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Neuroplastic alterations and variability of transcranial alternating current stimulation (tACS) on the human motor cortex among caffeine-adapted subjects

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Hiermit erkläre ich, die Dissertation mit dem Titel „Neuroplastic alterations and variability of transcrainal alternating current stimulation (tACS) on the human motor cortex among caffeine-adapted subjects“ eigenständig angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

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List of abbreviations

A1R	Adenosine Type 1 Receptor
A2R	Adenosine Type 2 Receptor
ANOVA	Analysis of Variance
Cz	Vertex Reference
d.f.	Degree of Freedom
ECG	Electrocardiography
FDI	First Dorsal Interosseus Muscle
GABA	Gamma-Aminobutyric Acid
LTD	Long-Term Depression
LTP	Long-Term Potentiation
M1	Primary Motor Cortex
MEPs	Motor evoked Potentials
MT1mV	1 mV peak-to-peak MEPs
NIBS	Non-invasive Brain Stimulation
NMDA	N-methyl-D-aspartate
PAS25	25ms Paired Associative Stimulation
PD	Pupil Diameter
PRE	Baseline MEPs
PST	Poststimulation effects
PUI	Pupillary unrest index
QPS	Quadripulse Transcranial Magnetic Stimulation
RMT	Resting Motor Threshold
rTMS	Repetitive Transcranial Magnetic Stimulation
tACS	Transcranial Alternating Current Stimulation
tDCS	Transcranial Direct Current Stimulation
tES	Transcranial Alternating Current Stimulation
TMS	Transcranial Magnetic Stimulation
tRNS	Transcranial Random Noise Stimulation

1 Introduction

Transcranial alternating current simulation (tACS), a non-invasive brain stimulation (NIBS) technique, can modulate brain plasticity as measured by motor evoked potentials (MEPs) generated by transcranial magnetic stimulation (TMS) on the motor cortex.

In this project, we compared the effects of caffeine and alertness on plasticity aftereffects induced by tACS or 25ms paired associative stimulation (PAS25) over the motor cortex between caffeine-naïve and caffeine-adapted subjects. This project consisted of two randomized, double-blinded, cross-over or placebo-controlled (caffeine) studies and was finalized with two publications (Zulkifly et al. 2021). Corticosteroid and caffeine concentrations were measured in saliva. This work is part of this project and includes only one of the above studies with focus on caffeine-adapted subjects.

The aim of this chapter is to introduce the reader to scientific background information on the techniques used in this work. The NIBS techniques, caffeine and vigilance are elaborated in Section 1.1, Section 1.2, and Section 1.3. The aims of the study are presented at the end of this chapter.

Caffeine consumers and caffeine-adapted subjects are used as synonyms in this work. The same is valid for vigilance and alertness.

1.1 Non -Invasive Brain Stimulation (NIBS)

1.1.1 NIBS techniques

In 2000, Nitsche and Paulus developed a tolerable weak transcranial direct current stimulation (tDCS) technique, followed by other transcranial electric stimulation (tES) techniques, such as tACS (Antal et al. 2008) or transcranial random noise stimulation (tRNS) (Ternery et al. 2008). These techniques induce neuroplastic effects in the brain with potential therapeutic use in the future.

TMS and tES are both known as NIBS technique (Paulus 2011).

The basic concept of TMS is that it produces a magnetic field which generates electric current in the brain (Figure 1a). This current, in particular it's voltage gradient of ~ 100 V/m, is capable to depolarize neurons and hereby may cause one or more action potentials. Furthermore, there are different application forms of TMS, such as single-pulse TMS, paired-pulse TMS and repetitive TMS (Vlachos et al. 2017). In this study,

single-pulse TMS will be used to induce MEPs and thus, to quantify the plastic aftereffects of tACS.

In opposition, tES applies a weak current for some minutes over the scalp. This, in turn, can interact with neural networks, modulate plasticity, and thus, modify behaviour (Bestmann and Walsh 2017). In the simplest and general terms, a sub-threshold current changes the membrane potential and consequently, modifies spontaneous firing rates and hereby neuronal networks (Figure 1b). At a microscopic cellular level, the involvement of the N-methyl-D-aspartate (NMDA) receptors (Liebetanz et al. 2002), or gamma-aminobutyric acid (GABA) and Glutamate-neurotransmitters release (Stagg et al. 2011; Hunter et al. 2015) may contribute to tDCS-induced long-term potentiation (LTP)/ depression (LTD) like aftereffects.

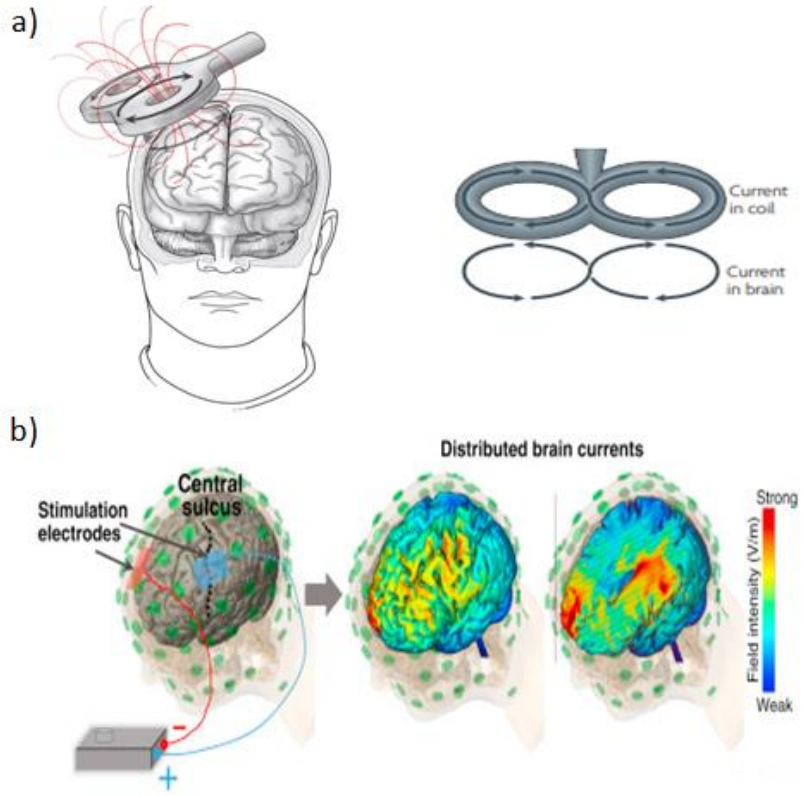

Figure 1: NIBS techniques. a) Illustration of magnetic field generated by TMS, which induces current in the brain. Adapted from (Vlachos et al. 2017; Ridding and Rothwell 2007). Used with the permission of De Gruyter. b) Illustration of tES over the primary motor cortex (M1). Adapted from (Bestmann and Walsh 2017). Used with the permission of Elsevier.

1.1.2 Transcranial alternating current stimulation (tACS) and brain plasticity

The first effects of tACS over the motor cortex (M1) were reported by Antal et al. (2008). The authors observed a significant effect of 10Hz tACS in learning during the serial reaction time task.

Furthermore, tACS can modulate cortical excitability (Moliadze et al. 2010), sensory motor integration (Feurra et al. 2011), or EEG activity (Zaehle et al. 2010). Cortical excitability is defined as the strength of the response of cortical neurons to a stimulus as measured by TMS-generated MEPs (Ly et al. 2016).

Particularly interesting for this study is the paper of Moliadze and colleges. The authors showed that frequency-specific (140 Hz) tACS at 1 mA over the M1 increases peak-to-peak MEP amplitudes (Moliadze et al. 2010). The authors hypothesized that the high frequency tACS interferes with ongoing brain oscillations and therefore, causes changes and modulates cortical excitability.

However, a basic understanding of the effects of tACS and NIBS techniques at a system level remains a challenge (Antal et al. 2022).

1.1.3 Motor evoked potentials (MEPs) and variability

Plasticity aftereffects of tACS over the M1 were measured by MEPs in the right first dorsal interosseous muscle (FDI) generated by TMS (Figure 2).

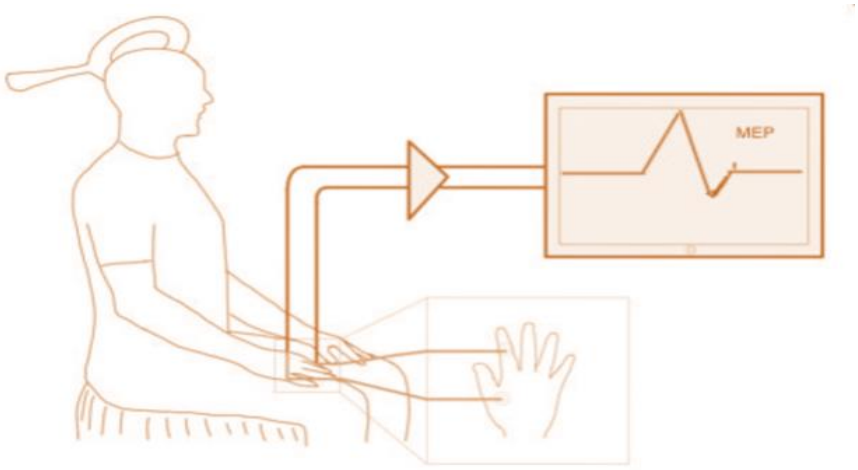

Figure 2: Illustration of a participant during a TMS measurement.

