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經皮神經電刺激應用於主要動作皮質對於動作學習時皮質興奮性與前額葉活化之影響：經顱磁刺激與近紅外線吸收光譜研究

Effect of Transcutaneous Electrical Nerve Stimulation on Primary Motor Cortex to Modulate Cortical Excitability and Prefrontal Activation during Motor Learning: A TMS and NIRS Study

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本論文係施政楷君（學號 r01428006）在國立臺灣大學物理治療學系暨研究所、所完成之碩士學位論文，於民國 103 年 7 月 11 日承下列考試委員審查通過及口試及格，特此證明

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碩士班兩年的生活對於我目前人生而言其實算是蠻大的轉折，碩士班這兩年我不斷的再學習，不管是待人處世還是論文實驗，但是對我來說最困難的永遠是人情世故這塊，我總是學習如何讓自己成為一個知情打理的人，而在過程中我也發現，不論是臨床或是學術表現，待人處事永遠需要具備來襯托所自己所奉獻的每一事物，而過程之中必然有許多的貴人，而要感謝的就是這些貴人所帶來的人生體悟。

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中文摘要

前言：根據先前的實驗結果所發現，經皮神經電刺激對於主要動作皮質的活性所造成的影響，無法與動作學習所產生的興奮性神經塑性有所區別，因此本實驗主要是為了瞭解頭顱經皮神經電刺激以及動作學習所產生去抑制現象之間的交互關係，而本實驗主要藉由量測在執行內隱式順序性動作學習的受試者主要動作皮質興奮性的改變，而本實驗另外使用近紅外線吸收光譜來量測前額葉在動作學習的過程中血液動力學反應的改變。**方法**：本實驗總共從社區以及大學徵招了48位年輕受試者，而受試者將會被隨機分配到電刺激組與電刺激伴隨動作學習測試組，而受試者需要完成兩次實驗，包含經皮神激電刺激和假刺激，而兩次測試則是隨機分配，試驗的間隔則需要大於一周，而本實驗成果測試項目包括：動作誘發電位、皮質內誘發、皮質內抑制、前額葉血液動力學變化以及順序性反應性動作測試中的反應時間長度，綜合以上神經生理或是行為科學上的量測來觀察經皮神經電刺激的效果。**結果**：在動作電位上則是發現有接受經皮神經電刺激的兩種試驗顯著上升，包含單純接受電刺激介入以及電刺激介入伴隨動作表現量測之情形，發現動作學習對於經皮神經電刺激所產生的效果產生協同作用，而在有動作執行的兩組(動作執行伴隨電刺激或假刺激)則是發現皮質內抑制所產生的抑制量明顯上升,而有電刺激伴隨動作執行量測的組別改變抑制的百分比顯著較假刺激明顯，而皮質內誘發則是在有電刺激的兩組(有無動作執行)顯著下降，但是組間的比較則是沒有明顯差異，而順序性反應時間測試中則是兩組(電刺激/假刺激)隨著練習的次數增加時明顯的降低反應時間，但是電刺激介入並沒有顯著影響動作表現，因此兩組間則是沒有顯著差異，而在前額葉血液動力學變化中則是只有在電刺激介入的情形有顯著效應，而在初始練習時發現有活性明顯上升的情形，而在30分鐘後測時發現顯著低於初始值的活性，因此代表電刺激的組別有一定的幫助學習固化的現象。**討論**：經皮神經電刺激顯著對皮質脊髓神經元產生明顯的興奮性誘發，但是同時間則發現皮質內抑制也有明顯增加的情形，而皮質內抑制上升則是

被認為會干擾皮質重組或是去覆蓋現象(unmasking)，而顯著動作的電位上升伴隨皮質內誘發明顯的下降則是有可能在皮質內誘發的量測中發生天花板效應，而在動作表現上則沒有發現明顯電刺激造成的效應，結論：本實驗證實經皮神經電刺激明顯增加主要動作皮質內之興奮性，而且動作練習對於經皮神經電刺激所產生的效果產生聯合反應，需要更進一步大於24小時的長期追蹤，或是增加電刺激介入的劑量，來進一步討論經皮神經電刺激的效應。

關鍵字：經皮神經電刺激、經顱磁刺激、近紅外線吸收光譜、內隱式順序性動作學習、神經塑性

ABSTRACT

AIM: The relationship between cranial transcutaneous electrical nerve stimulation (TENS) stimulation and cortical excitability change during motor learning process is unknown. This study aims to explore the effects of cranial TENS application on cortical excitability of primary motor cortex (M1) during implicit sequential motor learning process in normal subjects. Prefrontal activation pattern in learning process was also monitored by Near-infrared spectroscopy (NIRS). **METHODS:** 48 volunteers were recruited from colleges and communities. Subjects were randomized into TENS stimulation group (Stimulus-TENS/Sham) and TENS stimulation with motor task group (Motor-TENS/Sham). Subjects in both groups need to accomplish two trials (TENS or sham stimulation), the interval between trials was more than 1 week. Motor evoked potential (MEP), intracortical inhibition (ICI) and intracortical facilitation (ICF), Serial reaction time task and NIRS were measured to monitor physiological and behavioral change process in motor learning. **RESULTS:** MEPs amplitude in both Motor-TENS and Stimulus-TENS group increased significantly. MEP amplitude of Motor-TENS were significantly higher than Stimulus-TENS in followed up 60 mins. Motor task induced synergistic effect on enhancement of MEP amplitude. Higher suppression effects of ICI were also found in both Motor-TENS and Motor-Sham. Motor-TENS go higher suppression of ICI than Motor-Sham which revealed synergistic effect of TENS

stimulation. The ICF was also decreased in Motor-TENS and Stimulus TENS. However, between group comparison showed no significant different. In SRTT performance, reaction times significantly improved both in Motor-TENS/Sham but no difference between TENS and sham stimulation. Prefrontal activation showed significant time effect in TENS-Motor only. Increment activation in initial learning and following decrease activation in retention test was observed. Consolidation effect in Motor-TENS than Motor-Sham was also noted. **DISSCUSSION:** TENS intervention increased corticospinal neuron excitability. However, significant increase suppression induced by ICI may indicate disruption of cortical representation. Increment of ICF concurrent with increment MEP showed ceiling effect existed in ICF measurement. TENS intervention showed weak effect to altered motor learning process. **CONCLUSION:** TENS stimulation increase cortical excitability and inhibitory shift of intracortical circuits. Motor practice played a facilitation role to altered cortical excitability which induced synergistic effect on TENS intervention. Further study should be done to investigate the effect of TENS with long-term (more than 24 hour) effect or increase times of stimulus program.

Key words: Transcutaneous electrical nerve stimulation, transcranial magnetic stimulation, implicit sequential motor learning, near-infrared spectroscopy, neuroplasticity

LIST OF ABBREVIATIONS

tES	Transcranial electrical stimulation
ES	Electrosleep
EA	Electroanesthesia
TCES	Transcerebral electrotherapy stimulation
TCET	Transcranial electrostimulation treatment
NET	NeuroElectric therapy
tDCS	Transcranial direct current stimulation
tACS	Transcranial alternating stimulation
tPCS	Transcranial pulsed current stimulation
CES	Cranial electrotherapy stimulation
TENS	Transcutaneous electrical nerve stimulation
TMS	Transcranial magnetic stimulation
MT	Motor threshold
MEP	Motor evoked potential
ICI	Intracortical inhibition
ICF	Intracortical facilitation
fMRI	Functional magnetic resonance image
BOLD	Blood oxygen dependent level

PET	Positron emission tomography
SRTT	Serial reaction time task
NIRS	Near infrared spectroscopy
[O_2Hb]	Oxygenated hemoglobin
[HHb]	Deoxygenated hemoglobin
[tHb]	Total hemoglobin
[Hbdiff]	Hemoglobin difference
OD	Optical density difference
SCD	Scalp cortical distance
GABA	Gamma-Amino butyric acid
NMDA	N-methyl-D-aspartate
LTP	Long-term potentiation
EEG	Electrical encephalography
EMG	Electromyogram
M1	Primary motor cortex
DLPFC	Dorsal lateral prefrontal cortex
SMA	Supplementary motor area
FDI	First dorsal interosseous
ANOVA	Analysis of variance

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Chapter 1: Introduction

1.1 Background

Non-invasive brain stimulation (NBS) was one kind of central nervous system stimulation by small intensity of electrical current or magnetic field generated current to stimulate brain tissue. NBS can be classified into several types dependent on stimulation parameters and electrodes placements such as transcranial direct current stimulation (tDCS), transcranial alternating stimulation (tACS), transcranial magnetic stimulation (TMS) and cranial electrotherapy (CES)[1].

tDCS had much of evidences of its effect on cortical excitability modulation. Direct electrical current induced polarization effect on cortex tissue. Cathodal stimulation induced inhibitory effect on cortical excitability. Anodal stimulation induced excitatory effect on cortical excitability. Polarized electrical field would increase the difference of intracellular and extracellular membrane potential. The positive change of extracellular membrane potential induced by anodal stimulation would facilitate NMDA pathway[1]. However, single direction current may easily induce skin burn.

tACS was one kind of alternating current stimulation which commonly used biphasic sinusoid wave with low(10-50 Hz) and high frequency (kHz). tACS was believed to interrupt electroencephalic oscillation which may induce change of brain function[2]. In Electroencephalography of human, brain electrical oscillation was

observed. After Fast Fourier transform of the electrical signal, three level of frequency were identified. α wave was consist of signals which frequency range from 8-13 Hz, β wave was 13-30 Hz and γ was 30-100Hz. The Previous tACS study investigated the effect on cortical excitability, but the results were still controversial. β frequency range tACS (15-20Hz) was found more effective than other frequency band. Inhibitory effect which reduced intracortical facilitation(ICF) was reported[3]. Other studies also indicated that the stimulation would interrupt subject's motor performance[4,5]. In addition,, Kanai in 2010 applied 20 Hz tACS on visual cortex and found that the phosphate threshold was decreased[6]. Another study also reported that tACS can induce phosphate[7].

Cranial electrotherapy (CES) was developed over 20th century. CES stimulation can modulate neurotransmitter level which relieved insomnia, bipolar syndrome, depression or anxiety[8]. The electrodes were placed on supra-auricular and infra-auricular area where try to stimulate brain stem through cranial nerve or cervical nerve. Previous study revealed that the CES would enhance dopamine and norepinephrine level in rat's hypothalamic area[9]. General brain activity was significant decrease after CES stimulation also reported in fMRI study[10]. Connectivity of frontal lobe and parietal lobe was significant decrease that indicated the stimulation effect disrupted cortico-cortical network. CES also showed effect of interruption of neural

electrical oscillation status which lead to lower shift of median frequency.

Although the neurophysiological measurements reached statistical significant, but most of the studies didn't reach significant in functional task. Transcranial pulse current stimulation (tPCS) was another category of CES stimulation. This kind of stimulation used unidirectional or bidirectional symmetric rectangular current, so concept of stimulation was more related to tDCS.

One study reported the effect of tPCS on the gait pattern in Parkinson's disease. Significant improvements of strike length and gait velocity were noted[11]. However, polarization effect induced by tPCS was less than tDCS. Abhihek in 2012 compared different stimulation parameter [8]. He indicated the biphasic current charge imbalance current may have less effect on neuromudulation because of the symmetrically bidirectional current.

As we know, CES stimulation can effectively influence brain stem neurotransmitter level in animal study. Interruption of cortical connectivity also reported in fMRI study. Recent years, integrated therapy was gradually be emphasized. Combination of non-invasive brain stimulation with movement training would be more efficient of training effect. Recently, study showed that combination of constraint induce movement therapy with concurrent tDCS showed significant effect on increasing cortical excitability of affected hemisphere and reducing excitability of

unaffected hemisphere in stroke patients[12]. tDCS combined robotic assisted therapy also showed significant effect on spasticity control[13].

TENS was commonly used in pain management. Few of studies reported that cranial TENS intervention improve motivation of dementia population[14]. Parameter of Transcutaneous electrical nerve stimulation (TENS) was similar as CES. The electrical stimulation waveform was biphasic rectangular current with or without resting time Our study investigate the effect of cranial TENS intervention on cortical excitability. Because of feasible and easily approach of TENS, the effect of cranial TENS on movement performance or cortical excitability are important for further clinical using. Previous study showed increase cortical excitability after TENS intervention, but motor learning process itself may increase cortical excitability. The relationship between TENS intervention and motor learning process was unclear[15].

1.2 Purpose

This study investigated the effect of cranial TENS on cortical excitability and implicit sequential motor learning process. Our previous study revealed the effect of low frequency (15Hz) cranial TENS intervention. Significant increase of cortical excitability was observed. Although significant change of cortical excitability, we cannot clarify the excitatory effect was result of motor practice or TENS. We separated

the experiment into two groups. First group was cranial TENS stimulation group and second group was cranial TENS combined with serial reaction time task (SRTT). Independent measurement of cortical excitability help us compared the difference induce by motor practice. In addition, previous study showed no significant difference of thumb pinch accuracy task between TENS stimulation and sham stimulation. In this experiment we choose SRTT as functional task which was common design in motor learning studies. After repeated practice single number sequence, subjects would build up the linkage of each number they taped. Prevention of any information about exist sequence in the number they taped was important to ensure implicit leaning process.

We also measure cortical hemodynamic response of prefrontal cortex. Prefrontal cortex increase activation in initial SRTT practice then decrease activation in retention test was reported.

1.3 Question and Hypothesis

Question 1: Does cranial TENS intervention can modulate cortical excitability of primary motor cortex?

Null hypothesis: There is no significant difference of cortical excitability between cranial TENS trials and sham stimulation trials.

Alternating hypothesis: There are significant differences of cortical excitability between cranial TENS trials and sham stimulation trials.

Question 2: Are there any neurophysiological differences between the subjects with motor practice or not during cranial TENS intervention?

Null Hypothesis: After cranial TENS intervention, there are no significant differences of cortical excitability between the subjects with motor practice or not.

Alternating Hypothesis: After cranial TENS intervention, there are significant differences between the subject with motor practice or not.

Question 3: Is there any difference of prefrontal activity pattern during practice after cranial TENS intervention?

Null hypothesis: cranial TENS treatment do not change activation pattern of prefrontal cortex.

Alternating hypothesis: cranial TENS intervention significant decrease prefrontal activation after motor practice.

Question 4: Does subjects would get significant more improvement of reaction time in cranial TENS trial compare with sham stimulation trial in functional reaction time task?

Null hypothesis: There is no significant different improvement of reaction time between TENS trials and sham stimulation trials.

Alternating hypothesis: There is significant more improvement of reaction time in cranial TENS trials compared with sham stimulation trials.

1.4 Significance

TENS were common used in pain management in Taiwan. TENS devices were more feasible compared to tACS or tDCS in Taiwan. Cranial TENS intervention was lack of evidence about the treatment effect. This study investigated the effect of cranial TENS on cortical excitability of M1. Movement related disorder common change excitability of primary motor cortex. In Parkinson's disease, due to the impaired dopamine pathway which lead to imbalance of facilitated circuit or inhibit circuit.in basal ganglia[16]. Compensated change of increasing excitation of motor cortex was observed. In stroke patient, the cortical excitability was unbalance between affected hemisphere and unaffected hemisphere[17]. Unaffected hemisphere showed increasing but affected hemisphere showed decreasing of cortical excitability.

