Study Protocol – Version 5, March 30, 2023

NCT05503823

Project Title: Investigating Transcranial Alternating Current Stimulation (tACS) Preconditioning Effects on Resting and Active Motor Threshold

1. Summary of the Project

Transcranial Magnetic Stimulation (TMS) is a non-invasive brain stimulation technique which uses a large and rapidly changing magnetic field pulse for stimulation. Exposure to TMS has been associated with the improvement of the brain's plasticity and memory loss [1]. These after-effects of TMS are temporal and short-term. However, repetitive sessions of preconditioning the brain before TMS has the potential to extend the duration of these after-effects while also reducing the amount of exposure necessary for observable results. The proposed study focuses on providing some insight on the effects of priming the brain via Transcranial Alternating Current Stimulation (tACS) and Transcranial Random Noise Stimulation (tRNS) before applying TMS.

In this study, single pulse TMS is applied over the primary motor cortex, specifically for the hand area to find the resting and active motor thresholds. The Resting Motor Threshold (RMT) is the lowest intensity to produce involuntary hand twitches while the hand is at a relaxed position or when the motor evoked potential has a peak-to-peak amplitude of greater than 50μV for 5 of 10 trials [2][3]. The active motor threshold (AMT) is similarly the lowest intensity at which the participant shows involuntary hand twitches, but this time while the hand is contracted. AMT is also measured similarly as the lowest intensity at which the motor evoked potential has a peak-to-peak amplitude of greater than 100μV for 5 of 10 trials [4][5]. The electromyography (EMG) of the First Dorsal Interosseous muscle (FDI) is recorded before the application of tACS as a baseline for both RMT and AMT. Then the tACS/tRNS is applied at 40Hz for 10 minutes with the active electrode over the exact location of the TMS application (the hand area of the primary motor cortex) and the reference over the right supraorbital area. Then the EMG is recorded immediately after tACS/tRNS, 30 minutes after, and 1 hour after exposure to tACS/tRNS. These recordings are done both in resting and active states of the muscle. The objective is to observe a change in the motor evoked potentials (MEPs) after exposure to tACS or tRNS and analyze the time of the sustained MEP change.

The proposed study will be tested on 50 cognitively healthy participants from a diverse age group (18-95).

An increase in the peak-to-peak value of MEP post-tACS or post-tRNS could prove to indicate as a new technique to lower the RMT, resulting in lower intensity exposure to TMS. This will be helpful when TMS is used for treatments such as depression disorders. The goal of this study is to eventually find a preconditioning technique to increase the duration of TMS sessions to help in treatment of initial stages of Alzheimer's disease.

2. Background

Previous studies have investigated preconditioning the brain through electrical stimulation of the brain mainly using direct current application [6][7]. These studies show promising improvement of the motor evoked potential (MEP) when transcranial electric stimulation (tES)

techniques such as tDCS (transcranial direct current stimulation) are applied before a session of TMS [6][7]. This study attempts to extend the previous findings by investigating the effects of alternating current and random noise stimulations on the brain known as tACS and tRNS respectively.

tDCS

tDCS is electrical stimulation of the brain using direct current. The study by Lang et al [6] shows a prominent increase of motor evoked potential peak-to-peak amplitude caused by TMS after stimulation of the brain with tDCS over the motor cortex. This study concludes that preconditioning the brain with tDCS effectively improves the cortical plasticity during rapid TMS while also controlling the direction of TMS induced after-effects by changing the placement of the cathode and anode during application of electrical stimulation [6].

A second study conducted by Cosentino et al [7] also investigates the effects of priming the brain using tDCS in a similar setting as the previous study. Contrary to the previous study, this study shows that neither cathodal nor anodal stimulation caused a higher MEP than the baseline [7].

tACS

tACS is electrical stimulation of the brain using alternating current. This stimulation is bipolar therefore, it is not expected to see any difference between the cathodal and anodal after-effects of the priming. In this study we will extend the previous studies to investigate the effects of pre-conditioning the brain using tACS.

tRNS

tRNS is electrical stimulation of the brain using random noise. This stimulation is also bipolar therefore, it is not expected to see any difference between the cathodal and anodal after-effects of the priming. In the second part of this study, we will investigate the effects of pre-conditioning the brain using tRNS.