While the trial-to-trial variability of MEPs may be affected by pulse shape, stimulation intensity and coil orientation (Biabani et al. 2018; Cavaleri et al. 2017; Cuypers et al. 2014; Goldsworthy et al. 2016; Lewis et al. 2014), the variability between subjects is influenced by age, gender, time of day, previous history of plasticity, genetics, drugs, and attention (review by Ridding and Ziemann 2010). Ziemann and colleagues (2015) emphasize that more studies are needed to find the main factors of NIBS-induced

plasticity inter-session and inter-subject variability, because NIBS techniques have a great potential for therapeutic use, which is already realized by rTMS in depressive patients. These issues are addressed in this study.

1.2 Caffeine

1.2.1 Coffee and caffeine

Caffeine is the most widely consumed psychostimulant in the world. In US, almost 85% of the inhabitants consume daily coffee or caffeinated beverages, such as tea, cocoa beverages, chocolate bars, and soft drinks (Fredholm et al. 1999; Mejia et al. 2014; Mitchell et al. 2014).

Caffeine absorption from the gastrointestinal tract reaches 99% in humans in about 45 minutes after ingestion (Alsabri et al. 2018).

The participants of this study were moderate caffeine consumers. Cappelletti and colleagues (2015) have classified caffeine usage as low if the intake is less than 200 mg/day, moderate if it is 200-400 mg/day, and high if the caffeine intake is more than 400 mg/day.

1.2.2 Biochemical targets of caffeine in the brain

Caffeine not only induces a stimulant effect, but has also be claimed to induce neuromuscular changes and hereby improves exercise performance as shown by Mesquita et al. (2020). Moreover, caffeine via adenosine receptors may influence mood states (van Calker et al. 2019). The biochemical mechanism that caffeine acts in the brain depends on the concentration of caffeine in blood stream (Figure 3). The main physiological mechanism, even in lower doses, is via inhibition of the adenosine type 1 and 2 receptors (A1R and A2R) (Fredholm et al. 1999). In cortical neurons, caffeine can restore the effects of adenosine through blockade of A1R (Kerkhofs et al. 2018). It may also regulate calcium release or interfere with GABAergic synapses and suppress inhibitory neurotransmission (Isokawa 2016). However, this possibly occurs mainly in high doses of caffeine as shown in figure 3.

Corticosteroid and caffeine concentrations in saliva were also measured in this study. The results of the measurements are reported in the publication listed above. Nevertheless, they are not part of this dissertation.

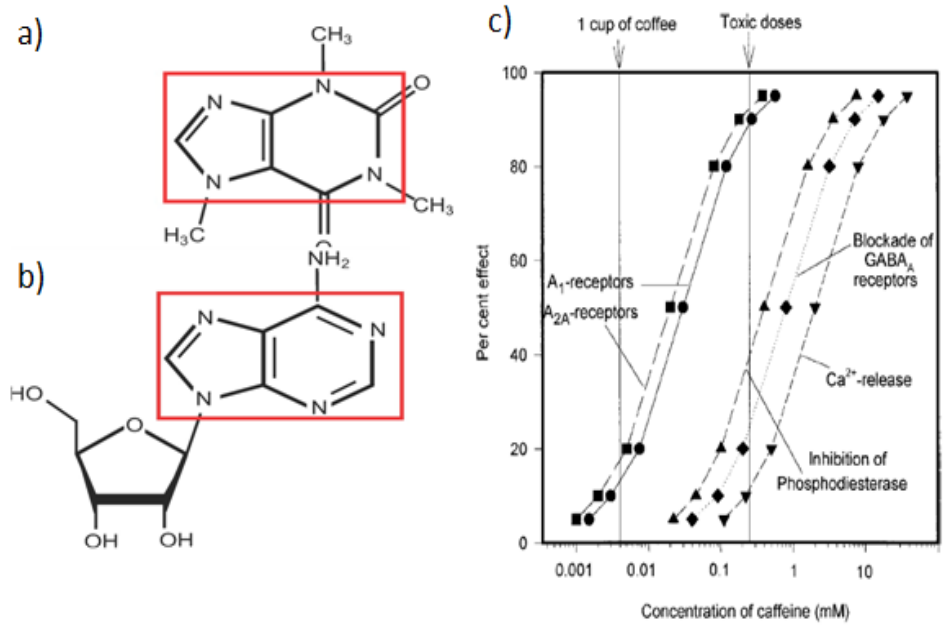

Figure 3: Caffeine. a) The chemical structure of caffeine (1,3,7-trimethylxanthineand and b) the chemical structure of adenosine. Adenosine and caffeine have similar basic structure, making caffeine also capable to bind to adenosine receptors. Adapted from (Kolahdouzan et al. 2017). Used with the permission of the authors.
c) Caffeine targets different biochemical structures in relation to its levels in humans. Adapted from (Fredholm et al. 1999). Used with the permission of Pharmacological Reviews.

1.2.3 Caffeine and brain plasticity

Hanajima et al. (2019) showed that caffeine reduces LTP-like aftereffects of quadripulse transcranial magnetic stimulation (QPS) two hours after intake of a 200 mg caffeine tablet, most likely via an antagonistic effect at the A2A receptor. Another study showed that administration of the receptor antagonist DCPCX to target adenosine A1 receptor reduces the LTD-like effect of cathodal tDCS (Marquez-Ruiz et al. 2012). These studies indicate that caffeine may play a role in the variability of NIBS-induced plasticity and thus, motivated us to first investigate the effects of espresso containing caffeine on the plasticity aftereffects of tACS (140Hz, 1 mA) on the human motor cortex in caffeine-naïve participants. We observed that MEPs decreased significantly after espresso with caffeine (Zulkifly et al. 2020). Based on these results, we aimed to further investigate and clarify this issue in this project.

1.3 Vigilance

1.3.1 Parasympathetic and sympathetic control of pupil size

Spontaneous pupil movements and pupil size are regulated by the autonomic nervous system (sympathetic and parasympathetic nervous system) via two antagonistic smooth muscle systems. These spontaneous oscillations reflect the level of central nervous activation (Wilhelm at al. 2001).

Briefly, the parasympathetic nervous system is responsible for near accommodation, convergence, and pupillary light reflex. Furthermore, the parasympathetic nervous system (narrow pupils) dominates in sleepy participants as the sympathetic central inhibition on parasympathetic nervous system decreases. In this case, the pupil diameter is small, unstable, and fatigue waves (frequency below 0.8 Hz) of spontaneous pupillary contraction and dilation can be detected. These fatigue waves were first described from Löwenstein et al. (1963).

In contrast, in darkness, in an alert participant, the sympathetic nervous system activity dominates and thus, the pupil diameter is large and stable (Figure 4).

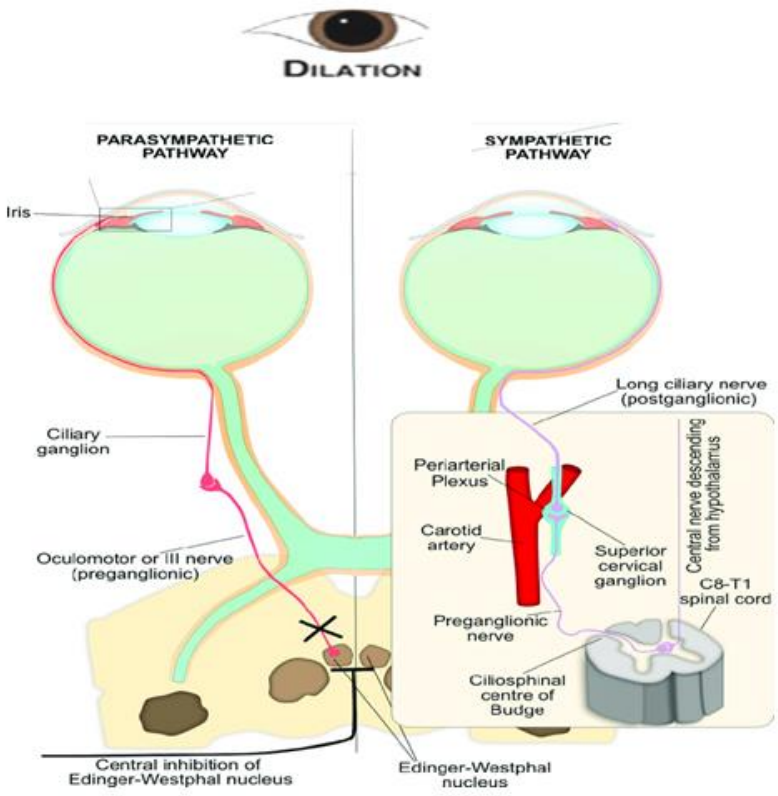

Figure 4: Parasympathetic and sympathetic nervous system on the pupil. The activation of sympathetic pathway and the central inhibition of parasympathetic pathway are both required for pupil dilation. Adapted from (Hall and Chilcott 2018; Wang and Munoz 2015). Used with the permission of Diagnostics (Basel) and Elsevier.