The future goal is to identify the pattern of cortical excitability change after cranial TENS and applied in neurological impaired population. For example, applied the cranial TENS on Parkinson's population or affected hemisphere of stroke patient to restore balance of cortical excitability.

Chapter 2 Literature Review

2.1 Historical View of Transcranial Electrical Stimulation

Transcranial electrical stimulation(tES) had been developed for more than century ago. The pioneer of transcranial electrical stimulation was Electrosleep (ES) and Electroanethesia (EA). The concept of EA was applying high frequency (3500-10000 Hz) transcranial electrical stimulation to serve as substitution for chemical anesthesia. Due to high frequency and high intensity of stimulation parameter, Electroanesthesia was less related to our study. In 1902, Louise Robinivitch designed another type of the transcranial electrical stimulation which was the first study of Electrosleep. The frequency was set between 30-100Hz with biphasic rectangular current with pulse which was more related to our experimental concept of stimulation parameter. In 1914, Electrosleep first served as clinical use and published first clinical report at the same time. Because of the relaxation effect, ES then be used as treatment of sleep disorder.. In 1950s, the Electrosleep reemerged to get public attention in Europe. In 1960, ES evolved to other names such as Transcerebral electrotherapy (TCES) in 1960s, NeuroElectric therapy (NET) in 1970s and Cranial electrotherapy (CES) in 2010. Such kind of transcranial electrical stimulation was used as treatment for anxiety, insomnia and depression[18].

Polarization electrical stimulation was developed based on ES with DC offset.

Initial polarization electrical stimulation was TCES combined DC bias, but latter the completely direct current developed in 1969 by Brown which stimulation parameter was same as contemporary tDCS. Transcranial pulse current stimulation (tPCS) was also based on polarization stimulation type. The stimulation parameter of tPCS was using the monophasic pulse current. tPCS has significant effect on improving gait performance in Parkinson's disease was reported in 2013[11].

Another contemporary type of tES was transcranial alternating stimulation (tACS). In 2011, tACS was developed. The stimulation waveform was sinusoidal biphasic current which was equal charge. Instead of polarization effect, tACS hypothesized that sinusoidal wave can disrupt current brain electrical oscillation status and change the brain oscillation pattern[19]. The effect of tACS on cortical excitability was still controversial. Large clinical trial of tACS for neurological disorders still lack of evidence. Even though, one fMRI study reported that β band frequency (15Hz) tACS have inhibit effect on cortical abnormal activity in idiopathic cervical dystonia patients[20].

2.2 Comparison Stimulation Parameter of Transcranial Electricl Stimution

In 2013, Berkan comprehensively compared different type noninvasive brain stimulation. Base on previous category of tES which included ES, EA, Neuroelectric therapy (NET), CES, tACS, tDCS. The stimulation setting of Electrosleep was place two active electrodes on bilateral frontal which ground placed on palm. Another stimulation pattern which focus on optical nerve stimulation for relaxation effect. In optical nerve stimulation condition, two active electrodes would place on bilateral eyes area and ground place on mastoid process which intend to make current run through eyes fossa. The intensity was between 0.1 to 0.5 mA.

NET was the precursor of CES. The electrodes setting shift from bilateral scalp in Electrosleep to bilateral ears which current was more focus on brain stem stimulation and tried to influence the pathway in brain stem including serotonergic and noradrenergic systems[21]. Electrodes number decrease from 3 electrodes to 2 electrodes that ground electrode was excluded. The intensity was smaller than CES which was about 600μA. Stimulation frequency was larger range (0.5- 100Hz) which was same as CES. In NET, electrodes placed on bilateral forehead which concept was not focus on brain stem stimulation. CES stimulation generated fixed electromotive in 5V with pulse current.

In Electroanesthesia(EA), four electrodes were placed on bilateral frontal and occipital area. EA was more intensive type of electrical stimulation. Stimulation was high which was about 40 mA with continue DC current. Another type of EA used biphasic current, but the stimulation intensity was related lower (about 10 mA) with frequency near 10000 to 20000 Hz.

In tDCS, the electrode placement was changed from brainstem stimulation concept to specific brain area stimulation. In M1 stimulation condition, the active electrode was placed on hotspot of M1 defined by tanscranial magnetic stimulation (TMS) mapping technique or placed on C3 and C4 in 10-20 EEG electrode system. The passive electrode was placed on contralateral supraorbital area which current flowed into unilateral motor cortex and premotor cortex. Stimulation intensity was set at 1-2mA. Electrode placement of tACS was also similar as tDCS. Depending on stimulation the target brain area, the tACS can alter subject's visual, cognitive, motor, sensory function. Previous montage study also show that the low frequency AC current was more efficient to induce electrical field change in cortex than DC current[22-24]. The electrical field induced in cortex by AC current was five times more than DC current in realistic head model which designed based on different tissue conductivity[22]. In our experiment, we combined the CES stimulation with contemporary setting of electrodes. We use TENS as intervention tools to induce neurotransmitter change and further

change cortical excitability. The current generated by TensMed 931 was symmetric biphasic current with pulse. The waveform of our stimulation tool was more close to the concept of CES, NET and TCES. The electrodes were place on M1 and contralateral supraorbital area.

2.3 Cranial Electrotherapy Stimulation

Cranial electrotherapy stimulation (CES) was developed from Electrsleep which mechanism was based on peripheral nerve stimulation to induce neurotransmitter level change central nervous system. Instead of using "transcranial" as term of description, CES used "cranial" as its term which emphasize the current not flow through the scalp[1]. The peripheral stimulation mechanism focuses on the stimulus transmit into CNS with change of antinociception reaction. The pathway of the stimulus may go from cranial nerve I-III and VIII to activate of brainstem center. Some of the CES emphasize the electrode need to place on bilateral eyes to induce peripheral pathway to influence central nervous system.

Second potential mechanism was CES may induce release of neurotransmitter. CES also have good effects on biological clock of brain which controlled in hypothalamic suprachiasmatic nucleus (SCN) in brain stem[21]. The serotonin level and noradrenaline level was also increase after CES intervention. Base on previous

physiological review, the serotonergic system was more response to stimulation with 10, 20 and 100Hz. TCET was one type of CES which was asymmetric biphasic current with equal charge. When TCET applied on rat, the dopamine and norepinephrine level were significant increase. The stimulation seems to induce synthesis of neurotransmitter in midbrain or hypothalamus. No neurochemical response was found in hindbrain neurotransmitter synthesis center which indicate the neurotransmitter synthesis response was localized not whole brain.[9]

Third potential mechanism was alternating current may interruption or disturbance ongoing cortical activity. Previous study also showed CES can alter EEG which showed lower shift median frequency of α wave. The frequency of CES was set at 0.5Hz and 100Hz. higher frequency has more obvious effect on interruption of α wave. Reduction median frequency of α wave was associated with more relaxation status[10]. α wave was also related to awake and sleep status change. Significant desynchronizing of α wave was found in sleep status[25].

2.3.1 Research in Animal Model

Some animal studies reveal how functional or structural change of the neural tissue after stimulated by alternating current. In hippocampus cell, high frequency electrical stimulation would induce long tern potentiation (LTP) which was long last

change of synaptic efficiency. Low frequency electrical stimulation do not induce LTP liked response or even resulted in long term depression. Recent study overthrows this concept and indicated that low frequency electrical stimulation (1Hz) would also produce LTP response with specific afferent system. The 0.5 Hz alternating pulse current induce CA1 LTP like response. However, low frequency stimulation on CA3 was ineffective[26].

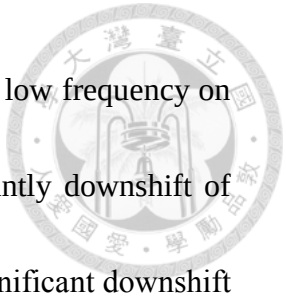
Whether the AC current actually can influence neuron is controversial especially when charge was balance. Transcranial electrostimulation treatment (TCET) was biphasic current with pulse which charge was balance. In the hypothalamic level of the rat, TCET significantly enhance dopamine and norepinephrine synthesis. In mid-brain of the rat, the serotonin and dopamine significant enhance synthesis. Although the dopamine and norepinephrine synthesis increase, the turned over rate not change which detected by measured the metabolite of neurotransmitter.

2.3.2 Research in Human Model

CES commonly used as treatment of depression, anxiety, insomnia, pain, migrant headache, pain of fibromyalgia and sleep-awake cycle disorder. Few of clinical trials focus on the rehabilitation effect of CES on movement disorder. Most of experiments of CES were focus on cognitive, biological clock, psychological problems. Malden's

study in 1985 indicated that the CES can enhance occupation therapy effect on gross motor performance in severe cerebral palsy children. Actually, the evidence level of motor function related study was not strong. CES also facilitates sensory motor integration in children. In Okoyey's in 1986, the motor accuracy and hand function significantly improved in minimal cerebral palsy children[27].

CES was also effective for pain relief. Most of the studies focus on how neurotransmitter of nociception system affected by CES. In another point of view that patients with chronic degenerative joint pain syndrome show abnormal peak and unsmooth pattern in EEG spectrum curve in bilateral frontal area. In contrast, normal healthy subject show relative more smooth of frequency spectrum curve than chronic degenerative joint pain patient. Both patient with pain and healthy subject received 20 mins of microcurrent stimulation with 0.5 Hz biphasic rectangular pulse current. The electrode placed on bilateral trapezius or earlobe. The electrode placed on trapezius muscle which was trying to induce neurotransmitter level change through cervical nerve. After CES intervention, the unsmooth pattern of EEG spectrum significant improved which was near normal spectral curve. Furthermore, pain score of patient also significant decrease[28,29]. Though the cortical electrical oscillation status change after CES intervention, the effect still believed derived from neurotransmitter modulate effect in subcortical area.



Another study compared the effects of high frequency CES and low frequency on EEG spectral curve. Low frequency CES (0.5Hz) showed significantly downshift of mean frequency of α band. High frequency CES (100Hz) showed significant downshift of mean and median α band frequency. α band frequency downshift was more related to relaxation status of subject which indicate the CES facilitation relaxation state. After 100Hz CES intervention the power fraction of β band frequency significant decrease. β band frequency was more related to stress, arousal, problem solving state. It seems that higher frequency of CES was more effective in brain oscillation modulation.[30] Another fMRI study reveal that CES stimulation disrupted cortico-cortical network such as connectivity between frontal lobe and parietal lobe[10]. General hemodynamic response of specific task was less in CES stimulation condition. Based on these findings, changes pattern of power spectral curve after CES was suspected the results of changes of neurotransmitter level in subcortical area.

2.4 Transcranial Magnetic Stimulation

In 1838 Micheal Faraday discovered the phenomenon of the area near electrical current would induce magnetic field change. Technique of transcranial magnetic stimulation (TMS) was based the Faraday's law of magnetic field. Based on concept of magnetic field drive electrical current, the initial machine of transcranial magnetic stimulation was developed by Barker in 1985.[31-33] Using magnetic field pass through the neuron membrane induced depolarization of motor cortex was more painless than tES. The direction of electrical field induced by TMS was penetrated perpendicularly to the coil. The current cause by TMS would parallel to the coil. Thus, TMS derived current was more focus on the cortex. By contrast, because of anode and cathode placement, the electrical field direction induced by tES would more focus on subcortical area[33]. The penetrated magnetic field generated electrical current on motor cortex which induce depolarization of the pyramidal neuron. The initial directly descending action potential was defined as "Direct Wave" which also called D-wave. Then follow late waves with interval 1.2-2.0 ms after D-wave were "Indirect Wave" which also called I-wave[31,34]. I-wave was more related to polysynaptic or synaptic recurrent network which represent excitability of motor neuron pool in cortical level or spinal level. The descending several I wave reach neuromuscular junction with temporal and spatial summation which reach threshold and leaded to muscle

contraction which was called motor evoked potential (MEP). MEP amplitude naturally fluctuated over time and affected by the integrity and excitability of cortico-spinal neuron. With voluntary contraction, the size of MEP increased which represented the higher level of excitability corticospinal motor neuron pool.

Safety issue of TMS had been discussed for several years. Metal implantation in brain or target stimulation area may potentially displaced or damaged under current generate through magnetic field including mental clips, deep brain stimulator, pace maker and cochlear implants. External body mental objects also need to be removed for any possible interaction such as glasses, watch and necklace. Some of side effect of TMS had been reported previously. Increase in auditory threshold due to the high pressure level sound derived from coil. In case of side effect of transient heavy hearing, earplug was suggested for auditory blocked. Another side effect may occur was seizure. Due to the electrical current generated in gray matter would induce imbalance of excitatory circuits and inhibitory circuit in some people which may leads to seizure when received TMS stimulation. The population probably induced seizure included subjects with multiple sclerosis, bipolar syndrome, major depression and have family history of seizure. Other side effect may exist such as, vascular syncope, increase heart rate, increase blood pressure, psychiatric change, interaction effect on drug of neuro-system.[34] Pregnancy should prevent from the stimulator coil more than 0.7 meter

for the purpose of that fetus may be affected by the magnetic field.[35]

2.4.1 Motor threshold

The definition of motor threshold (MT) or cortical motor threshold (CMT) was the lowest TMS stimulation intensity applied on motor cortex to induce muscle contraction of muscle which was defined by Rossini et al in 1994[36]. Motor threshold represents as the excitation status of pyramidal neuron and spinal neuron. Due to the fluctuation status of excitability of motor neuron, estimation of motor threshold needs validated method. The MT was defined as percentage of simulation intensity which with 50% success rate (5 times out of 10 times) to induce MEP more than 50μV peak to peak amplitude. Average 75 pulses needed to deliver to confirm the motor threshold by Rossini's method but relative shortness compare to other method[37]. Due to lack efficiency of Rossini's method, modified Rossini method was developed which use 3 times out of 6 times method was considered. However, No validity or reliability report of modified Rossini method was delivered.

2.4.2 Motor evoked potential

I wave with spatial and temporal summation would induce peripheral muscle contraction, the signal received from electromyogram called “Motor evoked potential”. Motor evoked potential not only reflect integrity of cortical spinal neuron, also represent excitability of corticospinal neuron. The higher intensity TMS stimulation, the more large size of motor evoked potential induced. While muscle slightly contraction status, the same intensity of magnetic field would lead to larger size of MEP than without muscle contraction. In slightly contraction status, the corticospinal neuron would increase excitability that cause the size of MEP was increase. Single pulse MEP also can be used as mapping technique to defined functional distribution of motor cortex. To monitor the change of cortical excitability of target muscle with specific stimulation point called “hotspot”.

