Protocol Title: Motor Evoked Potential and Cortical Silent Period in Migraine

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ISTINYE UNIVERSITY CLINICAL RESEARCH ETHICS BOARD RESEARCH PROTOCOL

1. Open title of the study: Investigation of Motor Evoked Potential and Cortical Silent Period in Migraine Patients

Basis and rationale for the study (Explain in detail): Migraine is a neurological disease that reduces individual quality of life and creates a significant economic burden on healthcare systems worldwide. While the pathophysiology of migraine has not been fully elucidated, recent research indicates that this disease is associated not only with vascular but also with neurological and cortical changes (Goadsby et al., 2017). There is increasing evidence of increased excitability and impaired inhibitory mechanisms, particularly in central nervous system regions such as the motor cortex (Coppola et al., 2007).

Transcranial magnetic stimulation (TMS) is a non-invasive and reliable neurophysiological method that examines muscle responses by stimulating the motor cortex. Parameters such as the motor evoked potential (MEP) and the cortical silence period (CSP) obtained with this method are used to measure motor cortex excitability and inhibitory control, respectively (Chen et al., 1999). CSP is widely used, particularly in the assessment of GABA-B-mediated inhibitory mechanisms. The hypothesis of decreased cortical inhibition in migraine patients is an important research question that can be tested with CSP measurements.

Some previous studies have reported findings such as shortened CSP duration and increased MEP amplitudes in migraine patients, which may indicate a state of cortical hyperexcitability (Brighina et al., 2002; Afra et al., 1998). However, these findings are not consistent, and how they differ across different subtypes of migraine (with or without aura) has not yet been fully elucidated.

Therefore, this study aims to reveal the changes migraine causes in cortical physiology using objective and quantitative methods, filling gaps in the literature and contributing to clinical assessments. Furthermore, by examining the relationship between these parameters and clinical characteristics such as migraine type, duration, and attack frequency, it is aimed to take a first step toward establishing individualized neurophysiological profiles**.**

1. **The main purpose of the research, any secondary purposes (Explain in detail):**

Primary Objective:

The primary objective of this study is to evaluate the level of inhibitory control in the motor cortex in migraine patients using objective and non-invasive methods. For this purpose, comparisons will be made between individuals with migraine and healthy controls by measuring Motor Evoked Potential (MEP) and Cortical Silence Period (CSP) parameters obtained with Transcranial Magnetic Stimulation (TMS). CSP duration has the potential to directly measure the effect of migraine on cortical inhibition, particularly because it is used in the assessment of GABA-B-mediated inhibitory mechanisms (Chen et al., 1999).

Secondary Objectives:

1. Motor output analysis: Peristimulus Time Histogram (PSTH) and Peristimulus Frequencygram (PSF) analyses will be applied to electromyographic signals to evaluate the temporal order

and frequency changes of motor unit responses after TMS. While PSTH analyzes the post-stimulation timing of individual motor units; PSF more sensitively displays the frequency change after stimulation (Türker & Powers, 2001). These analyses can reveal the reflection of cortical stimulation at the spinal level and its effect on motor output in more detail.

2. Biomarker potential: The potential use of TMS-based parameters such as CSP and MEP as biomarkers for the pathophysiology of migraine will be evaluated. This will aim to develop objective neurophysiological measures that can contribute to future diagnostic and therapeutic processes.

1. Expected benefits of the study:

At the end of this study, objective data on the inhibitory capacity of the motor cortex in migraine patients are expected to be obtained. TMS parameters, particularly cortical silence period (CSP) and motor evoked potential (MEP), can be used to understand the neurophysiological basis of migraine. These data offer the following potential contributions: They will contribute to a better understanding of the balance of cortical excitability and inhibition in the pathophysiology of migraine. The CSP and MEP data obtained may form the basis for the development of objective diagnostic biomarkers for migraine in the future. The study results may indicate the presence of cortical dysfunction in individuals with migraine and support the development of personalized treatment approaches. Evaluation of changes in motor output using electrophysiological methods such as PSTH/PSF, included in secondary analyses, can provide a more holistic understanding by demonstrating how the network from the cortical to the spinal level is affected. This study, in which volunteers did not undergo any invasive procedures, offers a non-invasive, safe, and reproducible research approach.