1.3.2 Vigilance and brain plasticity

Amongst the factors that modulate neuronal plasticity, the significance of the neuronal activation state on the effectiveness of NIBS hasn´t been addressed sufficiently. There is evidence that TMS effects are dependent on the brain state (Silvanto et al. 2008). Furthermore, Chen and Huang (2018) demonstrated that eyes-open or eyes-closed states also influence motor cortex excitability. Different brain states can, therefore, lead to a suboptimal intervention.

Still, vigilance itself could be influenced by caffeine and time of day. The stimulant effect of caffeine could be shown by the study of Wilhelm et al. (2014), where caffeine caused a decrease of pupillary oscillations in well-rested subjects.

Regarding the time of day, an earlier study of Wilhelm et al. (2001) stated that the pupillary unrest index (PUI) values, as a measure of pupil size instability, varied during the course of the day. The lowest values of PUI were at 09.00 h and 23.00 h. Higher PUI values were observed during the afternoon hours. Other parameters of attention (e.g. Visual Analogue or Stanford Sleepiness Scales) also follow a time-of-day alteration (Kraemer et al. 2000).

1.4 Aims of the study

The aim of this study is to better understand the factors that might cause variability in plasticity induction studies. More specifically, this thesis aims to examine the plasticity effects of tACS (140 Hz, 1 mA) over the M1 in caffeine-adapted subjects and to clarify the influence of caffeine, vigilance and time of day on plasticity aftereffects of tACS.

2 Materials and methods

This chapter provides information about the materials and methods used in this work. The Section 2.1 describes the criteria for recruiting participants in this study. The following sections describe TMS, tACS and pupillometry as techniques used in this work. The study design is elaborated in Section 2.5. And lastly, the methods for data analysis are described in Section 2.6.

The data is reported as mean ± standard error of the mean (SEM).

2.1 Participants

The study participants were healthy moderate caffeine consumers, mostly students of the Georg August University of Göttingen. 30 caffeine consumers (15 male and 15 females) participated in this study. The mean age of the participants was 23.8 ± 2.3 years and ranged between 19 and 29 years (Zulkifly et al. 2021a).

In a first interview, the researcher responsible for the study explored the medical and social background of each participant by putting an emphasis on exclusion criteria. During the conversation, the participants obtained information regarding the purpose of the study, including all possible risks of the experiments. A full medical history and clinical examination was obtained at the end of the meeting.

This study was approved by the ethics committee of the Medical Faculty of Göttingen University and was conducted conforming to the Declaration of Helsinki. It was registered ahead of the experiments in the ClinicalTrials.gov with ID: NCT04011670.

2.1.1 Inclusion Criteria

The study participants were right-handed (Oldfield 1971) and fully competent to give their consent. Electrocardiography (ECG) was recorded to exclude participants with any cardiac arrhythmias in case of caffeine adverse effects.

In order to avoid any confounding effects, the participants were asked to stop taking any caffeinated beverages or alcohol 24 hours before the experiment.

Smith et al. (1999) reported for the first time menstrual cycle dependency of cortical excitability. Thus, the female participants weren´t tested during menstruation phase, as low estrogen levels can have an influence on the NIBS-induced aftereffects (Lee et al.2018).

2.1.2 Exclusion criteria

Study candidates who fulfilled at least one of the following characteristics, were not recruited to participate in the experiments: Age < 18 or > 45 years old; Left hand dominant; Metallic implanted objects in the head (e.g. aneurysm clips, intravascular clips, intravascular stents, ear implants); Evidence of an internal disease or residues of a neurological disease; epileptic episodes; cardiac pacemaker; deep brain stimulation; A history of traumatic brain injury with loss of consciousness; A serious psychiatric pre-existing disorder; Alcohol dependence or substance use disorder; Drug administration; Receptive or global aphasia; Implication in another scientific study within the past four weeks; Gravidity; Breastfeeding; Participant with caffeine sensitivity experience; ECG-Abnormalities; Body weight < 60 kg.

2.2 Transcranial Magnetic Stimulation (TMS) measurements

Surface EMG electrodes for measuring MEPs were attached to the right FDI. The data were amplified and filtered (2Hz - 2000Hz). A micro 1401 AD converter (Cambridge Electronic Design Ltd., UK) was used to digitize the EMG signals at 5 kHz (Zulkifly et al. 2021a).

The MEPs were induced using a single pulse, monophasic TMS, specifically a Magstim 2002 magnetic stimulator (Magstim Co. Ltd., UK). A D70 coil was used to deliver TMS stimuli on the M1.

The TMS measurements were conducted strictly following the measurement protocol described below, in order to ensure a correct positioning of the TMS coil during the experiment.

First, the vertex reference (Cz) was marked on the scalp with a skin pencil. Next, a coil was located over the scalp in a posterior-anterior orientation and at a 45º angle in the direction of the right eye. The resting motor threshold (RMT) and the ~1mV threshold (MT1mV) were then determined as reported in Rossini et al. (2015).

RMT was here defined as the lowest TMS intensity necessary to produce peak-to-peak MEP of at least 50 µV in five of ten TMS trials. MT1mV is the stimulus intensity required to produce a MEP of 1mV from 25 trials.

The data was finally stored and used for statistical analysis.

2.3 Transcranial Alternating Current Stimulation (tACS)

tACS was generated by a battery-driven stimulator (NeuroConn GmbH, Illmenau, Germany). The current had a 140Hz sinusoidal waveform and the intensity was 1mA. The ramp-up and ramp-down time of tACS was five seconds. The Actual tACS stimulation lasted 10 minutes. In order to induce a blinding effect in context of similar skin sensation, the Sham stimulation duration was 30 seconds. The stimulation current conforms to the safety guideline published by Antal et al. (2017).

Transcranial alternating current was applied through a pair of conductive rubber electrodes as described by Moliadze et al. (2010; 2012). As a standard montage, one electrode (4 x 4 cm) was located over the area representing the right FDI muscle. A conductive paste (Ten20, D.O. Weaver, USA) was used for optimal contact and in order to maintain the impedance below 10 kΩ. The other electrode (5 x 7 cm), covered by a saline soaked sponge, was positioned contralaterally over the forehead, exactly above the right eye as shown in Figure 5.

Figure 5: A participant during stimulation wearing goggles providing a total dark condition.

2.4 Pupillometry

A pupillometer (F2D, AMTech Pupilknowlogy GmbH, Dossenheim, Germany) recorded the spontaneous and involuntary pupil movement in darkness to evaluate alertness. The records are based on the PUI, the main outcome parameter. Lüdtke et al.

(1998) define PUI (mm/min) as fluctuations of pupil diameter (PD) derived from mean values over a record of 11 minutes. Thus, lower PUI score indicates increased alertness.

The pupillometer consisted of goggles in the context of measuring pupillary motion of the left eye under light deprivation with an infrared video camera. The goggles were connected with a laptop in order to evaluate PUI (Figure 6).

The participants were seated in a comfortable chair with head and arm-rests. First, the tACS electrodes were placed over the scalp and forehead. Next, the participant wearied the pupillometer goggles. The participants were instructed to keep their eyes open and look in the direction of a green dot. The infrared camera continuously monitored the PD for eleven minutes. The first minute of the vigilance measurement was stimulation-free, followed by a ten-minute period with stimulation.

Figure 6: Pupillometry. The pupillometer consisted of goggles and an infrared video camera to evaluate PUI.

2.5 Study design

This study is a randomized, double-blind, cross-over study and consists of 8 sessions, with a minimum of five days between two experimental sessions (Table 1).

Table 1: Study design: Prospective, randomized, placebo-controlled, double blind study; 30 Participants; Healthy men and women between the ages of 18 and 45, moderate caffeine consumers.

Session	Stimulation type	Drug type	Time of the day	
			Morning	Afternoon
Session 1	tACS (140 Hz, 1 mA)	Caffeine (tablet 200mg)	X	
Session 2	tACS (140 Hz, 1 mA)	Caffeine (tablet 200mg)		X
Session 3	tACS (140 Hz, 1 mA)	Placebo (tablet)	X	
Session 4	tACS (140 Hz, 1 mA)	Placebo (tablet)		X
Session 5	Sham tACS	Caffeine (tablet 200mg)	X	
Session 6	Sham tACS	Caffeine (tablet 200mg)		X
Session 7	Sham tACS	Placebo (tablet)	X	
Session 8	Sham tACS	Placebo (tablet)		X

Notice the colours used in this table to associate the different combinations of stimulation type, drug type and time of day throughout the 8 sessions. The same colour coding will be used in the following chapters.