3. Research Instruments

The following instruments are used for the research:

1) Position sensor
2) Transcranial Magnetic Stimulation (TMS)
3) Electromyography (EMG)
4) Transcranial altering current stimulation (tACS)
5) Transcranial random noise stimulation (tRNS)
6) Sphygmomanometer
7) Oximeter
8) Two questionnaires:
    a. Montreal Cognitive Assessment (MoCA) for screening the participants of no cognitive impairment.

b. Questionnaire to exclude any participant using the exclusion criteria and gather information about possible confounding variables. This will be provided upon both visits.

Both questionnaires are attached to this document.

Position Sensor Camera

Vicra position sensor camera will be used to record the exact position, direction, and tilt of the TMS coil during each pulse. This is done by carefully determining facial landmarks and measuring the participant's scalp using a headband and a pointer equipped with position sensors. While the headband is around the participant's head, the RA will use the pointer to identify the nasion, notch above the tragus of the left and right ear, forehead, top of the head, back of the head, and the left and right sides of the head. The Brainsight software uses these positions marked by the pointer to track the position of all the stimulation pulses to accurately stimulate the exact same position for each RMT and AMT recording.

Transcranial Magnetic Stimulation (TMS)

TMS will be applied on each participant to measure RMT:

1) Before tACS/tRNS
2) Immediately after tACS/tRNS
3) 30 minutes after tACS/tRNS
4) 1 hour after tACS/tRNS

The following parameters will be applied for all eight applications of the TMS:

PARAMETER	DESCRIPTION/VALUE
DEVICE	Magstim Rapid2 Stimulator on single TMS pulse setting with the figure-of-eight AirFilm Coil
APPLICATION LOCATION	On C3 over the hand area of primary motor cortex

Electromyography (EMG)

EMG will be recorded during all TMS sessions using the following parameters:

PARAMETER	DESCRIPTION/VALUE
DEVICE	EMG Recording Pod, Brainsight 2
APPLICATION LOCATION	Two electrodes on First Dorsal Interosseous (FDI) muscle and the reference electrode on top of the wrist (head of ulna)
SAMPLING FREQUENCY	3 kHz
DURATION FOR EACH PULSE	50 ms before the pulse, 200 ms after the pulse

Transcranial altering current stimulation (tACS)

The following parameters will be applied during the tACS sessions:

PARAMETER	DESCRIPTION/VALUE
DEVICE	tES, Soterix Medical Inc. (Model: 2001)
FREQUENCY	40 Hz
APPLICATION LOCATION	Active electrode on C3 over the right-hand area of the motor cortex and reference on the contralateral motor cortex
INTENSITY	0.75 mA (1.5 mA peak-to-peak)
Waveform	Sinusoidal (bipolar)
DURATION	10 minutes

Transcranial random noise stimulation (tRNS)

The following parameters will be applied during the tRNS sessions:

PARAMETER	DESCRIPTION/VALUE
DEVICE	tES, Soterix Medical Inc. (Model: 2001)
APPLICATION LOCATION	Active electrode on C3 over the right-hand area of the motor cortex and reference on the contralateral motor cortex
INTENSITY	0.75 mA (1.5 mA peak-to-peak)
Waveform	Random Noise (bipolar)
DURATION	10 minutes

4. Participants

This research requires a total of 50 healthy participants who must meet the following inclusion criteria:

- Between the ages of 18 years and 95 years
- MoCA score ≥ 26
- Not diagnosed with any restricting conditions such as history of seizures, epilepsy, having pacemakers, being pregnant, having a diagnosed major psychotic disorder, having pain relief medication on a daily basis, or having any metallic implants that might cause a conflict while using TMS.
- Not being on any regular nervous system medication for depression, anxiety, sleep, etc.
- Not have had any brain injury

All participants will be assessed by MoCA and will fill out the questionnaire after signing the consent form.