2. Research method (Explain in detail):

Participants:

The study will include individuals aged 18–45, right-handed, and previously diagnosed with migraine. Migraine will be diagnosed according to the diagnostic criteria of the International Headache Society (IHS).

Data Collection Method:

This study will be conducted using surface electromyography (sEMG) and needle EMG methods, combined with non-invasive brain stimulation (TMS). Transcranial magnetic stimulation (TMS) will be applied to the motor cortex during both resting and voluntary muscle contraction. Simultaneously with TMS application, muscle responses will be recorded via surface EMG electrodes. Additionally, single motor unit (SMU) recordings will be made for more detailed analysis of motor neuron activity. For SMU recordings, needle EMG will be performed using special needles (approximately 70 µm in diameter, 25G) with sterile tips and Teflon-coated copper wire. After the needle is inserted into the FDI (first dorsal interosseous) muscle, it will be withdrawn, leaving only the wires inside. The curved tip of these wires, resembling a fishhook, facilitates their insertion into the muscle. After the needle is inserted, the subject will be asked to forcefully contract the target muscle several times, ensuring the wires are inserted into the muscle. This method allows monitoring the activity of a single motor neuron even during movement. All EMG signals will be collected using CED (Cambridge Electronic Design, UK) systems. EMG signals will be amplified using a CED 1902 four-channel amplifier (MKIII) and then digitized using a CED 3601 Power 1401 MKII

DAC unit. Recorded data will be analyzed using Spike2 7.20 software. SMU recordings will be taken in bipolar format, and the signals will be amplified 1,000-10,000 times. The signals will then be passed through a 200 Hz high-pass filter and digitized at a 20,000 Hz sampling rate.

Methods and Devices to be Used:

Transcranial Magnetic Stimulation (TMS) Application:

TMS applications will be performed using the Magstim 200^2 Monophasic Stimulator (Magstim Company Ltd, UK). A 70 mm diameter figure-of-eight (butterfly-type) double-ring coil is preferred for stimulation.

1. **Specify which of the following is the research design:**

Observational Studies

A. Descriptive research (case series are included in this group) ☐
B. Case-control studies (retrospective) ☐
C. Cross-sectional studies, surveys are included in this group) ☐
D. Cohort studies (prospective) ☐
E. Historical cohort studies ☐

2. Experimental Studies

A. Controlled trials
  I. Parallel controlled
    a. Randomized X
    b. No randomization ☐
  II. Sequential Controlled
    a. Self-controlled ☐
    b. Cross-over controlled ☐
  III. Externally controlled ☐
B. No control group ☐

3. Meta-analyses ☐

2. **The number of patients and volunteers to be included in the study, and their characteristics (age ranges, gender, handedness, etc.).**

In this study, the primary unit of analysis for measurements is not the "participant," but rather the motor unit activity. Since temporal and frequency analyses will be conducted by taking multiple motor unit measurements from each individual, the primary data is the motor unit count.

Nevertheless, for procedural reasons, the study plans to include a total of 20 volunteers (10 migraineurs, 10 healthy controls). Both groups will be matched for age and gender.

- Age Range: 18–45 years
- Gender: Both male and female participants will be included; however, due to the higher prevalence of migraine in women, the proportion of female participants may be higher.
- Handedness: Only right-handed individuals will be included.
- Diagnosis: The migraine group will consist of individuals who have been diagnosed with migraine for at least one year according to IHS criteria**.**

3. **Method for determining the number of volunteers to participate in the study and sampling method: (Where and how will the samples be obtained, and how will the selection be made in the clinic?)**

The total sample size was determined based on previous similar studies and power analyses, taking into account the level of efficiency and significance ($\alpha = 0.05$, power = 80%).

A minimum of 10 participants was anticipated for each group.

Purposive sampling will be used as the sampling method.

- **Migraine Group:** Individuals followed in neurology outpatient clinics and previously diagnosed with migraine will be included in the study if they are informed about the study and agree to volunteer.
- **Control Group:** Individuals with no history of neurological disease will be selected from university staff and students, matched by age and gender.