The participant was advised to sit comfortably and completely relaxed in a recliner.
The experiment followed the steps (Figure 7) as described below.

Step 1: Determining the motor threshold

First, surface EMG electrodes for measuring MEPs were attached to the right FDI muscle. The hotspot, the optimal representation of this muscle, was then identified using single pulse, monophasic TMS stimuli (see Chapter 2.2). Moreover, the coil position was marked on the scalp with a skin pencil. This mark was easily removed after the session. The RMT and the MT1mV were then determined. The last stimulus intensity was used to record the baseline MEPs (PRE).

Step 2: Oral admission of caffeine (200 mg) or placebo tablet

Next followed the oral admission of the tablet. Liguori et al. (1997) reported that the peak concentration of caffeine in saliva is reached after 67 ± 7 min. Thus, a 45-minute waiting period was necessary for the drug absorption. During these 45-minutes, the participants were permitted to read in order to stay alert.

Step 3: Application of tACS and pupillometry

Immediately after the waiting period, tACS was applied following respectively the study design (Table 1) in a randomized order. The excitatory or Sham tACS duration was ten minutes. The level of alertness was monitored by pupillometry for eleven minutes as described in Chapter 2.4. During this time, the participant was informed and awaked when PUI indicated a reduction in alertness or eyelids closure. The session appointments were scheduled at the same time of day, respectively for the morning and afternoon sessions (Table 1).

Step 4: Recording the aftereffects of stimulation

After stimulating the designated cortical area, poststimulation effects (PST), quantified as MEPs, were recorded every 5 minutes till 30 minutes immediately after stimulation.

Step 5: Questionnaire

At the end of the experiment, the participants were asked to fill in a questionnaire regarding short term adverse effects or stimulation-related sensations.

At the beginning and at the end of each experiment session, saliva samples were collected in order to measure caffeine and corticosteroid concentrations.

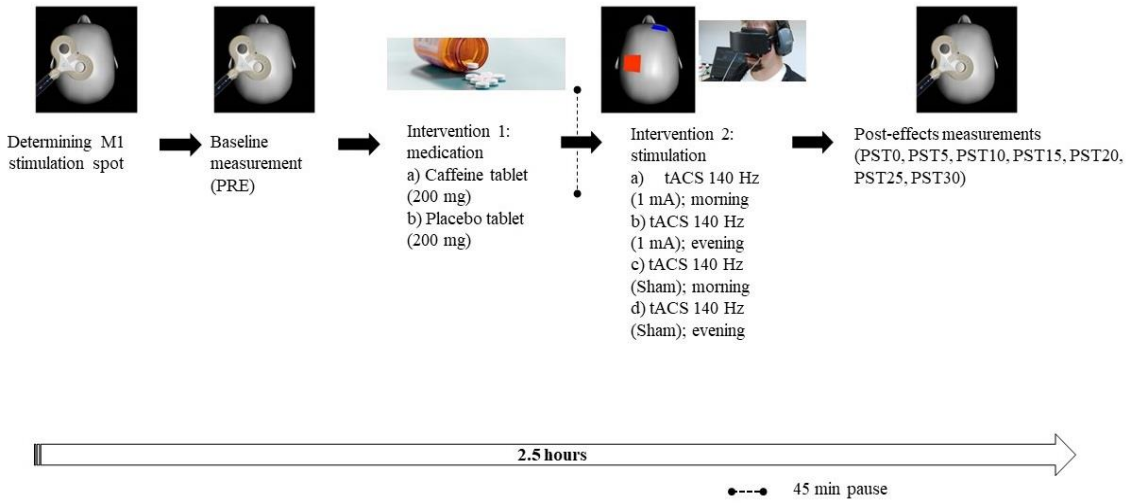

Figure 7: Experiment design. Adapted from (Zulkifly et al. 2021a).

2.6 Data analysis and statistics

The MEP amplitudes for each time-point were first analyzed via a Signal software script (Signal version 4.08, Cambridge Electronic Design Ltd., Cambridge UK). A time-point consisted of 25 MEP amplitudes. The mean values of data were then evaluated through a SPSS software (IBM SPSS statistics 26; NY USA).

Two rmANOVAs were conducted to compare the aftereffects of stimulation on cortical excitability. The first included all TIME-points recorded (8 levels), STIMULATION (2 levels), DRUG (2 levels) and DAY (2 levels) as factors. The time course consisted of the premeasurement (PRE) and seven postintervention measurements: PST0/PST5/

PST10/PST15/PST20/PST25/PST30 min after stimulation (Zulkifly et al. 2021a).

The second rmANOVA included the postintervention measurements in two time-points as pooled data set (POST 1: first 15 minutes after stimulation and POST 2: last 15 minutes after stimulation).

Mauchly´s test of sphericity was performed to validate ANOVA. Furthermore, the Greenhouse-Geisser correction was assessed to correct in case of violations of sphericity. For post hoc analysis were used the Bonferroni correction and paired t-tests.

The data are reported as mean ± SEM. A p-value ≤ 0.05 was used in this study.

Next, the perceived sensation and the correct identification of stimulation type was evaluated through a Chi-square test.

For Vigilance data analysis, the following applies: A lower PUI value indicates a greater alertness (Regen et al. 2013). Figures 8 and 9 demonstrate the pupillometry data of an alert and a sleepy participant respectively. Inaccurate data with more than 50% data lack were manually excluded.

Lastly, the mean PUI data was natural log transformed and a Pearson correlation coefficient was performed to assess a correlation between the level of vigilance and trial-to-trial variability of MEP.

Figure 8: Alert participant. Pupil diameter is stable and PUI is low.
Pupillendurchmesser = Pupil diameter (PD); Augenposition = eye position; Datenlücken = data gaps; Ergebn = Results

Figure 9: Sleepy participant. Pupil diameter is unstable and PUI is high.
Pupillendurchmesser = Pupil diameter (PD); Augenposition = eye position; Datenlücken = data gaps; Ergebn = Results

3 Results

This chapter presents the results of the study described in the previous chapter, with which we aim to examine the neuroplastic alterations and variability of tACS on the human motor cortex among caffeine-adapted subjects. These results have been published in the Journal of Clinical Neurophysiology (Zulkifly et al. 2021a) and were used here with a permission of the co-authors and the publisher (Elsevier).

The chapter is composed of four sections. First, Section 3.1 describes the stimulation perception and the participant´s identification of stimulation type. Next, the plasticity effects of tACS are presented in detail in Section 3.2. The roles of caffeine and vigilance on cortical excitability are finally introduced in Sections 3.3 and 3.4 respectively. The data analysis is described in the previous chapter, in Section 2.6.

3.1 Stimulation perception and participant´s identification of stimulation type

In a questionnaire, the participants were asked about the short-term adverse effects and stimulation-related sensations. No adverse effects from the 200 mg caffeine administered tablet were reported among the subjects (Zulkifly et al. 2021b). The most common side effects described from the participants were related to cutaneous sensations, such as burning or itching on the active electrode location.

A Chi-Square Test was performed to determine whether the proportion of participants who reported cutaneous sensations (Yes: 42.1%; No: 57.9%) differs by stimulus type. The results of the test were non-significant ($\chi 2$ (7) = 4.95, $p > 0.05$) (Zulkifly et al. 2021a). The same test was performed regarding the correct identification of stimulation (Actual stimulation: 40.6%, Sham stimulation: 30.3%, Don’t know: 29.1%). Similarly, the results were non-significant ($\chi 2$ (14) = 11.81, $p > 0.05$) (Zulkifly et al. 2021a). The number of participants reporting sensations during tACS and how they rated the stimulation quality is shown in detail in Table 2a) and 2b) respectively.

Table 2(a-b): Number of participants reporting sensations during tACS and their ratings of stimulation quality (Zulkifly et al. 2021a)

a) tACS sensation perception

	Yes, n (%)	No, n (%)
1) tACS (1.0 mA)		
afternoon, placebo	14 (46.7)	16 (53.3)
afternoon, caffeine	9 (30.0)	21 (70.0)
morning, placebo	13 (43.3)	17 (56.7)
morning, caffeine	15 (50.0)	15 (50.0)
2) tACS (Sham)		
afternoon, placebo	11 (36.7)	19 (63.3)
afternoon, caffeine	13 (43.3)	17 (56.7)
morning, placebo	15 (50.0)	15 (50.0)
morning, caffeine	13 (43.3)	17 (56.7)

b) Rating of stimulation type

	True stimulation, n (%)	Placebo stimulation, n (%)	Don't know, n (%)
1) tACS (1.0 mA)			
afternoon, placebo	10 (33.3)	9 (30.0)	11 (36.7)
afternoon, caffeine	9 (30.0)	9 (30.0)	12 (40.0)
morning, placebo	12 (40.0)	9 (30.0)	9 (30.0)
morning, caffeine	13 (43.3)	8 (26.7)	9 (30.0)
2) tACS (Sham)			
afternoon, placebo	13 (43.3)	11 (36.7)	6 (20.0)
afternoon, caffeine	9 (30.0)	11 (36.7)	10 (33.3)
morning, placebo	16 (53.3)	9 (30.0)	5 (16.7)
morning, caffeine	16 (53.3)	7 (23.3)	7 (23.3)

3.2 The plasticity effects of tACS in caffeine-adapted subjects

The morning sessions started at 09:41 ± 0:49 o'clock and ranged between 07:35 and 11:33 o'clock. The afternoon sessions started at 15:07 ± 1:05 o'clock and ranged between 12:00 and 18:03 o'clock (Zulkifly et al. 2021a).