5. Recruitment

Potential participants will be recruited by providing a poster on the supervisor's webpage https://home.cc.umanitoba.ca/~mousavi/ and Biomedical Engineering webpage of University of Manitoba at https://umanitoba.ca/engineering/biomedical-engineering. Furthermore, posters will be placed around the University of Manitoba campus with permission to recruit

participants. The posters will have information about the project and an email to contact the team to participate in the study.

6. Data Analysis

Motor Evoked Potential (MEP)

Motor evoked potential, as shown in Figure 1, is the involuntary response of the muscle due to the magnetic stimulation to the corresponding area of the brain, hand area for the primary motor cortex. In this study, we are collecting the FDI (first dorsal interosseous) EMG to observe the change in the peak-to-peak value of the MEP. A noticeable increase in the peak-to-peak value of the MEP, while a constant amount of TMS intensity is applied to the brain before and after priming via tACS/tRNS is used, suggests that the priming has been in fact successful, and the motor threshold has been reduced due to the priming.

The EMG recorded before and after tACS/tRNS will be compared to observe any change in the MEP.

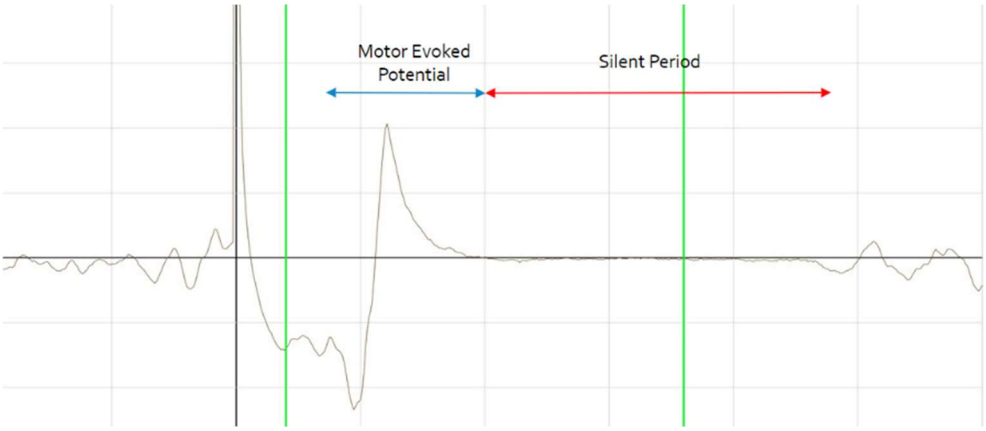

Figure 1. Active FDI EMG with MEP and cSP

Cortical Silent Period (cSP)

Cortical silent period (cSP) refers to the silent period that is followed immediately after MEP. This period is only observable in an active muscle EMG. By measuring the duration of cSP we can estimate the intracortical inhibition of pyramidal neurons caused by activation of $GABA_B$ (gamma-aminobutyric $acid_B$) [8]. A noticeable decrease in the cSP potentially indicates that the preconditioning stimulation via tACS/tRNS has been effective. In this study we will be collecting the active muscle EMG during TMS sessions before and after application of tACS/tRNS.

7. Informed Consent

A consent form will be provided to the volunteers at the beginning of the two-hour session to inform them about the requirements of the study, risks and benefits, withdrawal process, confidentiality policies, and debriefing procedure. The volunteers who wish to participate in the

study will sign the consent form and will be provided with a copy of the form for safekeeping. The original from will be locked along with the participants assigned code in a locked cabinet in the research supervisor's office.

8. Procedure

All volunteers will be contacted by the research assistant (RA) via phone or email. The RA will briefly explain the study while also setting a time slot for the volunteers to come in and read the consent form and discuss it. If the participant wishes to continue the study and signs the consent form, the RA can start the initial assessments by taking the MoCA test and having the participant fill out the questionnaire. Once the volunteers meet the criteria of the study, they will be assigned a code and enrolled to the study and all their data will be recorded under the assigned code. The consent forms which contain the participants code and contact information will be locked in a cabinet in the PI's office.