4. **List the inclusion and exclusion criteria for the study: Inclusion Criteria:**

Inclusion Criteria:

-Being between 18 and 45 years old
-Right-handed
-Having been diagnosed with migraine for at least 1 year (for the migraine group)
-Being a healthy individual without a migraine diagnosis (for the control group)
-Having signed a written informed consent form
-Having at least a primary school diploma (to be able to understand instructions))

Exclusion Criteria:

-History of epilepsy or seizures
-Presence of another neurological or psychiatric disease

-Having a metal implant, pacemaker, or similar conditions that contraindicate TMS
-Pregnancy
-Having previously experienced serious side effects from TMS or EMG
-Drug or alcohol addiction
-Severe visual or hearing impairment

5. If there is a control group, please describe its size and characteristics, and where and how it will be recruited:

Control group, migraine The study will consist of 10 healthy volunteers without a diagnosis of migraine. These individuals will be matched to the migraine group based on age, gender, and handedness. Individuals participating in the control group will be selected from among university staff and students through a volunteer call by the research team. Their eligibility will then be verified through a neurological assessment and brief medical history. The control group will be subjected to the same measurement protocol as the migraine group (TMS, EMG), allowing for objective comparisons between the two groups.

6. Tests, relevant laboratory, and other examinations to be performed:

Name of test or examination to be performed: Cortical Silence Period (CSP) measurement, Motor Evoked Potential (MEP) measurement, Surface Electromyography (sEMG) recording, Needle Electromyography (EMG), Motor unit analysis (analyzed with Spike2 software)
Name and surname of contact person: Prof. Dr. Kemal Sıtkı Türker
Address: The research was conducted at the Translational Dentistry Research Laboratory at the Faculty of Dentistry, Istanbul Gelişim University. This laboratory has the technical infrastructure to perform transcranial magnetic stimulation (TMS), surface and fine-wire EMG recording, and data analysis using Spike2 software.
Phone number: +90 532 598 77 91

7. What are the risks and process issues that may be encountered during the study? If you have any precautions or a plan B, please explain.

Possible Risks:

-Transient headache, dizziness, and occasional muscle twitches due to TMS application

Process Issues:

-Lack of participant cooperation
-Artifact formation during muscle activity
-Device stability issues due to laboratory temperature

Precautions:

-Detailed information and informed consent will be obtained from all participants.
-Participant comfort will be ensured before each test.
-Devices will be checked with pre-tests.
-Sessions will be canceled and rescheduled if necessary.

8. Which parameters, tests, and methods to be used in the study are routine and which are specific to the study:

Routine Applications:

-Surface EMG recording
-Needle EMG recording
-Motor cortex stimulation with TMS

Special Applications:

-Cortical silence period (CSP) measurement
-Motor unit extraction and analysis (using Spike2 software)
-Laser stimulation-induced pain protocol (within the specific procedure submitted to the ethics committee)

9. The anticipated study duration, with start and end dates:

-Planned Start Date: November 2025
-Planned Completion Date: May 2026
Total Duration: Approximately 6 months (including data collection and analysis)

10. What are the criteria for terminating the study?:

-Participant's willingness to withdraw voluntarily from the study
-Serious adverse effects or side effects (e.g., intolerance to TMS)
-Technically inadequate measurements (e.g., high artifact, low signal quality)
-If sufficient motor unit data cannot be obtained within the study period, the study may be terminated early.

11. The statistical methods to be used to evaluate the data obtained from the study should be explained:

-Descriptive statistics (mean, standard deviation, confidence intervals)
-For group comparisons:
-Independent samples t-test or Mann–Whitney U test (normal distribution)
-Repeated measures ANOVA (for time × group effect)
-Pearson or Spearman correlation analysis (for relationships between motor unit parameters and clinical variables)
-Multivariate regression analysis when necessary

All analyses will be conducted using IBM SPSS v27 and Spike2 software.

12. Resources:

Chen, R., et al. (2008). Cortical inhibition and silent period: Tools for understanding the pathophysiology of pain. *Clinical Neurophysiology*.

Türker, K. S., & Kahya, M. (2005). Cortical silent period evoked by painful laser stimuli. *Trends in Neurosciences*.

Rossi, S., et al. (2009). Safety, ethical considerations, and application guidelines for TMS. *Clinical Neurophysiology*.