The rmANOVAs were conducted to compare the aftereffects of tACS on cortical excitabilty. The results revealed that the main effect factors in the placebo sessions were TIME and STIMULATION. There was also a significant interaction effect between TIME x STIMULATION x DAY (Table 3) (Zulkifly et al. 2021a). In the caffeine sessions, the main effect factors were also TIME and STIMULATION but there were no significant interaction effects (Table 3).

Controlling for the factor DAY, there was a significant main effect of TIME in all sessions ($p < 0.001$). STIMULATION was a significant effect factor in the morning sessions, where there was also a significant interaction effect for TIME x STIMULATION. These effects were not seen in the afternoon sessions (Table 4).

In some sessions, the MEP amplitudes were reduced for 30 minutes. Specifically, MEP amplitudes were lower than baseline in the Sham sessions overall. Moreover, cortical excitability in the morning Sham sessions was significantly lower at PST10 – PST25 than in the morning 1.0 mA tACS sessions (Figure 10a).

Similarly, the MEPs were always significantly lower in POST 1 except in the morning, 1 mA tACS placebo sessions (Figure 10c).

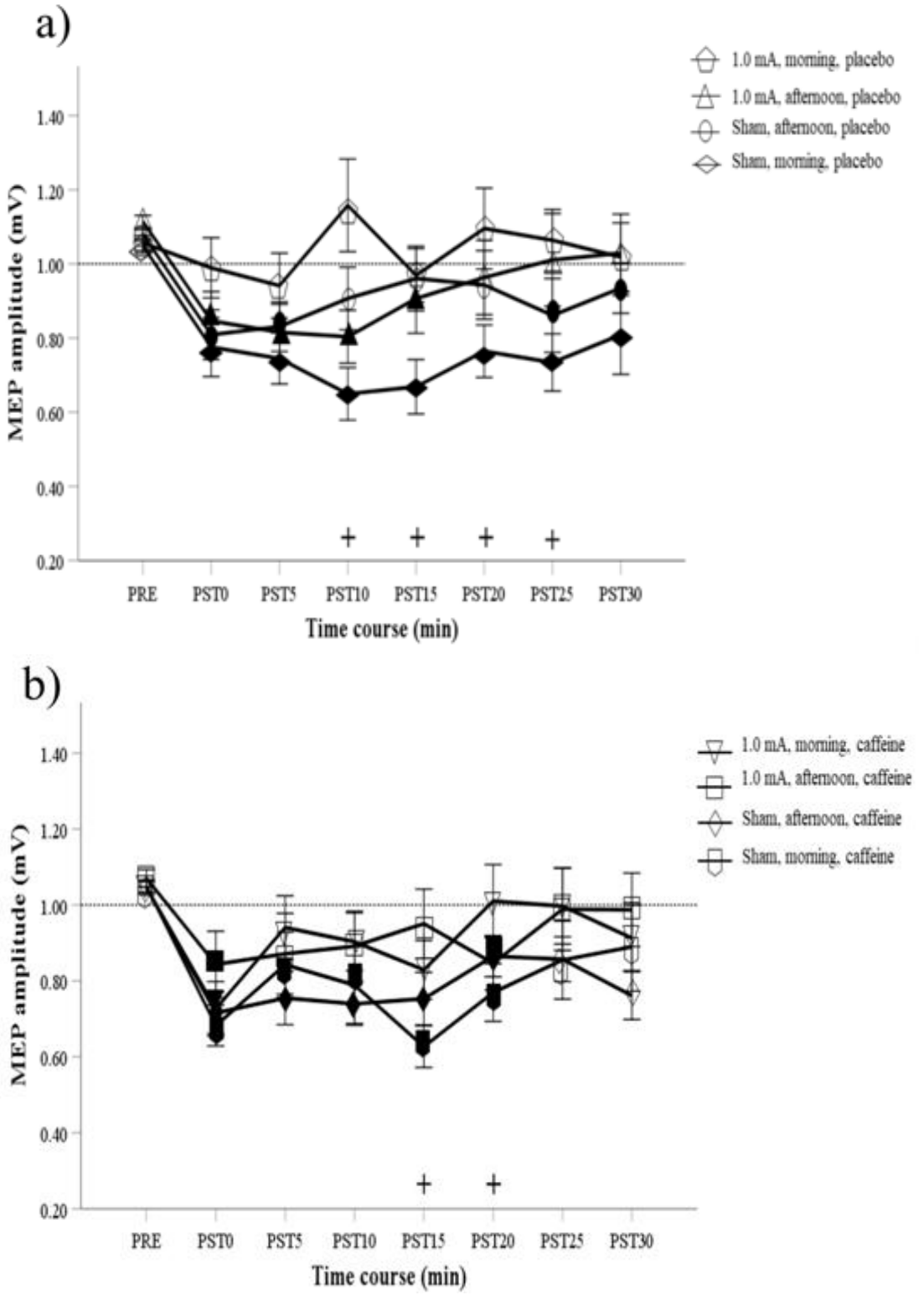

Figure 10(a-b): Neuroplastic alterations after placebo or caffeine administration. The aftereffects of Actual or Sham tACS a) in the placebo sessions or b) in the caffeine sessions (Zulkifly et al. 2021a). Data presentation: means ± SEM.

(+) = significant results between Actual and Sham stimulation in the morning placebo sessions;
Filled polygonal figures mean significant difference from PRE. PRE=baseline; PST0=0 min, PST5= 5min, PST10=10min, PST15=15 min, PST20=20 min, PST25=25min, PST30=30 min after stimulation.

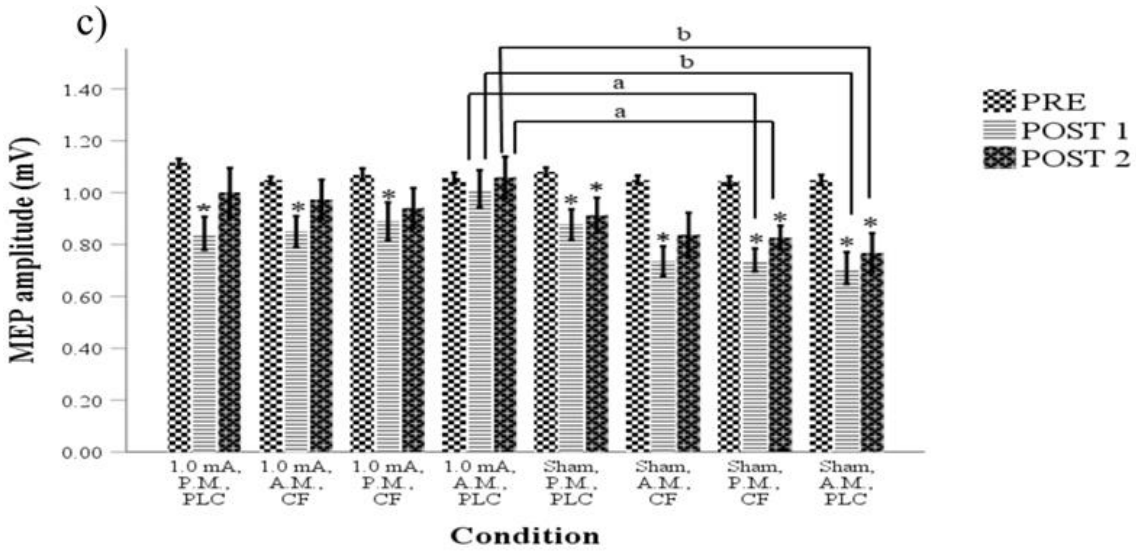

Figure 10c): Neuroplastic alterations after placebo or caffeine administration. c) The statistical analysis of pooled data of all data points per session demonstrated significant effects of TIME ($p < 0.001$) and STIMULATION ($p = 0.002$). There was a significant interaction between the factors TIME x STIMULATION ($p = 0.031$) and STIMULATION x DAY x DRUG ($p = 0.045$) (Zulkifly et al. 2021a). MEPs were smaller in the first 15 minutes after stimulation overall ($p < 0.05$).
(a) = significant difference between Actual tACS in the morning placebo sessions and Sham stimulation in the afternoon caffeine sessions (POST 1: $t(29) = 3.18$, $p = 0.004$; POST 2: $t(29) = 2.42$, $p = 0.02$).
(b) = significant difference between Actual tACS in the morning placebo sessions and Sham tACS in the morning placebo sessions (POST 1: $t(29) = 3.18$, $p = 0.004$; POST 2: $t(29) = 2.49$, $p = 0.02$).