Once enrolled, participants will be examined to achieve their RMT (resting motor threshold). To do this, first the Vicra camera is turned on and the scalp of the participant is measured and saved on the computer. Then single TMS pulses are applied with the coil positioned over the right-hand area of the primary motor cortex on the left hemisphere (C3) with initial intensity of 50% of the maximum intensity. The coil position will be adjusted over the primary motor cortex until the "hotspot" is found and an involuntary right-thumb twitch is observed. If no twitch was observed after few pulses, the intensity will be increased by increments of 5% until the hotspot is found. Once the "hotspot" is found, it will be marked as a target using the coordinating camera and the intensity will be reduced by decrements of 1% to the minimum intensity where a twitch is observed. The coordinating camera will be used to record the exact position and direction of the coil while a hotspot was achieved to help us accurately stimulate the hotspot once it is found. Similar procedure is applied to determine RMT for the right side of the brain, i.e. left hand movement.

Once the minimum intensity is recorded for the RMT, the participant gets a 10-minute break from the stimulation. During this time, the RA will measure the blood pressure and oxygen level and connect the electrodes over the FDI muscle of the right hand to record EMG and prepare the participant for the TMS session. During the first TMS session, 10 single pulses are applied to the participant over the hotspot recorded by the coordinating camera. The resting EMG of the corresponding muscle in a relaxed position will be recorded for further investigation under the participant code name. Immediately after, the participant will be asked to gently hold the arm rest of the chair and the active EMG will be recorded over 10 single pulses of TMS.

The participant will be given a 5-minute break, during which the RA place the tACS active electrode on the hotspot marked by the coordinating camera and the reference on the contralateral motor cortex. When the electrodes are all set up, tACS will be applied gradually from lower current intensity to higher for 1 minute to allow the participant to adjust to the current. The current is applied at 40 Hz frequency for the duration of 10 minutes. Immediately after the application of tACS the participant's resting and active EMG will be recorded similar to the pre-tACS procedure, with 10 pulses of TMS for each of the resting and active experiments.

Also, the blood pressure and oxygen level will be remeasured. A similar procedure will be followed to record the resting and active EMG after 30 minutes and after 1 hour from the initial application of tACS.

During the second session, the participant will be monitored by the exclusion questionnaire. If the participant passes the criteria, the RMT will be measured again and a similar procedure to the first session will be followed while tRNS is used instead of tACS. The second session can begin after 4 hours following the first session and may occur on a separate day depending on the participant's availability.

Each of these procedures will be applied once on each participant and the experiment for each participant will be done after one or two visits to the laboratory.

Data collection for the study will take place for a period of one year.

All the data will be deleted and destroyed 3 years after completion of the study.

9. Deception

There is no deception in this study.

10. Feedback/Debriefing

Participants can ask about their own performance and result of the study once the study is completed and published.

11. Risks and Benefits

Exposing the brain to magnetic energy via TMS might have some mild risks such as tingling in the jaw and the arm and mild headaches. However, participants who are pregnant, use pacemakers, or have any metallic implants (e.g., dental implants) will be excluded from the study to avoid any conflict while exposed to magnetic energy.

There are also some potential mild risks while preconditioning the brain using tACS/tRNS. These risks include mild pain, headaches, or fatigue. Participants may experience tingling, itching, burning sensation, or skin redness where electrodes are connected. Participants that have history of seizure or epilepsy will be excluded from the study as tACS and tRNS have limited information on the serious adverse effects.

There are no direct benefits concerning the participants from this study. However, the result of the study has the potential to provide sufficient means to treat early stages of memory loss for Alzheimer Dementia (AD) patients.

12. Anonymizing/Confidentiality

All participants' personal information, including their name and contact number will be remained confidential. Each participant will be assigned with a de-identified code and the master file containing the names and codes will be saved on a secure server, ECE server. The assessment data will be saved on the PI's BME server which is physically separate from the ECE server.