Motor learning was a process which included neuroplasticity which was functional change or structure change of neural system. Motor learning also induce long-term potentiation in motor cortex which cause increase of amplitude of MEP after learning process.[38,39]

2.4.3 Intracortical facilitation and intracortical inhibition

Intracortcial faciulitation(ICF) and intracortical inhibition(ICF) was related the techniques of pair pulse stimulation. Combination of different interval of sub-threshold stimulation following supra-threshold stimulation would induce inhibition or facilitation effect on motor cortex[33]. The first sub threshold stimulation was conditional stimulus and second stimulation was testing stimulus. With lower interval between 4-7 ms belong to ICI which represent excitation of inhibitory circuits and have inhibitory effect on cortical excitaiblity. Di Lazzaro et al. in 2000 revealed that ICI was more related to a-GABAergic pathway which was the primary inhibitory neurotransmitters in brain[40]. Excitatory circuitry can be evaluated effect in interstimulation interval between 7-20 ms which called intracortical facilitation. However, mechanism of intracortical facilitation was unclear. With intake of antagonist of N-methyl-D-aspartate (NMDA), ICF was significant decrease[41]. The suppressive effect of NMDA antagonist supported that the ICF was represent the glutamatergic transmission. Long-term potentiation which involving in increasing calcium ions level of dendritic spine was involved in NMDA pathway.[42] Due to the different mechanism of ICI and ICF, Some of the authors indicated that the circuitries involved may be independent.[42]

2.5 Near-Infrared Spectroscopy

Near-infared spectroscopy was used to measurement of blood flow of different tissue. When the blood flow increase, concentration of oxygenated hemoglobin [O2Hb] would increase, the concentration deoxygenated hemoglobin [HHb] would decrease and total hemoglobin would increase. With such phenomenon which called hemodynamic response can be applied on different tissue such as cerebral cortex, muscle belly and tendon. Near Infrared was mainly absorbed by melanin, so the hairy skin should prevent in measurement. Other tissue such as scalp, bone, cerebrospinal fluid would absorb infrared but related stable than cortex. Cortex would change blood flow by the time due to the metabolic rate increase of local area.

2.5.1 Mechanism of NIRS

The early experiment of NIRS was done by Jobsis in 1997 who found myocardiac and cortex had high penetration rate of near infrared.[43] Until 1980s, the first study of applied NIRS on human cortex to detected hemodynamic response was published. The near infrared can partial penetrated through tissue and partial reflex or absorbed by the tissue. Different kinds of molecules have different absorption spectra. For example, H2O was highly be absorbed from 1050 nm to more than 1300 nm of wavelength. To detect the hemodynamic response of cortex focus on concentration change of

oxygenated hemoglobin (Δ[O2Hb]) and deoxygenated hemoglobin (Δ[HHb]) between different events. HHb was high in absorption spectra when applied near infrared's wavelength between 600nm to 1000 nm. O2Hb was high absorption spectra when applied near infrared's wavelength between 700 nm to 1150 nm. 810 nm was equal absorption spectra of O2Hb and HHb The NIRS device often project two wavelength above and below 810 nm to calculus Δ[O2Hb] and Δ[HHb] by mathematics method of first degree polynomial in two variable.[44]

$$\Delta \mathrm{OD} = -\ln \frac{I_{\mathrm{Final}}}{I_{\mathrm{Initial}}} = \varepsilon B L \Delta C,$$

The formula show how to calculate level of Δ[O2Hb] and Δ[HHb] through optical density difference (ΔOD). ΔOD is change of optical density from initial to final. I_{Final} and $I_{Initial}$ are the measured density in initial and final. ΔC is the change of the concentration. L is distance from light source to detector. ε is the extinction Coefficient. B is differential pathlength factor which may be influenced by age effect. To calculus concentration change of Δ[O2Hb] and Δ[HHb], the formula also can rewrite into below:

$$\Delta \mathrm{OD}(\lambda) = (\varepsilon_{\mathrm{HbO}_2}(\lambda)\Delta[\mathrm{HbO}_2] + \varepsilon_{\mathrm{HbR}}(\lambda)\Delta[\mathrm{HbR}])B(\lambda)L$$

λ is particular wavelength. To measure the concentration change of Δ[O2Hb] and Δ[HHb] by this equation which need more than two wavelength light to determine the concentration of hemoglobin.

Neuro-activation would increase metabolic rate of neural tissue which was coupled with hemodynamic response. Increase metabolic demanding which drives vascular response to provide more oxygen to neuron. When increase blood perfusion of local neuron, [O2Hb] will increase and [HHb] will decrease. With change of hemoglobin concentration level which indicate neural activation status. Total hemoglobin concentration [tHb] also common measured in NIRS device which was sum of Δ[O2Hb] and Δ[HHb]. [tHb] can be serve as an index of blood perfusion of neural tissue.

The shape of infrared light penetrated area with perpendicular projection was described as banana shape or ellipsoid path. Penetration depth of infrared was about 2-3 cm.[45] Whether infrared can flow into cortex was depending on scalp cortical distance (SCD). Target tissue to estimate hemodynamic response as index of activation was focus on gray matter. By mathematic model of ellipsoid, Depends on different

SCD, the amount of gray matter volume which penetrated by infrared can be predict. With increase of SCD, measured the gray matter volume would decrease. Such as area near central sulcus where SCD would be too long to measure the cortical hemodynamic response.[45] For example, the area control lower extremity in primary motor cortex was located beneath central sulcus was not suitable for NIRS measurement. In this study, we measure activation of prefrontal cortex where SCD was related less which was feasible for NIRS measurement.

2.5.2 Psychometric studies of NIRS

Hemodynamic response of prefrontal cortex during motor practice was one of major outcome of this study. Reliability and validity of NIRS is important to explain outcome between trails variability. Gold standard of hemodynamic response of neural tissue was fMRI. Several psychometric studies have been published already. The limitation of fMRI was poor in temporal resolution and suitable task in restrict space when scanning was not functional. In contrast, NIRS is portable and high temporal resolution. Limitation of NIRS was poor spatial resolution and movement artifact signal may occur. Strangman in 2006 compare reliability of NIRS in different ways of data analysis. 19 subjecs were recruited to evaluation cortical hemodynamic response during complex thumb opposition task. Each trail consist of 16 sec moving time with

16 sec rest. Peak and trough of Δ[O2Hb] and Δ[HHb] were observed. Trial to trial reliability showd morderate correlated while Peason's correlation coeeficient was 0.33. Pairwise comparison of trials show first trial and 16th trial was most poor correlated. However, first trial was more correlated with second trial. The sesults indicated the longer interval between events the less reliabiltiy.

Blocke mean analysis of data showed imrpove measurement reliability. for example, average of 4 trials had better reliability than average with 2 trials. Based on two example above showed time effect on reliability of NIRS was exist[43].

Aging effect on decreasing hemodynamic response also be reported in 2002 by Mehagnoul-Schipper. He compare hemodynamic response of young population with elderly. Control event to measured activation was frequent finger tapping task. Subjects need to tap as fast as possible within time. Significant hemodynamic response was observed not only in young but also in elder, but significant lower response of Δ[O2Hb], Δ[HHb] and Δ[tHb] in elder population was observed. Based on the results, responsibility of NIRS was lower in elder population which may due to aging effect on poor vascular response.[46]

Validation study of NIRS common compared the blood oxygenate level dependent(BOLD) in fMRI with Δ[O2Hb], Δ[HHb] and Δ[tHb]. MRI compatible NIRS device was used to concurrently measure hemodynamic response during specific

event. Okamoto in 2004 compare correlation between ΔBOLD and Δ[O2Hb], Δ[HHb] ,Δ[tHb] during subjects doing apple peeling task. In multiple channel NIRS mapping technique found increase activation in M1, supplementary motor area (SMA) and premotor cortex. The activation area detected by NIRS was similar as fMRI. Compare Δ[O2Hb] with ΔBOLD by pearson's correlation show poor but significant correlated. (r= 0.2, P<0.05) Similar result also found when compared Δ[HHb] with ΔBOLD (r= - 0.19, P<0.05)[47]. Strangman et al in 2002 also compared NIRS and fMRI. However, different result found when using reciprocal ΔBOLD compared with Δ[O2Hb], Δ[HHb] and Δ[tHb]. High correlation was found in Δ[O2Hb] with 1/ΔBOLD and Δ[tHb] with 1/ΔBOLD, r value were between 0.8 to 0.9. Compared Δ[HHb] with 1/ΔBOLD showed poor to moderate correlation which r value were between 0.14 to 0.58.[44] The results support the relationship between concentration of Hb and ΔBOLD was non-linear.

2.6 Serial reaction time task

2.6.1 Implicit sequential motor learning process

Serial reaction time task(SRTT) had been developed by Nissen in 1986[48]. SRTT was used as tool to evaluation temporal organization of behavior by psychologist. Subject need to recognize four visual cues showed on screen independently with 12 words sequence. Each visual cue can find correspnding key on the keyboard. Number 1234 were common used as four visual stimulation. Subjects need to tap correspond key as fast as possible. Any information of exist seuquence in the number series they taped was prevented. Follwing several trials of practice was random number trial which indicated ability of trasfer skill to other condition. In random number trail, the expectation of next number from previous number was violate to aquired sequence.

Subject can built up connection of each number by repeated practice. The process was without awareness of the sequence that make sure learning type was implicit learning. To make sure the implicit learning process not turn into explicit learning pattern through recall test. Recall test often done in end of experiment. Subjects were ask “Whether any sequence exist in the number you taped ?”. If subjects answer that the numbers were seuqencial, they were ask to recall the memory about the sequence they tape.[49]

2.6.2 Brain Activiation pattern during SRTT

Berns in 1997 revealed the brain region where response for the novelty task with awareness. Based on SRTT task, positron emission tomography (PET) was used as monitoring brain functional activity tool. When subjects aquired new sequence, increase activation found in isplateral premotor cortex, isplateral anterior cingulate cortex and contralaeral venral straitum. Contralateral cerebellum and isplateral prenmotor cortex were more responsible for novelty of motor task which constant increas activation through all practice session. Dorsal lateral prefrontal cortex (DLPFC) was more responsible for the sequencial memory mentainence which showed decrease activation when switch from aquired sequence to novelty sequence and constant increase activation in repeated practice.[50]

Sequenctial motor memory was affected by several neural curcuits not only cortico-cortical curcuits but also subcortico-cortical curcuits. Activity of SMA was decrease after repeated TMS applied on M1 to interfered motor memory modification. Interfered motor memory also weaker correlation of activation between SMA, M1, cerebellium, anterior cyngulate cortex and straitum.[51]

Contextual interference of motor task also affected activation level of DLPFC, SMA and M1 in fMRI. Compare with repeatitive sequence(1112, 2223, 3334) or interleaved seuqence (2134, 4312, 1423) practice in SRTT, interleaved sequence

(difficult sequence) showed poor performance initially than repeatitive sequence(easy sequence). Related increase activation in more difficult condition were bilateral occipital lobe, temporal cortex, sensorimotor and premotor areas, premotor area, inderior and medial prefrontal area and medial temporal area.[52] In rentention test after practice session, interleaved sequecne seuqence showed significant better performance in reaction time. Significant increase M1 excitabiltiy was found in interleaced prctice which showed decrease of SICI and increase of ICF and control MEP. The neural curcuit shifted to more excitatory status with more difficult condition.[53] Excitation changed of M1 indicated more efficient of motor memory retrieve after preactice session. Region of increasing cerabral blood flow finally decreased in retention test were isplateral prefrontal, premorot cortex and inferior frontal areas compared to practice session[52]. Prefrontal cortex was more related to declarative memory system which may interaction with procedural memory system. Inhibit dorsal lateral prefrontal cortex (DLPFC) which would induce consolidation of procedural memory[54]. It seems that declarative memory reduce or even complete inhibit procedural memory. Decrease activation DLPFC, supplementary motor (SMA), medial frontal indicates more efficient of motor memory retrieval[52]. In addition to neurophysiological change, reaction time were significant improve compared to initial condition.

Chapter 3 Methods

3.1 Participants

This study was cross-over single blind design. Subject did not know which kind if intervention they received. Participant were randomized into two group, stimulation with motor execution group and isolately received stimulation group. Subject needed to acomplish two trials of experiment which were true stimulation trial and sham stimulation trial. Subjects were recruited from university or community. Inclusion criteria of participants: (1) age between 20 to 40.

Exclusion criteria: (1) Psychological disorder; (2) Have history or family history of epilepsy; (3) Have head trauma history, received brain surgery ,brain tumor, stroke and head metal implant; (4) Implant of pacemaker or electrical stimuator; (5) Vascular syncope or unknown reason syncope; (6) Intermittent headache; (7) Taking drug related to cognitive or emotional status. (8) Poor skin status.

Volunteers fulfilled criteria of above would be enrolled into this study. All subjects should sign on consent which was approved by Chang Gung Medical Foundation Institutional Review Board (see appendix). Subjects were allowed to reject further investigation without any reason. The experiment would immediately stop when uncomfortable status appeared during TENS intervention or receiving TMS such as headache, burn pain of skin, dizziness.

Sample size was estimated based on Liao's study[15]. The effect size was 0.72 calculated by treatment effect of TENS on MEP. Alpha level set at 0.05 and power was set at 80%. Sample size was 24 subjects for each groups which was estimated by G*Power 3.1.3. Total number of subjects need to enroll was 48.

3.2 Study Design

This study was randomized cross-over design. The subjects were randomized into TENS stimulation group and TENS stimulation with SRTT group. Participants needed to accomplish two trials of experiments which include TENS stimulation and sham stimulation with randomized order. Four conditions need to comparison included (1) Motor practice with TENS stimulation (Motor-TENS); (2) Motor practice with sham stimulation (Motor-Sham); (3) TENS stimulation (Stimulus-TENS) and (4) Sham stimulation (Stimulus-Sham). Wash-out period must to be more than 1 week (figure 1). Single blinded was designed in this study.

3.3 Experimental procedure

Basic data include age, gender, medical history, sleeping time were collected. Subjects were randomized into two groups. Permute block randomization was performed. Block number was four which include four conditions. Before start TENS

or sham stimulation, cortical excitability and prefrontal activation in SRTT were measured in baseline. Subject need to tap 7 blocks of SRTT concurrent measured the activation of prefrontal cortex after TENS applied on scalp for 5 minutes. After TENS intervention, Immediate TENS effect on cortical excitability was measured. Cortical excitability and prefrontal activation in SRTT was also measured in follow up test at 30 min and 60 min (Figure 2.).

During TMS assessment, subjects sit on comfortable seat with armrest to prevent any movement artifact in TMS assessment. Hand held circular coil was place on head which generated current in anterior-posterior direction on cortex. The TMS device used in this experiment was MagStim 200 stimulator. Surface electromyogram (EMG) of first dorsal interosseous was assessed by active electrode placed on muscle belly. To ensure no deviation of coil during assessment, dermatograph was use to mark on the hotspot.

In NIRS measurement, The PortaLite produced by Artinis Medical System was use. Detection optodes was placed on FP1 or FP2 in 10-20 EEG electrode system. FP1 and Fp2 were area approximately 20 to 30 mm above midpoint of eyebrow. The emission optodes were laterally placed which approximately F7 or F8. The average photon path from detection to emission cover right superior and middle frontal gyrus which mainly cover Brodmann's area 10[55,56]. The detection optode and emission

optode was covered by goggles with low opacity. Before measuring the concentration of hemoglobin, light leakage was test to ensure no light pass through goggles.

To perform SRTT, subjects need to place right hand on keyboard with finger correspond to key one the key board. Index finger was corresponding to 1; middle finger was 2; ring finger was 3; little finger was 4. A screen was place in front of subject with distance about 1 meter. Head position was with slight flex when looking at screen. Visual cue was at central of screen in identifiable size.