Data are reported as means ± SEM. (*) = $p < 0.05$; PRE= baseline; POST 1 = mean value of the first 15 after stimulation; POST 2 = mean value of the last 15 min after stimulation; PLC = sessions with placebo tablet; CF = sessions with caffeine tablet; A.M. = sessions in the morning; P.M. = sessions in the afternoon.

Table 3: Results of ANOVAs (control for factor DRUG)

	Parameters	d.f.	F	η_p^2	P
1) Placebo					
All data	Time	7, 203	5.10	0.15	<0.001*
	Stimulation	1, 29	6.00	0.17	0.021*
	Day	1, 29	0.19	0.01	0.665
	Time x Stimulation	7, 203	1.36	0.05	0.225
	Time x Day	7, 203	0.91	0.03	0.501
	Stimulation x Day	1, 29	4.71	0.14	0.038*
	Time x Stimulation x Day	7, 203	2.38	0.08	0.023*
2) Placebo					
Pooled data	Time	1, 29	24.40	0.46	<0.001*
	Stimulation	1, 29	7.28	0.20	0.011*
	Day	1, 29	1.18	0.04	0.286
	Time x Stimulation	1, 29	3.94	0.12	0.057
	Time x Day	1, 29	0.24	0.01	0.63
	Stimulation x Day	1, 29	4.22	0.13	0.049*
	Time x Stimulation x Day	1, 29	4.94	0.15	0.034*
3) Caffeine					
All data	Time	7, 203	9.48	0.25	< 0.001*
	Stimulation	1, 29	5.86	0.17	0.022*
	Day	1, 29	0.01	0.00	0.922
	Time x Stimulation	4.56, 132.26	0.70	0.02	0.673
	Time x Day	7, 203	1.20	0.04	0.302
	Stimulation x Day	1, 29	0.01	0.00	0.91
	Time x Stimulation x Day	7, 203	1.29	0.04	0.26
4) Caffeine					
Pooled data	Time	1, 29	27.38	0.49	<0.001*
	Stimulation	1, 29	8.09	0.22	0.008*
	Day	1, 29	0.06	0.00	0.815
	Time x Stimulation	1, 29	3.27	0.10	0.081
	Time x Day	1, 29	0.01	0.00	0.919
	Stimulation x Day	1, 29	0.08	0.00	0.776
	Time x Stimulation x Day	1, 29	0.02	0.00	0.878

(*) = $p < 0.05$; degree of freedom (d.f.); partial eta square (η_p^2).

Table 4: Results of ANOVAs (control for factor DAY)

	Parameters	d.f.	F	η_p^2	P
1) Afternoon					
All data	Time	7, 203	7.47	0.21	< 0.001*
	Stimulation	1, 29	2.38	0.08	0.134
	Drug	1, 29	7.80	0.03	0.380
	Time x Stimulation	7, 203	0.89	0.03	0.516
	Time x Drug	7, 203	0.34	0.01	0.934
	Stimulation x Drug	1, 29	1.15	0.04	0.292
	Time x Stimulation x Drug	5.20, 150.78	0.98	0.03	0.446
2) Afternoon					
Pooled data	Time	1, 29	32.17	0.53	<0.001*
	Stimulation	1, 29	3.94	0.12	0.057
	Drug	1, 29	1.68	0.06	0.205
	Time x Stimulation	1, 29	0.67	0.02	0.421
	Time x Drug	1, 29	0.06	0.00	0.804
	Stimulation x Drug	1, 29	0.93	0.03	0.344
	Time x Stimulation x Drug	1, 29	1.30	0.04	0.264
3) Morning					
All data	Time	4.92, 142.56	6.56	0.18	< 0.001*
	Stimulation	1, 29	9.62	0.25	0.004*
	Drug	1, 29	0.61	0.02	0.442
	Time x Stimulation	7, 203	2.77	0.09	0.009*
	Time x Drug	7, 203	1.70	0.06	0.110
	Stimulation x Drug	1, 290	1.64	0.05	0.211
	Time x Stimulation x Drug	4.66, 135.20	0.92	0.03	0.468
4) Morning					
Pooled data	Time	1, 29	33.41	0.54	<0.001*
	Stimulation	1, 29	10.58	0.27	0.003*
	Drug	1, 29	0.66	0.02	0.423
	Time x Stimulation	1, 29	7.69	0.21	0.010*
	Time x Drug	1, 29	0.43	0.02	0.516
	Stimulation x Drug	1, 29	1.80	0.06	0.190
	Time x Stimulation x Drug	1, 29	1.29	0.04	0.27

(*) = $p < 0.05$; degree of freedom (d.f.); partial eta square ($\eta p2$).

3.3 The roles of caffeine on cortical excitability

Concerning possible interaction effects between stimulation and caffeine, no STIMULATION x DRUG interaction was demonstrated neither in the morning nor in the afternoon sessions (Table 4).

Furthermore, MEP amplitudes in the first 20 minutes after stimulation were lower than baseline in almost all the caffeine sessions (Figure 10b).

3.4 The roles of vigilance on cortical excitability

The graphic presented in figure 11 was composed to compare the level of alertness in the placebo and caffeine sessions. The mean values of PUI in the placebo sessions were significantly higher than the mean values of PUI in the caffeine sessions (Figure 11). Thus, we can conclude that caffeine increased alertness.

In contrast, the graphic as shown in figure 12 was composed to compare the pupil diameter in the caffeine and placebo sessions. The results revealed that mean pupil diameters were constant overall (Figure 12). The pupil diameters observed in this study ranged from 6.7 ± 1.2 mm to 7.0 ± 1.3 mm (Zulkifly et al. 2021a).

A Pearson correlation coefficient was performed to assess the relationship between $\log_{10}$ PUI and POST MEPs. There was a significant negative correlation between the two variables only in 1 mA tACS placebo sessions (Figure 13a), suggesting a positive effect between increased alertness during stimulation and tACS aftereffects. There were no significant correlations between the two variables in any other session (Figure 13b-13d).

Regarding the quality of data, six low quality data were manually detected and excluded.

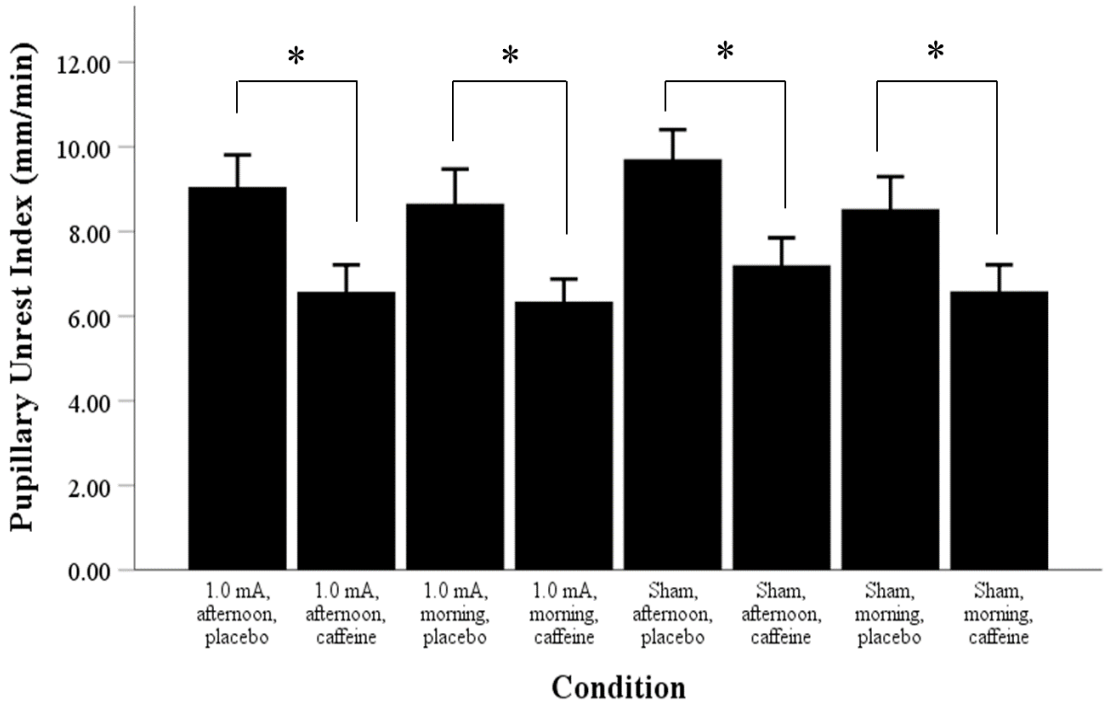

Figure 11: Pooled data for pupillary unrest index (PUI) during Actual or Sham stimulation. Comparison of mean values of PUI in the caffeine and placebo sessions (Zulkifly et al. 2021a). Data presentation: means ± SD; (*) = $p < 0.05$.