13. Compensation

This study does not provide any compensation for the participants.

14. Dissemination

The study result will be presented in relevant seminars, conferences, and journal paper publications.

15. References

[1] S. J. Boniface, U. Ziemann, Plasticity in the Human Nervous System: Investigations with Transcranial Magnetic Stimulation. Cambridge, University of Cambridge Press, UK, 2003, Ch. 1-2, pp. 1-61. [Online]. Available: https://web-p-ebscohost-com.uml.idm.oclc.org/ehost/detail/detail?vid=0&sid=c2c48531-d39d-4217-b80f-ff0b8b8d2840%40redis&bdata=JnNpdGU9ZWhvc3QtbGl2ZQ%3d%3d#AN=120392&db=e000xna. [Accessed: June 20, 2022]

[2] M. Schecklmann, M. Schmaußer, F. Klinger, P. M. Kreuzer, L. Krenkel, and B. Langguth, "Resting motor threshold and magnetic field output of the figure-of-8 and the double-cone coil," *Scientific Reports*, vol. 10, no. 1, 2020. [Online]. Available: https://www.nature.com/articles/s41598-020-58034-2. [Accessed: June 20, 2022]

[3] J. J. Borckardt, Z. Nahas, J. Koola, and M. S. George, "Estimating resting motor thresholds in transcranial magnetic stimulation research and practice," *The Journal of ECT*, vol. 22, no. 3, pp. 169–175, 2006. [Online]. Available: https://oce-ovid-com.uml.idm.oclc.org/article/00124509-200609000-00003/HTML. [Accessed: June 20, 2022]

[4] H. Huang, W. Cai Liu, and Y. H. Song, "Effects of repetitive transcranial magnetic stimulation on masseter motor-neuron pool excitability," *Archives of Oral Biology*, vol. 73, pp. 289–294, 2017. [Online]. Available: https://www-sciencedirect-com.uml.idm.oclc.org/science/article/pii/S0003996916302928. [Accessed: June 20, 2022]

[5] A. Kirton, G. deVeber, C. Gunraj, and R. Chen, "Cortical excitability and interhemispheric inhibition after subcortical pediatric stroke: Plastic Organization and effects of RTMS☆," Clinical Neurophysiology, vol. 121, no. 11, pp. 1922–1929, 2010. [Online]. Available: https://www.sciencedirect.com/science/article/abs/pii/S1388245710003743?via%3Dihub. [Accessed: June 20, 2022]

[6] N. Lang, H. R. Siebner, D. Ernst, M. A. Nitsche, W. Paulus, R. N. Lemon, and J. C. Rothwell, "Preconditioning with transcranial direct current stimulation sensitizes the motor cortex to rapid-rate transcranial magnetic stimulation and controls the direction of after-effects," *Biological Psychiatry*, vol. 56, no. 9, pp. 634–639, 2004. [Online]. Available: https://www-sciencedirect-com.uml.idm.oclc.org/science/article/pii/S0006322304008157. [Accessed: June 20, 2022]

[7] G. Cosentino, B. Fierro, P. Paladino, S. Talamanca, S. Vigneri, A. Palermo, G. Giglia, and F. Brighina, "Transcranial direct current stimulation preconditioning modulates the effect of high-frequency repetitive transcranial magnetic stimulation in the human motor cortex," *European Journal of Neuroscience*, vol. 35, no. 1, pp. 119–124, 2012. [Online]. Available: https://onlinelibrary-wiley-com.uml.idm.oclc.org/doi/full/10.1111/j.1460-9568.2011.07939.x. [Accessed: June 20, 2022]

[8] B. Poston, S. N. Kukke, R. W. Paine, S. Francis, and M. Hallett, "Cortical silent period duration and its implications for surround inhibition of a hand muscle," *European Journal of Neuroscience*, vol. 36, no. 7, pp. 2964–2971, 2012. [Online]. Available: https://onlinelibrary-wiley-com.uml.idm.oclc.org/doi/full/10.1111/j.1460-9568.2012.08212.x. [Accessed: June 20, 2022]