The TENS current was generated by "Enraf-Nonius Muscle stimulator, TensMed 931". The current waveform was biphasic rectangular current with pulse duration was 200 μs. Current was deliver through pair of rubber electrodes (6X8 cm^2) placed on Hotspot and contralateral supraorbital area. Elastic bandages were used to make electrode well contact on skin. Intensity was 2 mA which was below sensory threshold stimulation. Frequency was set at 15Hz based on previous experiment[15] The TENS device was controlled behind subject, so the subjects doesn't know the device was on or not. Any uncomfortable feeling reported by subject or adverse effect such as burn pain of skin, would be recorded.

3.4 Experimental Assessments

Basic data were initially retrieved from subjects included age, gender, current or previous medical history.

TMS stimulations were generated by MagStim 200. To locate hotspot of primary motor cortex respond to FDI muscle, 40 to 50% of TMS intensity was used. The area where induce most high amplitude of MEP was defined as hotspot. After locate hotspot, TMS intensity was decrease in 2% gradient. MT was defined as the smallest intensity which can induce MEPs amplitude more than 50μV in 50% success rate (5 out of 10). Single pulse MEP amplitude which represents cortical excitability was measured at 120% intensity. Pair-pulse stimulation was used to assess intracortical inhibition(ICI) and intracortical facilitation(ICF). Condition stimulation's intensity was set at 70% of MT which was subthreshold level. Following testing stimulation's intensity was set at 120% of MT which was suprathreshold. To assess ICI in this study, inter-stimulation interval was set at 2 and 3ms. To assess ICF, inter-stimulation interval was set at 7, 10, 15 ms. Due to the excitatory or inhibitory effect of pair-pulse stimulation, order of 2 ms, 3 ms, 7 ms, 10 ms and 15ms would be randomized. All TMS assessment was measured at baseline, immediate after TENS stimulation and follow up 30, 60 min.

Motor performance at baseline was assessed by SRTT program generated in Matlab 8.0 version. Three sequence consist of 12 words was used in this study

(2431-2314-1432, 1243-1432-3124, 4213-2431-3142). Random number also used to detect any transfer effect of motor learning. Each trial consists of 120 times of tapping. Inter-trial interval was 30 minutes. Subject need practice 2 block sequence trial and single number sequence trial at baseline, After TENS applied for 5 minutes, subjects need to accomplish 7 block sequence trials. Two block sequence trials and single random number trial were also measured immediate after TENS stimulation and at follow up 30 min, 60 min. NIRS was concurrent measured with SRTT. Δ[O2Hb], Δ[HHb], Δ[tHb] was measured in 10Hz sample rate.

3.5 Statistical Analysis

All statistic were done with Statistical Package for Social Science 17.00 (SPSS, Inc, Chicago, IL, USA). Outcome measurement and demographic data were expressed by mean and standard deviation (mean ± SD).

Basic data were express by descriptive statistic. The Shapiro-Wilk's test was conducted to exam normality of outcome variables. To check the data were fulfilled assumption of homogeneous of variance, Leaven's test was conduct first. Independent t test was used to compare basic data between TENS intervention group and TENS intervention with SRTT group. If variable not fulfilled assumption, non-parametric test was used for further statistical analysis such as Wilcoxon signed-rank tests.

4x4 two-way repeated analysis of variance (ANOVA) was used to analysis MT, MEP, ICI, ICF, Δ[O2Hb], Δ[HHb] and Δ[tHb]. Four conditions need to comparison included (1) Motor practice with TENS stimulation; (2) Motor practice with sham stimulation; (3) TENS stimulation and (4) Sham stimulation. Four evaluation time were compared includes baseline, immediately after TENS intervention and follow up 30 min, 60 min.

SRTT were analysis by two-way repeated ANOVA included reaction time (RT) and correction response rate. 2x15 two-way repeated ANOVA was used to analysis reaction time and correction response rate with sham stimulation condition and TENS intervention condition at 15 block sequence trials. 4X4 two-way repeated ANOVA was used to analysis reaction time and correction rate at 4 random number trials.

To exam normality and homogeneous of data Shapiro-Wilk's test and Mauchly's test of sphericity were used. Post-hoc test would be conduct to analysis main effect or interaction. If data was not fulfilled assumption of two-way ANOVA, non-parametric test would be conduct. Fridemann's test woud be used to analysis data. Wilcoxon signed-rank test would use to pairwise comparison if any effect was found.

To avoid type 1 error, alpha level was set at 0.05.for all variable. Intention to treat method was used when there was any missing data.

Chapter 4 Results

4.1 Basic data and baseline measurements

49 subjects were recruited to this study. 1 subject was excluded due to psychiatric disorder. 25 subject were allocation into TENS intervention with SRTT group. 23 subject were allocated into TENS intervention group. There was no significant difference of sex, age, between two groups at baseline (Table 1). There was no difference of MT or MEP, ICI, ICF, between four testing conditions at baseline (Motor-TENS, Motor-Sham, Stimulus-TENS and Stimulus-Sham). There was no significant difference of reaction time of SRTT in block practice/random practice or [Hbdiff] between Motor-TENS and Motor-Sham at baseline. (Table 2). All data were not fulfilled assumption of parametric test, the analysis were based on nonparametric test. Friedman's test and Wilcoxon sign rank's test were used to analysis effect and post hoc multiple comparisons.

4.2 Effect of cranial TEN Intervention

4.2.1 Results of motor evoked potential

Friedman's test showed significant time effect in Motor-TENS ($P<0.001^*$) and Stimulus-TENS ($P< 0.001^*$) but not in Motor-Sham ($P=0.236$) or Stimulus-Sham ($P=0.484$) (Table 3; Figure 3). Subjects in Motor-TENS trial got significant increase

amplitude of MEP at Immediate effect, follow up 30 mins and 60 mins compared to baseline (P<0.001*; P<0.001*; P<0.001). In Motor-Sham trial, subjects also got increment of MEP amplitude but not reach statistically different. Subjects in Stimulus-TENS trial got significant increase amplitude of MEP at immediate effect, follow up 30 mins and 60 mins compared to baseline (P<0.001*; P<0.001*; P<0.001*) There was no trend or statistical different in Stimulus-Sham trial. Between group comparison show significant higher of MEP in Motor-TENS than Motor-Sham, Stimulus-TENS and Stimulus-sham at follow up 60 mins (P=0.001*, P=0.048*, P0.003*) (Table 3). There was no significant different between Stimulus-TENS and Stimulus-Sham showed weak effect of TENS intervention solely on MEP amplitude (P= 0.543). Combination of Motor practice showed synergistic effect to increase MEP amplitude than Stimulus alone (Table 3). Significant higher amplitude of MEP in Motor-TENS than Stimulus-TENS was observed at follow up 60 min.

4.2.2 Results of pair pulsed stimulation

Significant decrement of ICI was observed in Motor-TENS (P=0.041*) and Motor-Sham (P=0.003*) but not in Stimulus-TENS (P=0.213) or Stimulus-Sham (P=0.211)(Table 4; Figure 4). Motor-TENS trial showed significant decrement of ICI at immediate effect (P=0.024*) compared with baseline. Motor-Sham trial showed

significant decrement of ICI at follow up 30 mins (P= 0.001*) and 60 mins (P=0.04*) compared with baseline. Motor-TENS trial got more decrement of ICI than Motor-Sham at immediate effect (P= 0.037*) and follow up 60 mins (P= 0.032*). Motor-TENS significantly lower of ICI amplitude than Stimulus-Sham in followed up 60 mins (P= 0.042*).

Significant decrease of ICF was observed in Motor-TENS trial (P<0.001*) and Stimulus-TENS (P=0.004*) trial but not in Motor-Sham (P=0.302) or Stimulus-Sham trial (P=0.286) (Table 5; Figure 5). In Motor-TENS trial, significant decrement of ICF was observed at immediate effect (P=0.026*), follow up 30mins (P=0.009*) and 60 mins (P=0.008*) compared with baseline. In Stimulus-TENS trial, significant decrement of ICF were observed at follow up 30 mins (P=0.014*) and 60 mins (P=0.04*) compared with baseline. No significant between group difference was found showed weak effect of TENS or motor practice on ICF modulation.

4.2.3 Results of prefrontal hemodynamic response

Friedman's test showed significant time effect of [Hbdiff] in Motor-TENS trial (P=0.013*) but not in Motor-Sham. Significant increment of [Hbdiff] at intermittent of practice session was observed (P=0.014*) which indicate increase activation in

prefrontal cortex in initial stage of motor learning. In Retention test, decrements of [Hbdiff] at three follow up trials were observed. Significant decrement of prefrontal activation at follow up 30 mins (P=0.007*) compared with baseline which showed consolidation effect of practice (Table 6; Figure 6). Between group comparison showed Motor-TENS trial got significant lower of [Hbdiff] in follow up 30 mins compared to Motor-Sham trial (P=0.03*).

4.2.4 Results of serial reaction time task

The data of Reaction time do not fulfilled the assumption of ANOVA (Sphericity test). Friedman's test showed significant time effect in reaction times in Motor-TENS (P<0.001*) and Motor-Sham (P<0.001*) which indicated improvement reaction time in both trials. However, there was no between group difference. (Figure 7). In transfer task, significant improvements of reaction time were found in Motor-TENS group (P<0.001*) but not Motor-Sham group (P= 0.07). There was no significant different of reaction time between Motor-TENS and Motor-Sham in transfer task (Figure 8).

Chapter 5 Discussion

5.1 Neurophysiological outcomes after cranial TENS intervention

Cranial TENS intervention showed significant effect to modulate cortical excitability. Based on our previous study, concurrent with motor practice and TENS intervention induce excitatory shift of NMDA pathway with disinhibition of GABA-a pathway. However, with independently experiment design of motor task and TENS stimulation, the relationship between motor practice and TENS on cortical excitability were clear. The higher MEPs amplitude Motor-TENS than TENS-Stimulus which was more correlated to motor learning disinhibition hypothesis[57]. Motor practice induce excitatory shift of cortical neuron facilitated the increment of MEP induced by TENS stimulation. Synergistic effect of Motor practice on facilitation effect induced by TENS stimulation was noted. Besides, the results of ICI also showed similar pattern. Motor practice significantly reduced ICI amplitude especially with motor practice. Significant lower of ICI was observed in Motor-TENS than Motor-Sham. TENS Showed Synergistic effect on motor task induced increment activity of inhibitory circuit. Not only TENS but also motor task showed weak effect on ICF modulation indicated that TENS primary involved in inhibitory circuit activity

In NIRS measurement, the results correlated to most of fMRI paradigm of motor learning. Our study revealed that increment of hemodynamic response in initial

practice stage. After 7 blocks of practices, significantly decrease of hemodynamic response in retention test which showed consolidation stage of motor learning. The behavioral outcome also showed significant decrease of reaction time in retention test but no significant different of behavioral outcome was found between Motor-TENS and Motor-Sham.

5.1.1 Motor Evoked Potential

In SRTT paradigm, significantly increment of MEP amplitude after motor practice had been well published.[57-61] Motor practice induced disinhibition of cortical neuron. Increase amplitude of MEP indicated that the increment of cortical excitability. Single pulse TMS measurement majorly detects the strength and excitability of descending cortical spinal tract. Our results showed significant increment of MEP in Motor-TENS and Stimulus-TENS. The cranial TENS intervention facilitated the excitability of descending pathway. Increment of MEP was more correlated to our expect which also seen in other procedural learning paradigm.[58,60] The excitation effect of TENS on MEP also reported by our study previously. However, the results of our study were controversial which showed no correlation with inhibitory circuit or excitatory circuit measurements. Poor correlation between MEP, ICI and ICF also had been reported by other studies. Independent system of MEP, ICI and ICF which indicate the descending

cortical-spinal neuron may not be modulated by cortico-cortical neural excitatory or inhibitory modulation[61].

5.1.2 Intracortical Inhibition

Disinhibition hypothesis of peocedural learning implicated that the decrease activation of GABA-a circuits which lead to cortical representation[59]. The disinhibition mechanisms implicated unmask of existed cortical neuron. This kind of responses can be seen in novel task learning[62]. However, disinhibition of neural circuits may not be observed in well acquainted task. Contrast to our prediction, ICI significantly decrease after motor practice in our study but not increase.

Strafella A. P. in 2011 revealed that amount of suppression induced by ICI were correlated with change of cerebral blood flow[63]. Separated neural circuits induced hemodynamic response indicated that the independent neuron system of ICI and ICF. In our study, ICI significantly increase of suppression after TENS intervention which may represent increase activation of primary motor cortex. Sidhu in 2013 reveal that sustain cycling exercise increase the amount of ICI suppression especially at the final 5 minutes of exercise program[64]. Fatigue effect which lead to increment of ICI which may relate to reduction of cortical drive. In our study, we still cannot excluded the effect of fatigue existed in the motor task.

Previous study showed CES intervention disrupted cortico-cortical connectivity in frontal parietal network. The stimulation parameter was more close to our study concept. TENS intervention showed inhibitory synergistic effect on primary motor cortex which significant decrease amplitude of ICI. Significant increase of ICI indicated that TENS intervention disrupt cortical representation after motor practice.

5.1.3 Intracortical facilitation

ICF was believed to measurement NMDA pathway activity. NMDA was more related to long-term potentiation (LTP) which was increase strength of synaptic transmission. Increase activation of excitatory pathway was observed in motor learning paradigm. However, lack of strong evidence about the role of ICF in motor learning induced plasticity. In our study, increment of MEP and decrement of ICF implicated that existing ceiling effect of on ICF measurement[59]. In medication study, NMDA antagonist medication also result in disruption of motor learning but not recall acquainted task.[65] Our Study showed weak evidence of TENS on ICF modulation eventually to affect motor performance.

5.1.4 Prefrontal hemodynamic response

Motor sequential learning involving in multiple cortical network included dorsolateral prefrontal cortex, premotor cortex, supplementary motor area and primary motor cortex[52]. In initial practice session, initial action selection and inhibit other selections lead to increase hemodynamic response such as superior frontal area and inferior frontal area. After several block of repeated sequence practice, the action selection and inhibition of other selections would be easier than initial stage. Prefrontal hemodynamic responses significantly decrease in retention test which indicated consolidation of sequential motor learning. Previous study showed that DLPFC play an important role in learning consolidation. With disruption by repeated TMS on DLPFC showed significant attenuated of learning effect in the end of acquaintance.[66] The goal directed task with higher cognitive component results in more memory related consolidation which may rely on higher order of motor area such as DLPFC. The consolidation were start after initial learning practice within five hours.[67] In our study, Motor-TENS trial showed significant consolidation effect of learning in prefrontal activation pattern but no Motor-Sham trial. However, motor performance was not different between Motor-TENS and Motor-Sham trial. It seems that TENS intervention reduce high level process demand of aquatinted motor task.

5.1.5 Serial reaction time task

SRTT was related less complexity motor task which was common use as tool about procedural learning paradigm. tACS now still controversial about its effect to modulate cortical excitability as well as behavioral measurement. β band range of frequency seems more effect to altered motor performance. However, our study results also showed no significant difference between Motor-TENS or Motor-Sham. The duration of TENS was much less than contemporary tACS parameter. The weak electrical field induced by TENS may be the cause. Other transcranial electrical stimulation studies with SRTT commonly follow up more than 24 hours. Our study did not test effect of TENS on off-line learning. Need further long-term follow up to reveal the effect of TENS on behavioral outcome.