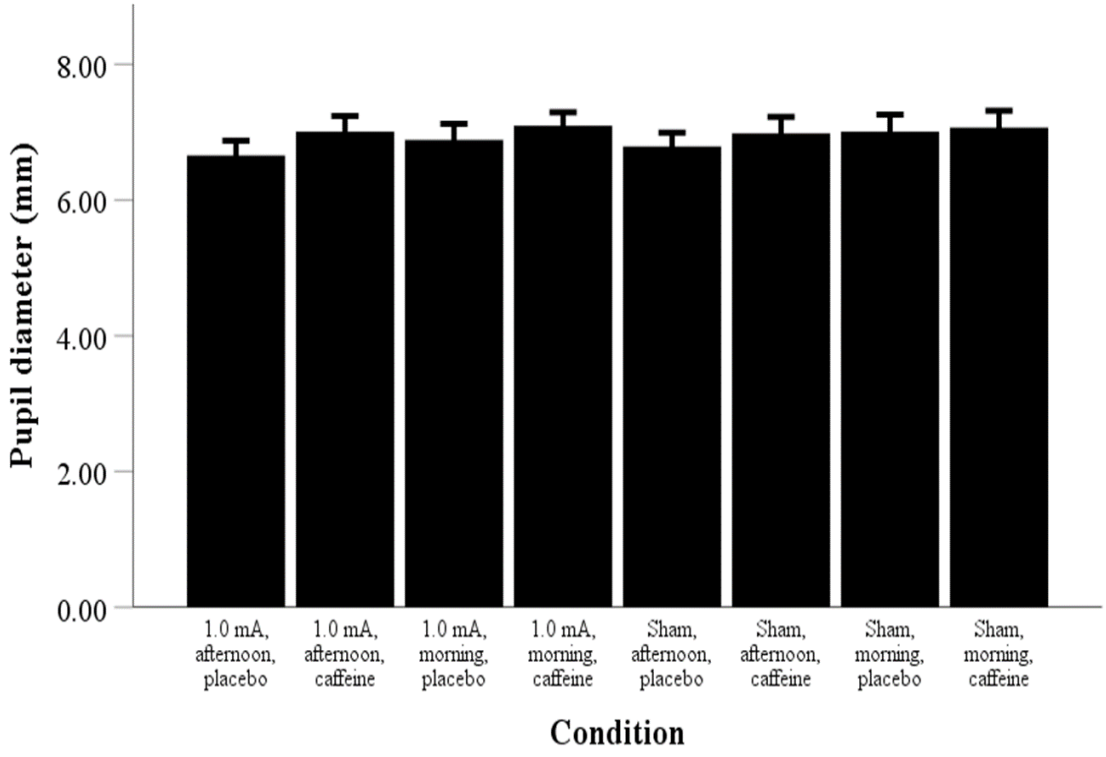

Figure 12: Pooled data for mean pupil diameters during Actual or Sham stimulation. Comparison of pupil diameters in the placebo and caffeine sessions (Zulkifly et al. 2021a).
Data presentation: mean ± SD; (*) = $p < 0.05$.

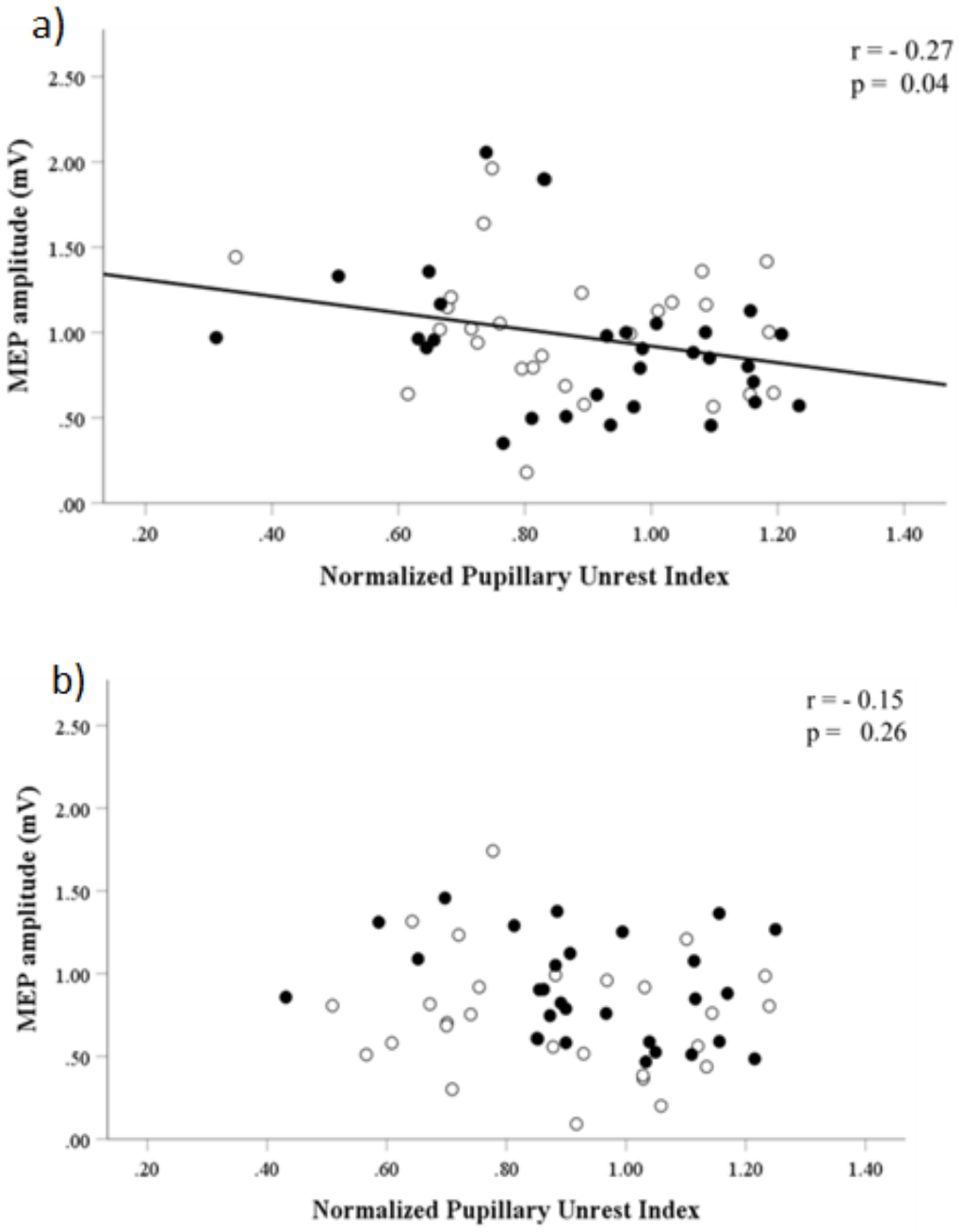

Figure 13(a-b): Correlations between normalized PUI and MEP amplitudes. a) Correlation between normalized PUI and MEP amplitudes in the Actual tACS placebo sessions. Alertness showed a significant positive correlation with cortical excitability. b) Correlation between normalized PUI and MEP amplitudes in the Sham stimulation placebo sessions (Zulkifly et al. 2021a).
Unfilled symbols = sessions in the morning; filled symbols = sessions in the afternoon.

