excitability were observed, with a general trend of reaching the first peak in the first 3 min, then slowly levelling off and starting the next peak, and then showing a decreasing trend again at around 26 min. A meta study also summarized tDCS impact on cortical excitability before 2014, on the basis of which we analysed the normalized cortical excitability at the end of tDCS for all papers, and the results showed that the response curve of cortical excitability tended to rise and then fall during the increasing duration of tDCS, with the peak (Normalized MEP: 1.38) was around the studies with a duration of 1-7 min, and the cortical excitability facilitation was reduced for studies with a duration of more than 14 min (Normalized MEP: 1.3) [113]. Meanwhile, similar results were found in studies preceding the trough period occurring at 26-28 min, and studies by Monte-Silva [96] and Hassanzahraee [10] showed that after 26 min of anodal tDCS stimulation, cortical excitability shifts from a facilitatory to an inhibitory effect, possibly due to the fact that spill over caused by prolonged Ca^{2+} stimulation may activate counteracting potassium channels, thereby limiting Ca^{2+} influx, which then produces an inhibitory effect. These findings suggest that simply increasing the intensity or duration of tDCS stimulation does not inevitably result in a corresponding increase in its effectiveness. In fact, it is possible that such modifications may even alter the direction of the observed effects. This raises the hypothesis that there might exist a "ceiling effect" for a single stimulation protocol in healthy individuals, wherein further intensification of the stimulation does not yield additional benefits. However, repeated stimulation protocols could be considered as potential approaches for enhancing the efficacy of stimulation [114]. Additionally, studies have demonstrated that pharmacological interventions can extend the after-effects of tDCS for approximately one day beyond the stimulation period [115].

3.10.3 Variability of response to tDCS for different subjects

When comparing the response curves of different subjects, it is noticeable that the moment of peak excitability varies for each individual. This variability among individuals in response to tDCS can be attributed to the fact that tDCS affects people differently, as previously observed by Tremblay. In their study, significant differences in response to tDCS were observed among subjects under identical stimulation conditions. Furthermore, even for the same tDCS parameters applied to the same individual, there were significant differences in their average response[116]. Furthermore, individual differences in cerebrospinal fluid (CSF) thickness may also play a role, with a thicker layer of CSF resulting in decreased electric field strength [117].

3.11 Proposed Mechanisms

3.11.1 Mechanism of Online Effects of tDCS

tDCS works by decreasing the concentration of GABA in the stimulation target area, which increases the excitability of neurons and improves the body's ability to perform motor functions. In this study, real-time tDCS stimulation was found to evoke cortical excitability in the M1. Studies conducted by Bachtiar et al [118] and Stagg et al [119] showed that anodal tDCS stimulation of the M1 decreased GABA concentration, resulting in a cortical inhibition reduce and an increase in neuronal excitability [120]. Hence, the immediate impact of tDCS is likely to improve cortical excitability through a reduction in GABA concentration.

3.11.2 Mechanism of tDCS Aftereffects

tDCS can alters cerebral blood flow, regulates neurofunctional functional connectivity and neuronal activity, and enhances cortical excitability. Study demonstrated that tDCS stimulation resulted in heightened cortical excitability. Jamil et al [121] found that anodal tDCS stimulation of the left M1 area increased blood flow in the M1 below the stimulated target area by 15.3%. A study conducted by Polania et al. [122] revealed that anodal tDCS led to an augmentation in functional connectivity between the MC regions. Additionally, they found that anodal tDCS applied to the left M1 area resulted in heightened functional connectivity between the stimulated region and the distal M1. Similarly, Rosso et al. [123] revealed a positive relationship between the strength of functional connectivity between the and premotor cortex and the level of MC excitability. Additionally, their findings demonstrated that heightened excitability of the MC has a favourable effect on human motor performance. Furthermore, a

study by Martin et al [124] provided evidence that tDCS may improve behavioral abilities associated with functional networks by improving neurofunctional network connections. Therefore, the aftereffects of tDCS may improve human locomotor performance by increasing motor cortex blood perfusion, functional connectivity between M1 and secondary motor cortex, and increasing cortical excitability.

4 Conclusion

4.1.1 Summary of findings

In summary, our study found the following results:

- The application of 1 mA tDCS for 30 minutes can effectively increase cortical excitability, and this effect can last for at least 30 minutes after stimulation.
- Cortical excitability can be measured by the MEP value evoked by TMS during tDCS stimulation. Our findings suggest that the association of cortical excitability and the duration of tDCS stimulation is not characterized by a linear relationship, with two peaks observed at 2-5 minutes and 15-25 minutes, respectively. A trough in cortical excitability is then observed at 26-28 minutes.
- Our findings indicate that the response to tDCS varies between subjects, and the relationship between cortical excitability and tDCS duration is not consistent across individuals.

4.1.2 Implications and significance of the study

In this experiment, the significance of our research is divided into three main parts:

- Previous studies have measured cortical excitability after tDCS. However, this study is the first to investigate the online effect of tDCS by simultaneously applying TMS and tDCS to the human motor cortex. We used TMS to measure cortical excitability during tDCS, which provides guidance for exploring the online mechanism during tDCS.
- Drawing on the benefits of real-time measurement of cortical excitability in our experimental design, we devised a more extensive scheme to investigate the correlation between tDCS duration and cortical excitability. In the same experimental setup, we observed a reverse association between the duration of tDCS stimulation and cortical excitability, thus enhancing our comprehension of the response curve between these variables.
- During the study, it was observed that different subjects had specific responses to tDCS, which suggests that different tDCS parameters should be considered for future tDCS treatments or regulations in order to tailor the approach to individual subjects.

4.1.3 Limitations and suggestions for future research

The research protocol for this study was developed after extensive discussion, the pre-experimental effects were carefully studied and judged, and the experimental procedures were strictly implemented according to the established criteria. Nonetheless, there are two limitations to this experiment.

Firstly, there was a single choice of stimulus intensity. While we observed a certain effect of tDCS stimulation during the stimulation duration and after tDCS stimulation, the effect was still relatively weak. It is possible that the conventional tDCS stimulation did not stimulate the motor cortex in a very focused manner, resulting in a relatively small actual current acting on the motor cortex. Thus, future tDCS studies could use a higher current stimulation intensity (2mA) or a more focused stimulation (HD-tDCS) to achieve a better stimulation effect.

Secondly, the effects induced by transcranial electrical stimulation are specific. Both previous studies of transcranial electrical stimulation and the results of this study show that the effects of transcranial electrical stimulation vary for different subjects, and we need to recruit more subjects to determine a relatively uniform pattern. Future studies need to include more samples to find relatively stable patterns.

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