5.2 Possible clinical application of cranial TENS intervention

MEP was found significantly reduced in some neurological patient such as stroke, Parkinson's disease, dystonia, Alzheimer's disease, Tourette's syndrome and schizophrenia. Especially movement disorder which kind of diseases related to impaired motor system. Abnormal cortical excitability was suitable for clinical transcranial electrical stimulation to deliver. Two Major neurophysiological changes after Cranial TENS intervention were (1) Increase excitability of corticospinal neuron;

(2) Increase intracortical inhibition which was related to increase GABA-a pathway activation. Cranial TENS intervention is suitable for Parkinson's disease. Parkinson's disease showed abnormal imbalance of inhibitory and excitatory circuits. The feature of cortical excitability was decrease of ICI and ICF with increasing of MEP[69,70]. The increase MEP size was related to compensatory mechanism of imbalance between direct pathway and indirect pathway. Decrease substantial nigra secret of dopamine lead to imbalance between direct pathway and indirect pathway. Use biphasic rectangular current stimulation on animal study showed increase dopamine synthesis. Dopamine medication showed increase suppression of intracortcial inhibition and increase MEP size[71]. The cranial TENS intervention may help Parkinson's population normalized intracortical circuits and need further study to prove this concept.

5.3 Limitation

The non-correlated change pattern of ICI, ICF and MEP were still difficult to discuss the structure or physiological condition of cortical neuron. Prefrontal activation showed consolidation effect in retention test. However, the consolidated effect occur within 5 hour after motor practice but our study showed consolidation effect occurred after TENS for 30 mins. In follow up 60, there was no between trials difference. In 60 min follow up, with more blocks of practice, consolidation effect should be greater

than follow up 30 mins. However, there was no difference of prefrontal activation between TENS or Sham. Controversial prefrontal activation pattern still cannot well explain.

5.4 Future Studies

Our study clarified the relationship of motor task and cortical excitability. However, lack of strong evidence about the effect of cranial TENS intervention on motor performance. The pattern of cortical excitability changed after TENS intervention still controversial due to the non-correlated between MEP and ICI. Need further longer follow up (>1day) to reveal the off –line learning effect with or without TENS intervention. Prefrontal cortex activation pattern fulfilled initial activation and late consolidation process. However, TENS intervention was focus on M1 region where may not similar pattern as prefrontal cortex in motor learning process. Tools of NIRS were easily light leakage due to not well cover of optode which may be less reliable tools to measure cortical hemodynamic response. fMRI was more suitable and reliable tool to measuring hemodynamic response in different cortical region with execution of SRTT. Need further comparison of M1 activation and M1 excitability in TENS intervention.

Chapter 6 Conclusion

TENS stimulation can increase cortical excitability and increase GABAnergic activity. Motor task showed synergistic effect on TENS induced increment of cortical excitability and increment of GABAnergic activity. Lower hemodynamic response of prefrontal cortex in retention test indicated TENS reduced high level cortex process demand in acquainted motor task. However, there was no significant different of motor performance with or without TENS intervention.

Reference

1. Zaghi S, Acar M, Hultgren B, Boggio PS, Fregni F. Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. Neuroscientist 2010;16:285-307.

2. Schutter DJ, Hortensius R. Brain oscillations and frequency-dependent modulation of cortical excitability. Brain Stimul 2011;4:97-103.

3. Zaghi S, de Freitas Rezende L, de Oliveira LM, El-Nazer R, Menning S, Tadini L, et al. Inhibition of motor cortex excitability with 15Hz transcranial alternating current stimulation (tACS). Neurosci Lett 2010;479:211-214.

4. Antal A, Boros K, Poreisz C, Chaieb L, Terney D, Paulus W. Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. Brain Stimul 2008;1:97-105.

5. Wach C, Krause V, Moliadze V, Paulus W, Schnitzler A, Pollok B. Effects of 10 Hz and 20 Hz transcranial alternating current stimulation (tACS) on motor functions and motor cortical excitability. Behav Brain Res 2013;241:1-6.

6. Kanai R, Paulus W, Walsh V. Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. Clin Neurophysiol 2010;121:1551-1554.

7. Turi Z, Ambrus GG, Janacsek K, Emmert K, Hahn L, Paulus W, et al. Both the cutaneous sensation and phosphene perception are modulated in a frequency-specific manner during transcranial alternating current stimulation. Restor Neurol Neurosci 2013;31:275-285.

8. Datta A, Dmochowski JP, Guleyupoglu B, Bikson M, Fregni F. Cranial electrotherapy stimulation and transcranial pulsed current stimulation: a computer based high-resolution modeling study. Neuroimage 2013;65:280-287.

9. Warner RL, Johnston C, Hamilton R, Skolnick, M.H., Wilson OB. Transcranial electrostimulation effects on rat opioid and neurotransmitter levels. Life Sci. 1994;54:481-490.

10. Feusner JD, Madsen S, Moody TD, Bohon C, Hembacher E, Bookheimer SY, et al. Effects of cranial electrotherapy stimulation on resting state brain activity. Brain Behav 2012;2:211-220.

11. Alon G, Yungher DA, Shulman LM, Rogers MW. Safety and immediate effect of noninvasive transcranial pulsed current stimulation on gait and balance in Parkinson disease. Neurorehabil Neural Repair 2012;26:1089-1095.

12. Bolognini N, Vallar G, Casati C, Latif LA, El-Nazer R, Williams J, et al. Neurophysiological and behavioral effects of tDCS combined with constraint-induced movement therapy in poststroke patients. Neurorehabil

Neural Repair 2011;25:819-829.

13. Ochi M, Saeki S, Oda T, Matsushima Y, Hachisuka K. Effects of anodal and cathodal transcranial direct current stimulation combined with robotic therapy on severely affected arms in chronic stroke patients. J Rehabil Med 2013;45:137-140.

14. Cameron M, Lonergan E, Lee H. Transcutaneous electrical nerve stimulation (TENS) for dementia. Cochrane Database Syst Rev 2003:CD004032.

15. Liao YH. Effect of trancutaneous electrical nerve stimulation on cortical excitability and motor performance in the primary motor cortex. School and graduate institude of physical therapy: National Taiwan Univiersity, 2013

16. Bergman H, Deuschl G. Pathophysiology of Parkinson's disease: from clinical neurology to basic neuroscience and back. Mov Disord 2002;17 Suppl 3:S28-40.

17. Traversa R, Cicinelli P, Pasqualetti P, Filippi M, Rossini PM. Follow-up of interhemispheric differences of motor evoked potentials from the 'affected' and 'unaffected' hemispheres in human stroke. Brain Res. 1998;24:1-8.

18. Guleyupoglu B, Schestatsky P, Edwards D, Fregni F, Bikson M. Classification of methods in transcranial electrical stimulation (tES) and evolving strategy from historical approaches to contemporary innovations. J Neurosci Methods

2013;219:297-311.

19. Feurra M, Bianco G, Santarnecchi E, Del Testa M, Rossi A, Rossi S. Frequency-dependent tuning of the human motor system induced by transcranial oscillatory potentials. J Neurosci 2011;31:12165-12170.

20. Angelakis E, Liouta E, Andreadis N, Leonardos A, Ktonas P, Stavrinou LC, et al. Transcranial alternating current stimulation reduces symptoms in intractable idiopathic cervical dystonia: a case study. Neurosci Lett 2013;533:39-43.

21. Scherder E, Knol D, van Someren E, Deijen JB, Binnekade R, Tilders F, et al. Effects of low-frequency cranial electrostimulation on the rest-activity rhythm and salivary cortisol in Alzheimer's disease. Neurorehabil Neural Repair. 2003;17:101-108.

22. Lopes S, Davies N, Toumazou C, Grossman N. Theoretical Investigation of Transcranial Alternating Current Stimulation using Laminar Model. Conf Proc IEEE Eng Med Biol Soc. 2012:4152-4155.

23. Manoli Z, Grossman N, Samaras T. Theoretical Investigation of Transcranial Alternating Current Stimulation using Realistic Head Model. IEEE EMBS, 2012

24. Neuling T, Wagner S, Wolters CH, Zaehle T, Herrmann CS. Finite-Element Model Predicts Current Density Distribution for Clinical Applications of tDCS

and tACS. Front Psychiatry 2012;3:83.

25. Manganotti P, Formaggio E, Felice AD, Storti SF, Zamboni A, Bertoldo A, et al. Time-frequency analysis of short-lasting modulation of EEG induced by TMS during wake, sleep deprivation and sleep. Front Hum Neurosci 2013;7:767.

26. Habib D, Dringenberg HC. Alternating low frequency stimulation of medial septal and commissural fibers induces NMDA-dependent, long-lasting potentiation of hippocampal synapses in urethane-anesthetized rats. Hippocampus 2009;19:299-307.

27. Kirsch DL, Smith RB. The use of cranial electrotherapy stimulation in the management of chronic pain: A review. NeurorRehabilitation. 2000;14:85-94.

28. Heffernan M. The effect of variable microcurrent on EEG spectrum and pain control. canadian journal of clinical medicine 1997;4:4-11.

29. Heffernan MS. Comparative effects of microcurrent stimulation on EEG spectrum and correlation dimension. Integr Physiol Behav Sci. 1996;31:202-209.

30. Schroeder MJ, Barr RE. Quantitative analysis of the electroencephalogram during cranial electrotherapy stimulation. Clin Neurophysiol. 2001;112:2075-2083.

31. Hallett M. Transcranial magnetic stimulation: a primer. Neuron

2007;55:187-199.

32. Horvath JC, Perez JM, Forrow L, Fregni F, Pascual-Leone A. Transcranial magnetic stimulation: a historical evaluation and future prognosis of therapeutically relevant ethical concerns. J Med Ethics 2011;37:137-143.

33. Kobayashi M, Pascual-Leone A. Transcranial magnetic stimulation in neurology. The Lancet Neurology 2003;2:145-156.

34. Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. Clin Neurophysiol 2012;123:858-882.

35. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMSCG. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 2009;120:2008-2039.

36. Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol 1994;91:79-92.

37. Tranulis C, Gueguen B, Pham-Scottez A, Vacheron MN, Cabelguen G, Costantini A, et al. Motor threshold in transcranial magnetic stimulation:

comparison of three estimation methods. Neurophysiol Clin 2006;36:1-7.

38. List J, Kubke JC, Lindenberg R, Kulzow N, Kerti L, Witte V, et al. Relationship between excitability, plasticity and thickness of the motor cortex in older adults. Neuroimage 2013;83:809-816.

39. Elahi B, Hutchison WD, Daskalakis ZJ, Gunraj C, Chen R. Dose response curve of Associative Plasticity in human motor cortex and interactions with motor learning. J Neurophysiol 2013

40. Di Lazzaro V, Oliviero A, Meglio M, Cioni B, Tamburrini G, Tonali P, et al. Direct demonstration of the effect of lorazepam on the excitability of the human motor cortex. Clin Neurophysiol. 2000;111:794-799.

41. Ziemann U, Chen R, Cohen LG, Hallett M. Dextromethorphan decreases the excitability of the human motor cortex. Neurology. 1998;51:1320-1324.

42. Butler AJ, Wolf SL. Putting the brain on the map: use of transcranial magnetic stimulation to assess and induce cortical plasticity of upper-extremity movement. Phys Ther 2007;87:719-736.

43. Strangman G, Goldstein R, Rauch SL, Stein J. Near-infrared spectroscopy and imaging for investigating stroke rehabilitation: test-retest reliability and review of the literature. Arch Phys Med Rehabil 2006;87:S12-19.

44. Strangman G, Culver JP, Thompson JH, Boas DA. A Quantitative Comparison

of Simultaneous BOLD fMRI and NIRS Recordings during Functional Brain Activation. NeuroImage 2002;17:719-731.

45. Haeussinger FB, Heinzel S, Hahn T, Schecklmann M, Ehlis AC, Fallgatter AJ. Simulation of near-infrared light absorption considering individual head and prefrontal cortex anatomy: implications for optical neuroimaging. PLoS One 2011;6:e26377.

46. Mehagnoul-Schipper DJ, van der Kallen BF, Colier WN, van der Sluijs MC, van Erning LJ, Thijssen HO, et al. Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. Hum Brain Mapp 2002;16:14-23.

47. Okamoto M, Dan H, Shimizu K, Takeo K, Amita T, Oda I, et al. Multimodal assessment of cortical activation during apple peeling by NIRS and fMRI. Neuroimage 2004;21:1275-1288.

48. Nissen MJ, Bullemer P. Attentional requirements of learning: evidence from performance measures. Cogn. Psychol. 1987;19:1-32.

49. Robertson EM. The serial reaction time task: implicit motor skill learning? J Neurosci 2007;27:10073-10075.

50. Berns GS. Brain Regions Responsive to Novelty in the Absence of Awareness.

Science 1997;276:1272-1275.

51. Censor N, Dayan E, Cohen LG. Cortico-subcortical neuronal circuitry associated with reconsolidation of human procedural memories. Cortex 2013

52. Lin CH, Knowlton BJ, Chiang MC, Iacoboni M, Udompholkul P, Wu AD. Brain-behavior correlates of optimizing learning through interleaved practice. Neuroimage 2011;56:1758-1772.

53. Lin CH, Chiang MC, Wu AD, Iacoboni M, Udompholkul P, Yazdanshenas O, et al. Age related differences in the neural substrates of motor sequence learning after interleaved and repetitive practice. Neuroimage 2012;62:2007-2020.

54. Galea JM, Albert NB, T. D, Miall RC. Disruption of the Dorsolateral Prefrontal Cortex Facilitates the Consolidation of Procedural Skills. J Cogn Neurosci. 2010;22

55. Paul F, Vermeij A, van Beek AHEA, Olde Rikkert MGM, Claassen JAHR, Kessels RPC. Effects of Aging on Cerebral Oxygenation during Working-Memory Performance: A Functional Near-Infrared Spectroscopy Study. PLoS ONE 2012;7:e46210.

56. Okamoto M, Dan H, Sakamoto K, Takeo K, Shimizu K, Kohno S, et al. Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping.

NeuroImage 2004;21:99-111.

57. Rosenkranz K, Kacar A, Rothwell JC. Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. J Neurosci 2007;27:12058-12066.

58. Camus M, Ragert P, Vandermeeren Y, Cohen LG. Mechanisms controlling motor output to a transfer hand after learning a sequential pinch force skill with the opposite hand. Clin Neurophysiol 2009;120:1859-1865.

59. Chen R. Interactions between inhibitory and excitatory circuits in the human motor cortex. Exp Brain Res 2004;154:1-10.

60. Perez MA, Wise SP, Willingham DT, Cohen LG. Neurophysiological mechanisms involved in transfer of procedural knowledge. J Neurosci 2007;27:1045-1053.

61. Marker RJ, Stephenson JL, Kluger BM, Curran-Everett D, Maluf KS. Modulation of intracortical inhibition in response to acute psychosocial stress is impaired among individuals with chronic neck pain. J Psychosom Res 2014;76:249-256.

62. Smyth C, Summers JJ, Garry MI. Differences in motor learning success are associated with differences in M1 excitability. Hum Mov Sci 2010;29:618-630.

63. Strafella AP, T. P. Cerebral blood-flow changes induced by paired-pulse

transcranial magnetic stimulation of the primary motor cortex. J Neurophysiol. 2001;85:2624-2629.

64. Sidhu SK, Lauber B, Cresswell AG, Carroll TJ. Sustained cycling exercise increases intracortical inhibition. Med Sci Sports Exerc 2013;45:654-662.

65. Donchin O, Sawaki L, Madupu G, Cohen LG, R. S. Mechanisms Influencing Acquisition and Recall of Motor Memories. J Neurophysiol. 2002;88:2114-2123.

66. Kantak SS, Sullivan KJ, Fisher BE, Knowlton BJ, Winstein CJ. Neural substrates of motor memory consolidation depend on practice structure. Nat Neurosci 2010;13:923-925.

67. Shadmehr R. Neural Correlates of Motor Memory Consolidation. Science 1997;277:821-825.

68. Williams PS, Hoffman RL, Clark BC. Cortical and spinal mechanisms of task failure of sustained submaximal fatiguing contractions. PLoS One 2014;9:e93284.

69. Vacherot F, Attarian S, Eusebio A, Azulay JP. Excitability of the lower-limb area of the motor cortex in Parkinson's disease. Neurophysiologie Clinique/Clinical Neurophysiology 2010;40:201-208.

70. Ni Z, Bahl N, Gunraj CA, Mazzella F, R. C. Increased motor cortical

facilitation and decreased inhibition in Parkinson disease. Neurology. 2013;80:1746-1753.

71. Bares M, Kanovsky P, Rektor I. Disturbed intracortical excitability in early Parkinson's disease is l-DOPA dose related: a prospective 12-month paired TMS study. Parkinsonism Relat Disord 2007;13:489-494.

FIGURES

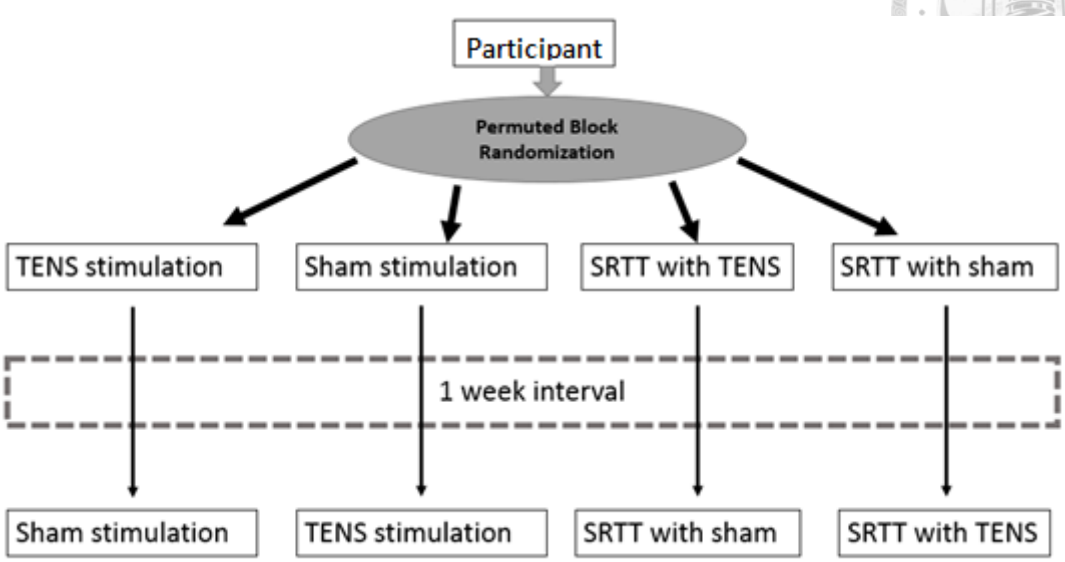


Figure 1. Randomized Crossed-over designed in this study. Subjects were randomized into 4 testing conditions. Subjects received another trials of sham stimulation or TENS intervention with 1 week interval.

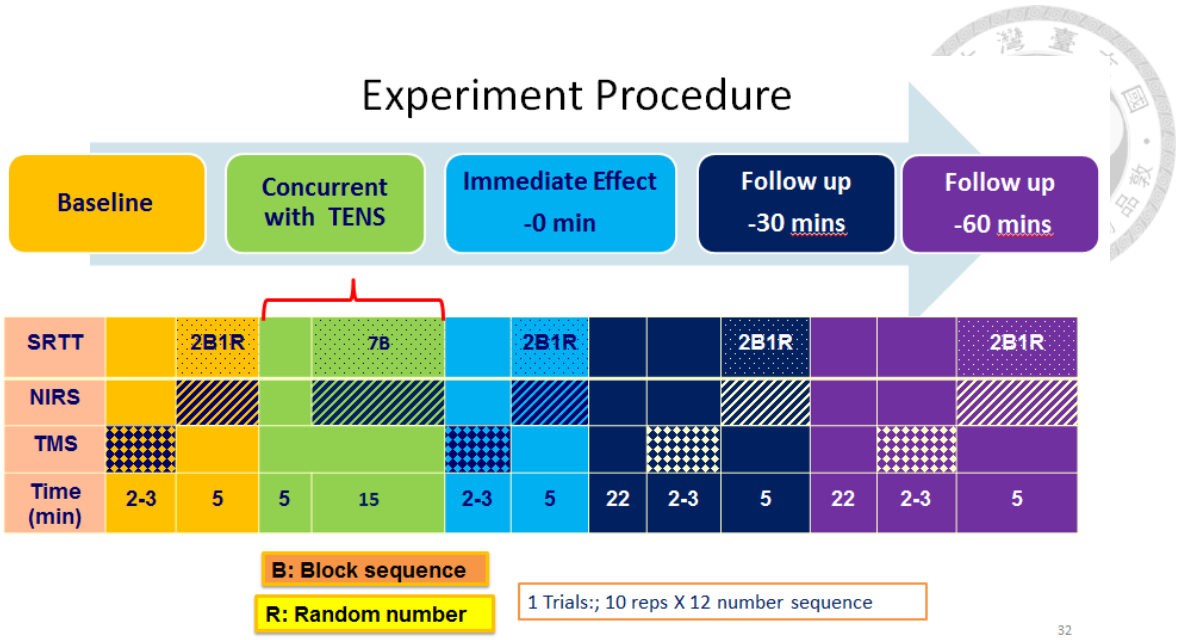


Figure 2. The figure shows experimental procedure of this study. NIRS and SRTT measurement only delivered in Motor-TENS trial and Motor-Sham trial. Intermittent TENS intervention was concurrently measurement Prefrontal cortical hemodynamic response.

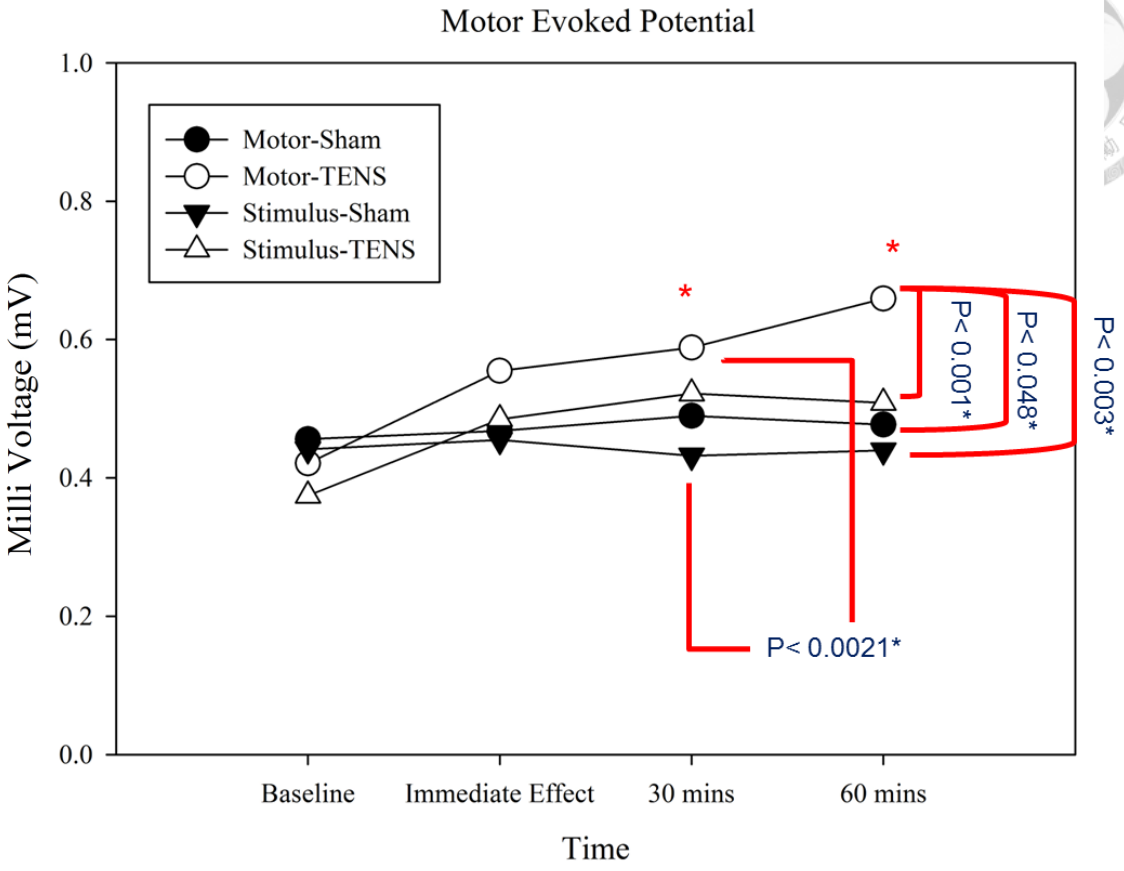


Figure 3.Motor-TENS and Stimulus-TENS showed significant increment of MEP size Motor-TENS showed the superior excitatory response. However, there was no significant different of MEP size change in Motor-Sham and Stimulus-Sham.

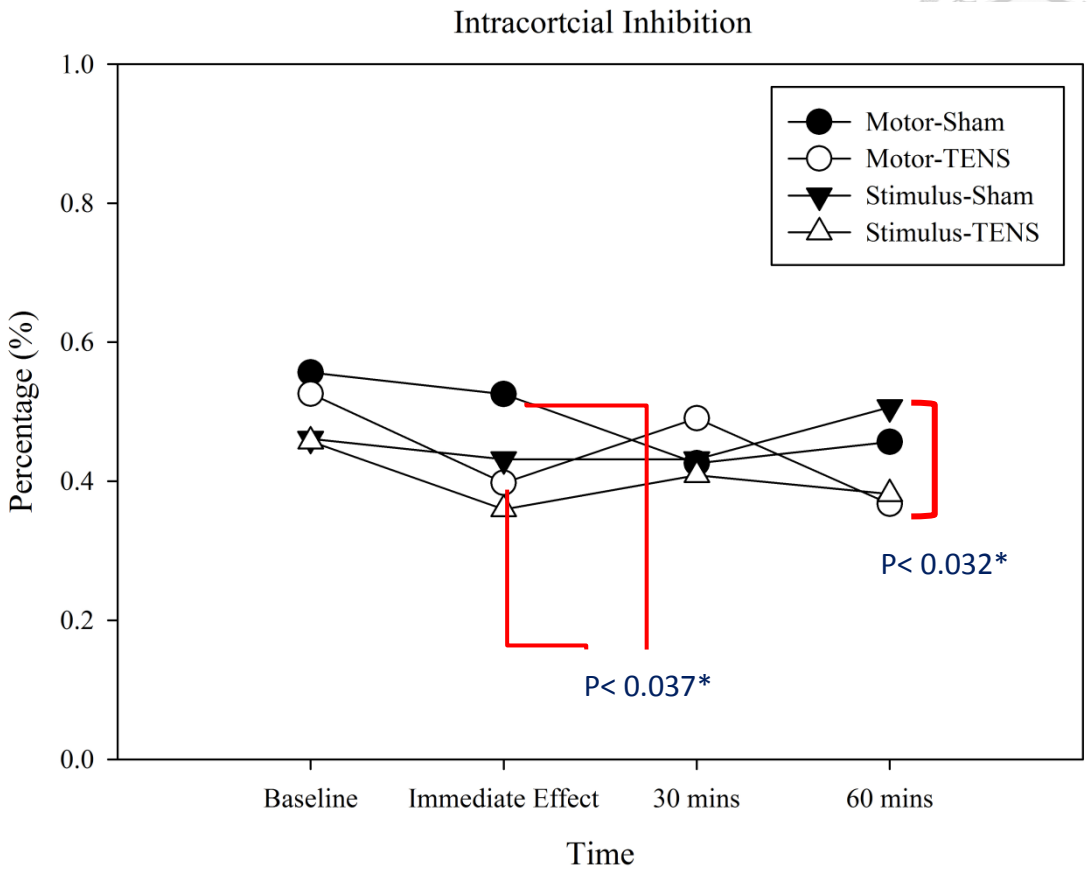


Figure 4 Motor-Sham and Motor-TENS showed significant declined of ICI. There was no significant different of ICI in Stimulus-Sham and Stimulus-TENS. Motor-TENS got significant lower ICI than Motor-Sham indicated that TENS increase suppression induced by ICI.

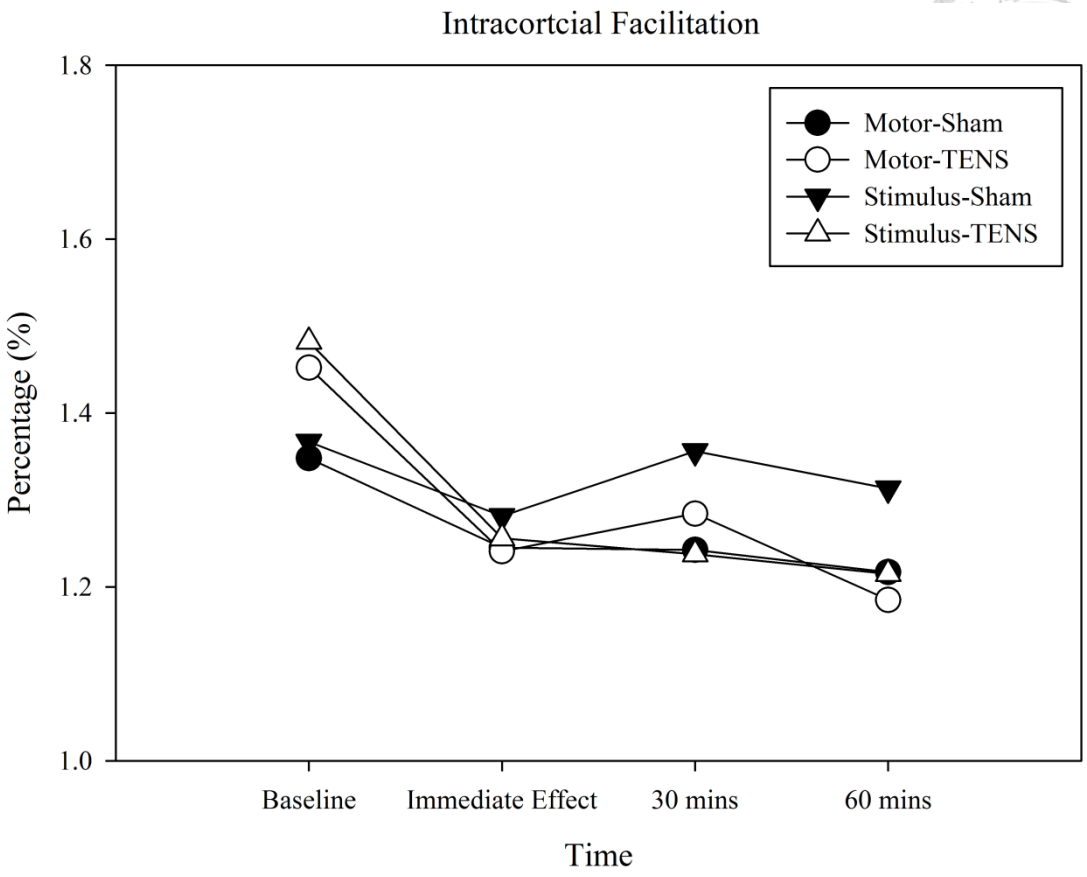


Figure 5 Significant decrease of ICF in Motor-TENS and Stimulus-TENS. However, there was no significant difference between Motor-TENS, Motor-Sham, Stimulus-Sham and Stimulus-TENS in immediate effect or follow up measurements. Indicated that poor evidence of ICF decrease after TENS intervention.

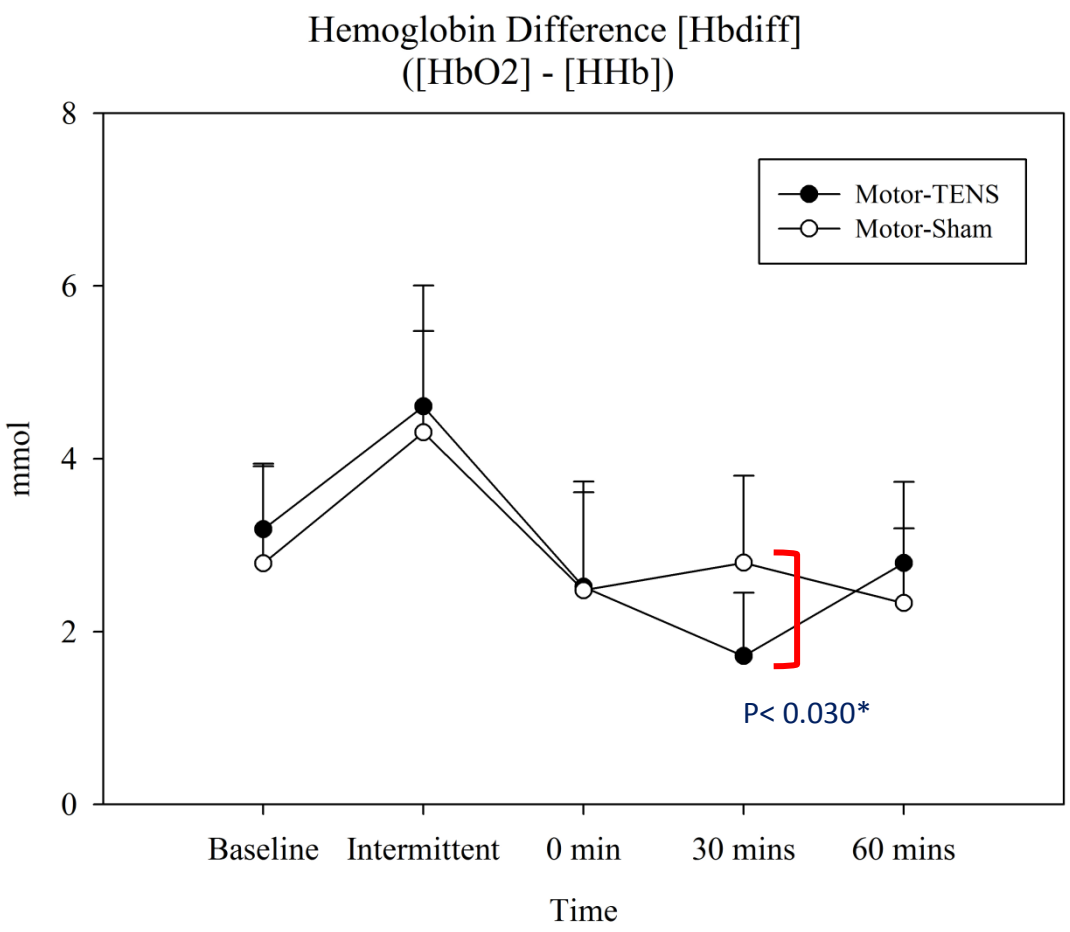


Figure 6. This figure showed hemodynamic response during motor practice. Change of hemoglobin difference means cerebral oxygenation status which implicated level of hemodynamic response. Prefrontal hemodynamic response significantly increase in intermittent of TENS intervention and motor practice. Following significant decrease activation in retention task in Motor-TENS trial indicated the TENS reduce higher level cortical demand. Motor-TENS got significant lower of [Hbdiff] in follow up 30 mins

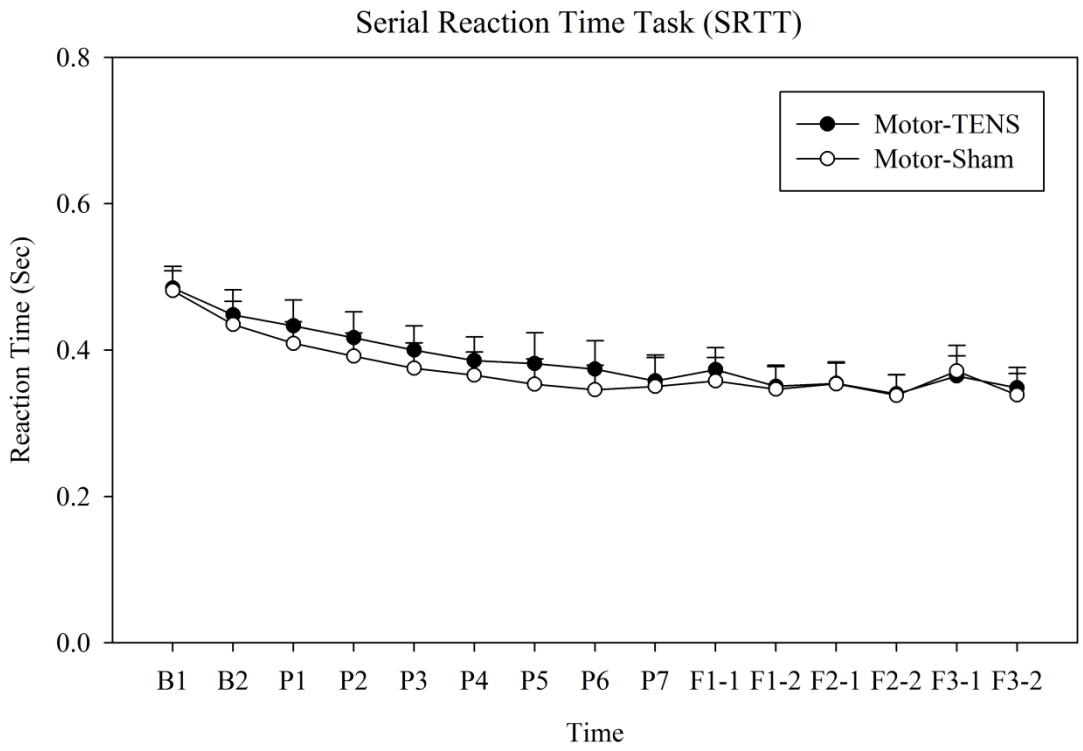


Figure 7. There was no significant different of reaction time of Motor-TENS and Motor-Sham in all practice trials. A trend of reduce decrease reaction time was found in Motor-TENS during concurrent TENS with motor execution.

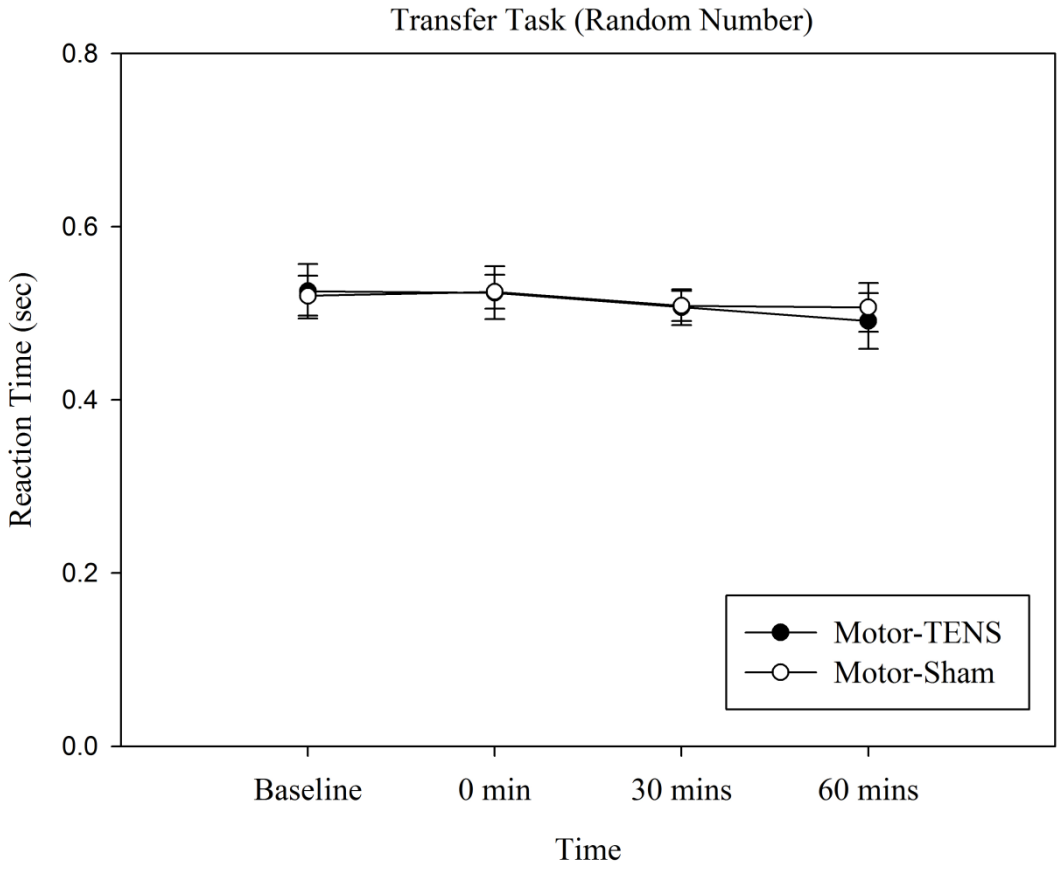


Figure 8. In transfer task (random number trials) showed no significant different between Motor-TENS and Motor-Sham in immediate effect and follow up. Both group showed decrease of reaction time after practice.

TABLES

Basic Data/Group	Motor-TENS/Sham	Stimulus-TENS/Sham	Independent t test
Gender (F:M)	12:13	13:10	
Age	22.4±1.7	21.9±1.5	P= 0.309

Table1. The basic data of participant. No significant different of sex and age between two groups

Neurophysiological Measurement	Motor-TENS	Motor-Sham	Stimulus-TENS	Stimulus-Sham	Independent t Test
MT (%)	54.52±8.0	53.6±8.0	56.7±11.5	56.4±10.2	>0.05
MEP (mV)	0.42±0.21	0.46±0.22	0.37±0.20	0.44±0.20	>0.05
ICI (2, 3ms)	0.53±0.24	0.56±0.21	0.46±0.17	0.46±0.26	>0.05
ICF (7, 10, 15ms)	1.35±0.34	1.45±0.30	1.37±0.29	1.48±0.45	>0.05
[Hbdiff] (mol)	2.79±2.92	3.18±1.84			0.932
RT-block sequence (sec)	0.48±0.07	0.48±0.06	-	-	0.695
RT-Random number (sec)	0.53±0.07	0.52±0.05	-	-	0.683

Table 2 The baseline of all outcome measurements. There was no significant different of MT, MEP, ICI, ICF between four testing trials. In motor related outcome measurements, there was also no significant different of initial prefrontal activation ([Hbdiff]), RT in block sequence practice and RT in random number practice between Motor-TENS and Motor-Sham.

Motor Evoked Potential (㎷)	Baseline	Immediate Effect	30 mins	60 mins	Friedman's Test (P value)
Motor-TENS	0.42±0.22	0.55±0.24	0.59±0.25	0.66±0.24	>**0.001***
Motor-Sham	0.46±0.22	0.47±0.22	0.49±0.21	0.48±0.21	0.236
Stimulus-TENS	0.37±0.20	0.48±0.25	0.52±0.30	0.51±0.31	>**0.001***
Stimulus-Sham	0.44±0.20	0.45±0.22	0.43±0.21	0.44±0.21	0.484
Between group comparison (Wilcoxon sign rank test; p value)					
Motor-TENS/Motor-Sham	0.753	0.189	0.076	**0.001***	
Stimulus-TENS/Stimulus-Sham	0.089	0.715	0.144	0.543	
Motor-TENS/Stimulus-TENS	0.447	0.346	0.362	**0.048***	
Motor-TENS/Stimulus-Sham	0.784	0.073	**0.021***	**0.003***	

Table 3 This table showed the results of MEP. Significant increase MEP size was found in Motor-TENS and Stimulus-TENS. There was no significant difference in Motor-Sham and Stimulus-Sham. Between trials comparison showed MEP amplitude of Motor-TENS significant higher than Motor-Sham in follow up 60 mins. Besides, Motor-TENS also got higher MEP amplitude than Stimulus-TENS in follow up 60 mins. It seems that Motor-TENS trail got superior facilitation than received TENS intervention alone. .

Intracortical Inhibition (%)	Baseline	Immediate Effect	30 mins	60 mins	Friedman's Test (P value)
Motor-TENS	0.53±0.24	0.40±0.18	0.49±0.21	0.37±0.16	0.003*
Motor-Sham	0.56±0.21	0.53±0.18	0.43±0.16	0.46±0.15	0.041*
Stimulus-TENS	0.46±0.26	0.36±0.19	0.41±0.23	0.38±0.24	0.211
Stimulus-Sham	0.46±0.17	0.43±0.18	0.43±0.16	0.51±0.23	0.213
Between group comparison (Wilcoxon sign rank test; p value)					
Motor-TENS/Motor-Sham	0.391	**0.037***	0.290	**0.032***	
Motor-TENS/Stimulus-Sham	0.429	0.627	0.330	**0.042***	

Table 4. This table showed the results of ICI. Significant increase suppression induced by ICI was found in Motor-TENS and Motor-Sham. Between trials comparison showed Motor-TENS significant increase more ICI suppression than motor-Sham in follow up 60 mins. Based on these findings, TENS intervention facilitated ICI suppression induced by motor task.

Intracortical Facilitation (%)	Baseline	Immediate Effect	30 mins	60 mins	Friedman's Test (P value)
Motor-TENS	1.45±0.30	1.24±0.35	1.28±0.25	1.18±0.18	>0.001*
Motor-Sham	1.35±0.34	1.24±0.24	1.24±0.24	1.21±0.3	0.302
Stimulus-TENS	1.48±0.45	1.26±0.35	1.24±0.28	1.21±0.24	0.004*
Stimulus-Sham	1.37±0.29	1.28±0.32	1.36±0.30	1.31±0.26	0.286
Between group comparison (Wilcoxon sign rank test; p value)					
Motor-TENS/Motor-Sham	0.317	0.475	0.607	0.732	
Motor-TENS/ Stimulus-Sham	0.287	0.563	0.503	0.094	
Stimulus-TENS/Stimulus-Sham	0.808	0.761	0.274	0.107	

Table 5. This table showed the results of ICF. Significant decreases of ICF were found in Motor-TENS and Stimulus-TENS. Based on the results, TENS intervention decrease amount of facilitation induced by ICF. There was no significant different between four different trial indicated weak effect of TENS on ICF modulation.

[Hbdiff] (mmol)	Baseline	Intermittent	0 min	30 mins	6o mins	Friedman's Test (P value)
Motor-TENS	3.18±1.84	4.61±3.54	2.52±3.10	1.72±1.86	2.79±2.38	0.013*
Motor-Sham	2.79±2.91	4.30±2.96	2.48±2.87	2.79±2.55	2.33±2.19	0.053
Between group comparison (Wilcoxon sign rank test; p value)						
Motor-TENS/Motor-Sham	0.932	0.864	0.757	0.030*	0.493	

Table 6. This table showed the results of prefrontal hemodynamic response. Significant increase activation in initial learning and following decrease activation in retention test. Between trials comparison showed Motot-TENS got significant lower of [Hbdiff] in follow up 30 mins.

APPENDIX

Permission of Institutional Review Board and Consent Form

長庚醫療財團法人人體試驗倫理委員會
臨床試驗同意證明書

地　址：333 桃園縣龜山鄉舊路村頂湖路123
傳　真：03-3494549
聯絡人及電話： 邱春櫻 03-3196200 ext. 3714
電子郵件信箱： cci@cgmh.org.tw

試驗名稱： 經皮神經電刺激應用於主要動作皮質區對於皮質活性與內隱性動作學習時皮質血液動力學反應之影響：經顱磁刺激與近紅外線吸收光譜研究

本院案號： 102-2266A3

試驗期間： 102年08月12日起至103年07月11日止

主持人： 林口物理治療學系張雅如副教授

共同主持人： 陸哲駒、黃美涓

執行機構： 長庚大學

同意計畫書版本： 版本一 20130607

同意之受試者同意書版本： 版本二 20130715

通過日期： 102年08月12日

通過會期： 102年07月31日

※請於到期前二個月繳交期中報告以利本會進行審查※

長庚醫療財團法人
人體試驗倫理委員會謝燦堂主席

中　華　民　國　102　年　08　月　15　日

人體試驗倫理委員會
核准日期
102. 08. 12
長庚醫療財團法人

長庚大學
受試者同意書

一、 試驗主題：經皮神經電刺激應用於主要動作皮質區對於皮質活性與內隱性動作學習時皮質血液動力學反應之影響：經顱磁刺激與近紅外線吸收光譜研究

二、簡介

近來非侵入型腦刺激包括了經顱直流電刺激、經顱交流電刺激、經顱磁刺激，而目前經顱直流電刺激目前已經有相當多的實驗證實，藉由給予大腦具有極性的刺激可有效的調節大腦皮質興奮性，陽極刺激可以有增加皮質的興奮性，而陰極則可以抑制皮質的興奮性，這樣的陰陽及差異可以應用在調節特定大腦區域活性來增進如決策、語言、記憶、感知覺和動作表現等。

而目前經顱交流電刺激對於皮質興奮性影響的討論則尚未有一致結論，但是可以從不同的實驗當中發現不一樣電流刺激的頻率可以造成不同的效果，概念為藉由不同頻率的電刺激，影響大腦本身的震盪現象，交流電的使用相較於直流電是較為安全，而本實驗概念較接近經顱交流電刺激，藉由使用經皮神經電刺激在主要動作皮質，來討論低頻率的脈衝式雙極方波電刺激對於大腦皮質興奮性的影響。

近紅外線吸收光譜目前已廣泛應於量測組織血流量，相較於功能性磁振照影成本相較之下是較低的，而也有文獻將這樣的技術證實可有效量測大腦血流量的變化，近紅外線吸收光譜藉由提供微弱不同波長的紅外線，紅外線在穿過不同組織後會有不一樣的吸收程度，藉此量測大腦皮質之血流量，因此本實驗將會在動作測試的同時量測您大腦血流量的變化，而所投射的近紅外線為低能量無感覺的。

在同意參加這項研究計畫之前，研究主持人會向您說明這份受試者同意書，請您充分了解此份同意書的內容。這份同意書內容包括試驗目的、方法與程序和可能的益處、危險、不舒服及注意事項。同意書也提及其他的治療方法和您可以隨時終止參加本研究的權利。如果您自願參加並同意參加本研究，您將會收到這份同意書的副本。

您參加的這項研究計畫將會全程介入約兩次、每次約兩小時，全部預計約有 48 位受試者將參與這項研究。

三、試驗基本資料　　病歷號碼：_____

1. 計畫編號：
IRB 案號：102-2266A3
2. 執行單位： 長庚大學 復健科學研究所
3. 委託單位： 無
4. 主要主持人：張雅如　　服務單位：長庚大學 物理治療學系
職稱：副教授　　電話：03-2118800 *5515
共同主持人：黃美涓　　服務單位：長庚醫院 桃園分院復健科
職稱：教授　　電話：03-3196200 *2376
協同主持人：陸哲駒　　服務單位：臺灣大學 物理治療學系
職稱：助理教授　　電話：02-33668133
5. 受試者姓名：
性別：　　出生日期：
通訊地址：
聯絡電話：

四、試驗目的

本研究計畫的目的是為了要了解：使用經皮神經電刺激作為一介入工具的可行性，期望能藉此了解使用低電流之經皮神經電刺激是否對健康成人的大腦皮質興奮性、動作學習過程、大腦血流量是否有任何影響。

五、試驗方法與程序說明

（一）如果您符合下列的納入/排除條件才有資格參與本試驗

1. 納入標準：二十歲到四十歲之健康成年人
2. 排除條件：
(1) 不穩定之精神、心理或健康狀況
(2) 本身或家族中有癲癇病史者
(3) 腦部曾受過創傷，如手術開刀、腦瘤、中風或植入顱內金屬物者
(4) 體內裝有心律調節器或其他電刺激器
(5) 曾因不明原因昏厥、常頭痛者
(6) 服用影響認知或情緒之相關藥物

（二）方法及程序說明

若您決定加入本研究且簽署這份同意書後，我們將會對您進行基本資料的收集(包括年齡、性別、用藥情形等)。本試驗的地點在長庚大學復健科學研究所的神經肌肉學實驗室中進行，全程試驗分二次，每次進行時間約為 2 小時，其中接受 20 分鐘經皮神經電刺激。

人體試
102
長庚

您將會被隨機分配到實驗一與實驗二

實驗一：

第一次經顱磁刺激評估→ 經皮神經電刺激介入 20 分鐘 →0 分鐘第二次評估 → 30 分鐘第三次評估 → 30 分鐘第四次評估

實驗二：

第一次經顱磁刺激評估 + 動作評估與腦部血流量偵測 →經皮神經電刺激介入 20 分鐘 → 0 分鐘第二次評估 → 30 分鐘第三次評估 → 30 分鐘第四次評估

本試驗將收集 48 位受試者，並使用「隨機單盲」的方式進行研究。「隨機」的目的是確保研究結果不被人為因素影響，(15 赫茲、無電流輸出的順序)與(是否質性動作測試)將以抽籤的方式隨機決定，「單盲」的意思為在試驗進行過程中您將不被告知目前正在介入方式是那一種。

實驗流程如下圖：

進行介入時，我們會將經皮神經電刺激的電極片一擺放在左側顱骨的位置，一擺放在左側前額眉毛上方處，並使用彈性綁帶固定海綿電極片，經皮神經電刺激器提供持續 2 毫安培的微量無感電流。

另外，進行評估時會將圓形線圈會擺放在頭顱位置約為中間的位置，並以防水筆標記在頭皮上，確保在試驗過程中皆以此標記作為經顱磁刺激的線圈是否在相同的位置。提醒您，經顱磁刺激的評估過程是非侵入性的，而每一次的磁場發射皆會有類似空氣槍的聲音，請您不用過於擔心，但請配合：評估過程中請保持清醒，以避免因打瞌睡而影響試驗結果。而近紅外線吸收光譜也為非侵入型的量測工具，將探頭放置在頭頂的部分即可量測到大腦血流變化，測試過程中不會有任何感覺。

以上臨床研究您可自由選擇參與或不參與，若您在試驗過程中有任何原因不想繼續進行，您可以不需提出任何理由，隨時要求退出研究。

倫理委員會
准日期
08. 12
療財團法人

六、可預期之風險、副作用、發生率及處理方法：

您參加本研究計畫可能會有一些風險。本試驗所使用的經皮神經電刺激已通過衛生署核可，目前已屬於可安全上市之醫療器材，在醫院診所均廣泛使用。而使用電刺激可能的風險主要是電流強度過大和電極片的擺放不當所引起的皮膚燙燒，本試驗參考過去文獻的電流設定，皆為低強度(小於 2 毫安培)的無感電流，且過去試驗中所有受試者皆可接受其刺激、皆無受試者因不適而中斷試驗，唯有兩篇指出因電極片刺激引起的些微灼熱感和輕微燙燒的例子，**而在過去文獻中產生電燒傷的比率約占所有受試者 4%到 9%**，但試驗後追蹤一至兩周後皆無造成持續傷害，**除此之外並沒有發現其他不良反應**。因此，本試驗過程中，會確實注意電極片是否正確的

擺放，並在過程中隨時留心受試者是否有不適的情形發生，以降低發生皮膚燙燒的危險。

另外，經顱磁刺激也是通過衛生署核可，屬於可安全上市之醫療器材，正常使用下無安全疑慮，一般感到不適的情形多在治療過程中頭皮或臉部會有輕微的收縮或刺痛感，也可能有輕度短時間的頭痛或頭暈。關於經顱磁刺激可能會導致癲癇發作、精神上的異常症狀等風險，是使用重複性刺激的模式，而本試驗使用的單脈衝和雙脈衝刺激的模式，過去文獻回顧中罕有類似風險例子。而且，若參考與本試驗相同使用經顱磁刺激做為評估工具的相關研究，皆無報告指出因刺激上肢之大腦動作皮質區而有不適情形的例子。

提醒您，本試驗可預期的不良反應包含頭昏、頭痛、嘔吐，脖子痛，疲倦，刺激部位是否發癢、刺痛、發紅等問題，試驗進行中我們將會隨時監測，若過程中您對經皮神經電刺激或經顱磁刺激有產生明顯不適的反應或任何其他異常反應，我們將會立即停止實驗介入並進行處理。

七、其他可能之治療方式及說明

本試驗參加的受試者皆為健康人，所以無需其他的治療方式。

八、本試驗之禁忌與限制，請您務必要充分配合之事項

本試驗無任何特殊的禁忌活動。唯請配合：評估過程中請保持清醒。

九、預期試驗效果

預期結果是期望在使用經皮神經電刺激提供雙向脈衝型電刺激介入後，大腦皮質興奮性上之改變有顯著差異，是否會影響動作學習與動作執行中的大腦血流量變化，而研究的結果將可提供更多有效之臨床試驗，以利於未來回饋至臨床復健的訓練上。

十、緊急狀況之處理

若您發現您有因為研究經皮神經電刺激或經顱磁刺激的使用而感到不舒服的情形，**請立即告知實驗執行者，若是發生昏厥與癲癇發作等緊急事件時，緊急處理後將會送往長庚醫院桃園分院做後續處置，並通報**主持人張雅如(03-2118800 轉 5515)、共同主持人陸哲駒(02-33668133、0968-662939)。

十一、補助、費用負擔與損害補償：

1. 補助：本試驗將會補助您來參與試驗時的交通費用每次新台幣五百元整(三次共新台幣一千五百元整)。
2. 費用負擔：參與本試驗您不需繳交或承擔任何費用。
3. 損害補償：如依本研究所訂臨床試驗計劃因而發生不良事件或造成您的

人體試驗
核
102.
長庚醫

損害，由本試驗主持人負補償責任，但本受試者同意書上所記載之預期不良反應，不予補償。除上述之補償外，本研究不提供其他形式之補償。您不會因為簽署本同意書而喪失在法律上的任何權利。若您不願意接受這樣的風險，請勿參加試驗。
十二、保護隱私與機密性 我們將會有一個試驗代碼代表您的身分，此代碼不會顯示您的姓名。請您亦瞭解若簽署同意書即同意您的原始醫療紀錄可直接受監測者、稽核者、研究倫理委員會及主管機關檢閱，以確保臨床試驗過程與數據符合相關法律及法規要求。上述人員並承諾絕不違反您的身分之機密性。
十三、試驗之退出與中止 受試者或立同意書人有權在無任何理由情況下，隨時要求終止參與試驗，此將不會減損您的正當醫療權益與法律權利。試驗主持人亦可能於必要時中止該試驗之進行。
十四、受試者權利 如果您在試驗過程中對試驗產生疑問；對身為患者之權利有意見或懷疑因參與研究而受害時；可與本院之人體試驗倫理委員會聯絡請求諮詢，其電話號為：(03)319-6200 轉 3703。
十五、試驗成果及權益歸屬 如本試驗計畫成果產生學術文獻發表、實質效益或衍生其他權益時，亦同意無償捐贈給本研究團隊作為疾病預防、診斷及治療等公益用途。
十六、聲明 本試驗內容及同意書已經　　完整口頭告知及說明，受試者本人或/及法定代理人已充分瞭解並同意。

倫理委員會
日期
8. 12
財團法人

*本同意書一式二份，XX(人員)已將受試者同意書之副本交給您。

A. 受試者：_____(正楷)

B. 立同意書人/法定代理人/(有同意權人)：

_____(正楷)

_____(簽名)　日期：____年____月____日

與受試者之關係：_____

C. 見證人　：_____(正楷)

_____(簽名)　日期：____年____月____日

與受試者之關係：_____

D. 研究主持人：

人體試驗倫理委員會
核准日期
102. 08. 12
長庚醫療財團法人

_____(正楷)

_____(簽名) 日期：____年____月____日

E. 取得同意書人：

_____(正楷)

_____(簽名) 日期：____年____月____日

依據醫療法第 79 條：醫療機構施行人體試驗時，應善盡醫療上必要之注意，並應先取得接受試驗者之書面同意；接受試驗者以有意思能力之成年人為限。但顯有益於特定人口群或特殊疾病罹患者健康權益之試驗，不在此限。

＊前項但書之接受試驗者為限制行為能力人，應得其本人與法定代理人同意。(滿 20 歲為成年人。)

＊接受試驗者無行為能力人，應得其法定代理人同意。

＊依據人體試驗管理辦法第 5 條：依據醫療法第 79 條第一項但書招募之成年或已結婚未成年之受試者，主持人應依下列順序取得其關係人之同意：

一、配偶

二、父母

三、同居之成年子女

四、與受試者同居之祖父母

五、與受試者同居之兄弟姐妹

六、最近一年有同居事實之其他親屬

前項關係人之同意，不得違反受試者曾表示之意思。

＊依據人體研究法第 12 條：為 12 條第一項但書之成年人時，應依下列順序取得其關係人之同意：

一、配偶。

二、成年子女。

三、父母。

四、兄弟姊妹。

五、祖父母。