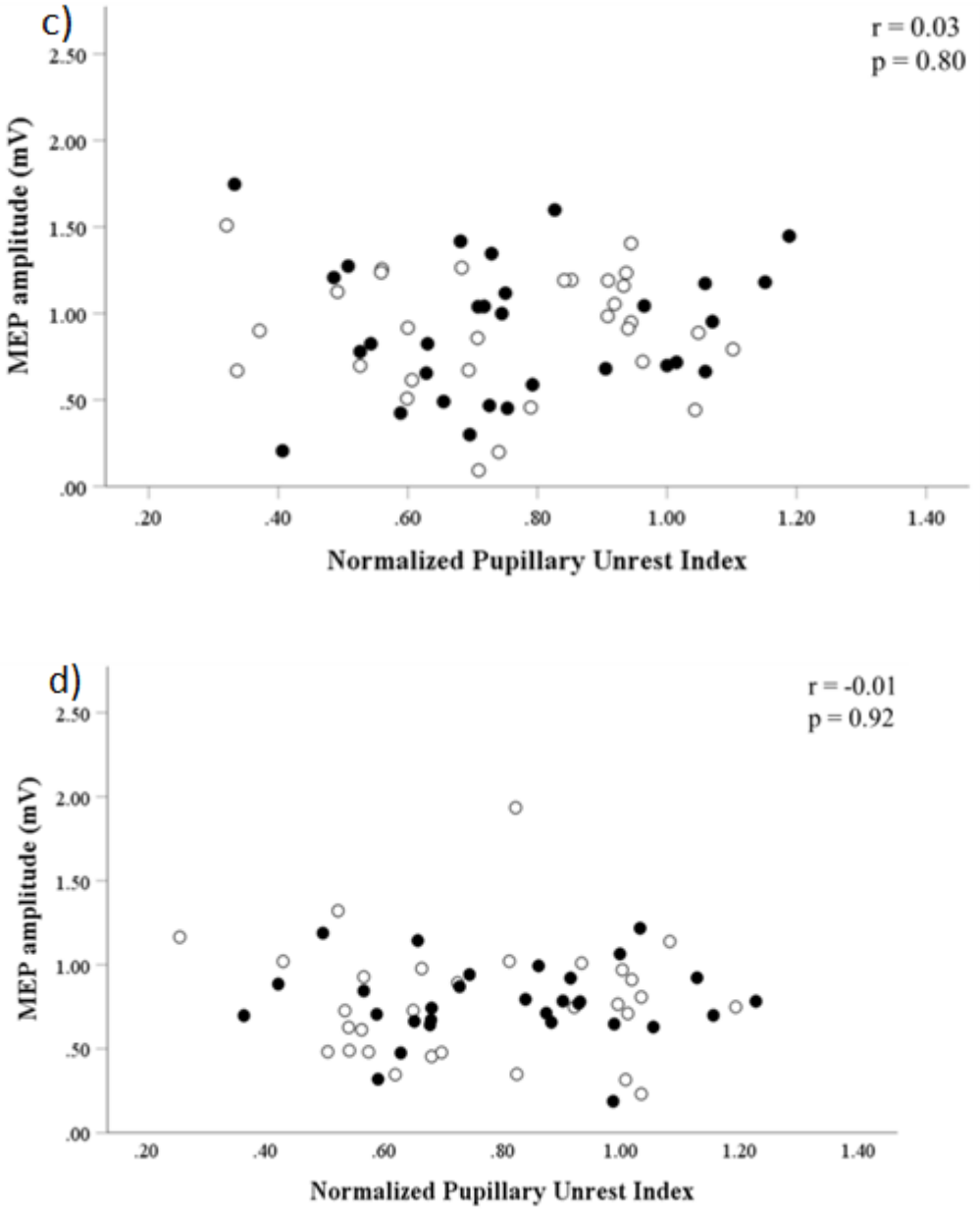

Figure 13 (c-d): **Correlations between normalized PUI and MEP amplitudes.** c) Correlation between normalized PUI and MEP amplitudes in the 1.0 mA tACS caffeine sessions. d) Correlation between normalized PUI and MEP amplitudes in the Sham caffeine sessions (Zulkifly et al. 2021a). Unfilled symbols = sessions in the morning; filled symbols = sessions in the afternoon.

4 Discussion

The main observation in this study was the stimulant effect of caffeine. This will be discussed in Section 4.1. Among others, light deprivation and caffeine can be regarded as confounding factors in plasticity induction studies as described in Section 4.2. and 4.3 respectively. Next, the plasticity effects of tACS are addressed in Section 4.4. Finally, the discussion concludes with the limitations of this work.

4.1 Caffeine increases alertness

It is well known that caffeine increases alertness. Interestingly, the effects of caffeine were first described in myths. One myth is that Muhammad could "unhorse 40 men and make 40 women happy" after the coffee consumption (Fredholm 2011). Furthermore, present knowledge of the effects of caffeine still depends heavily on the 1960s discovery of adenosine and its A2A receptors (Jacobson et al. 2022).

Nowadays, the effect of caffeine on alertness can very well be evaluated by pupillometry. PUI was lower and pupil fluctuations were less after caffeine intake as shown in Figure 3. Wilhelm et al. (2001) reported a mean daytime PD of 7.24 ± 0.40 mm. In conclusion, the PUI and the pupil diameters in this study match perfectly with those described in literature.

4.2 Light deprivation as a confounding factor

We observed a consistent reduction in cortical excitability (except tACS placebo morning), most likely due to the stimulation being carried out in light deprivation conditions, mandatory for obtaining a reliable reading from the pupillometry. Surprisingly, the light deprivation is possibly a confounding factor in this study (admittedly not considered as one, while we designed the experiment). This makes the interpretation of the following results difficult.

However, there is evidence to confirm our result with reference to MEP reduction due to light deprivation. Cambieri et al. (2017) investigated the effects of dark adaption on cortical excitability via rTMS. The authors showed that 30 minutes of visual deprivation reduced the facilitation effects induced by rTMS.

Leon-Sarmiento et al. (2005) however reported controversial results. 30 minutes of visual deprivation increased the MEP amplitudes compared to the eyes-open state.

These studies highlight that light deprivation plays a role on cortical excitability. This raises, immediately, the question how the light deprivation affects the motor cortex excitability.

The answer could be found in the link between the primary visual and the primary motor cortex. Strigaro et al. (2015) demonstrated, via paired TMS between first visual and then motor cortex stimulation with an interstimulus interval in the range 12-40 ms, that this connection is most likely moderated by inhibitory interneurons in the M1.

And lastly, if attempting to understand the significance of light in the brain oscillations, the "Berger effect" must be recalled. In the 1930s, Hans Berger, the pioneer of EEG, made the observation of "alpha blocking". The EEG oscillations in the alpha band were reduced in amplitude as the participants opened their eyes. The Berger effect is generally considered to implicate a desynchronisation of activity in different neurons. Thus, stimuli may affect the phase of brain oscillations (Kirschfeld 2005) and it is not a surprise that light deprivation plays a role in cortical excitability. Kirschfeld (2005) emphasizes that understanding neural synchronisation and the origin of "alpha blocking" is very important for the development of a theory of sensory and cognitive processing.

By the way, light itself could be seen as a neuromodulator when light-mediated neuromodulation with the aid of optogenetics is used. The latter is a biological technique to control the activity of cells and neurons with light (Delbeke et al. 2017). The future seems full of great perspectives.

4.3 Caffeine as a confounding factor

We found that there is a consistent reduction in cortical excitability in all caffeine sessions. Here, it is difficult to differentiate between caffeine and light deprivation effects. We can, however, see caffeine as a confounding factor, because of the difference in the excitatory effects of tACS between placebo and caffeine. Concretely, we observed that, while tACS induced excitatory effects compared with Sham in the placebo morning sessions, there is no significant difference between Actual or Sham tACS in the caffeine sessions.

Additionally, the results of the parallel study (Zulkifly et al. 2021a) of the project are also worth mentioning here. In caffeine-naïve subjects investigated in normal room lighting, different from caffeine-adapted subjects, there was a caffeine-induced increase in cortical excitability under tACS. These different behaviours between caffeine-naïve subjects and caffeine-adapted subjects indicate that chronic caffeine consumption may contribute to the response variability of plasticity induction between individuals. The

main mechanism might be due to altering the A1/A2A AR function and balance (Karcz-Kubicha et al. 2003).

Finally, adenosine itself may have an endogenous protective role in our body. This raises the question whether the consumption of moderate amounts of caffeine as an adenosine receptor antagonist is risky (Jacobson et al. 2022). Messina et al. (2015) mention that coffee may be seen as a functional food. According to their review, many studies suggest that caffeine intake is beneficial, may have protective effect via A2A AR on developing Alzheimer´s disease, and is inversely associated with risk for various diseases, such as type 2 diabetes, colon cancer, liver cirrhosis and gallstone. Regarding the last point, the authors emphasise that association does not prove causation and thus, more studies are needed to clarify this issue. Intriguing and still unclear is also an association with coffee consumption and reduced overall mortality in Europa (Gunter et al. 2017; Jacobson et al. 2022). Furthermore, Messina et al. (2015) do not forget to mention a few negative effects of caffeine consumption, such as an increase in vascular constriction or blood pressure and reduced control of fine motor movements.

4.4 The plasticity effects of tACS

Actual tACS increased MEPs compared with Sham stimulation in the morning sessions. This is in line with the excitatory effects of the protocols showed by previous studies (Moliadze et al. 2010; 2012). However, this is not satisfactory enough, as we were inefficient to reproduce the excitatory aftereffects of tACS exactly as shown by Moliadze et al. (2012). This may have the following explanations. Firstly, the light deprivation as a confounding factor and secondly, the long duration of experiment (2,5 hours) followed by the lack of physical activity, may have influenced the plasticity aftereffects as similarly reported also by Huang et al. (2017). In a review, Antal et al. (2022) are also attempting to explain why NIBS techniques may fail to produce excitatory effects. According to the authors, a reason might be the stimulation intensity. Both, too low and too high intensity can fail to induce inhibition or excitation.

Importantly, in the morning sessions, tACS induced an increase of MEPs compared with Sham. As mentioned above, our observations showed no reduction in cortical excitability in the tACS placebo morning sessions. This indicates that time of day may also be a confounding factor and thus, cortical excitability may be modulated by circadian phase. Automatically, this raises the question whether the endogenous corticosteroid levels contribute to this effect, as it is well known that cortisol is regulated by circadian rhythm. For this reason, we measured corticosteroid levels in saliva. Nevertheless, endogenous corticosteroid concentrations in saliva didn't correlate with tACS postmeasurements (Zulkifly et al. 2021b).

This is in contradiction with the study of Sale et al. (2008). However, it should be emphasised that they are using PAS, which is supposed to be synapse specific, and not tACS, which is supposed to be synapse unspecific, as NIBS technique (Kuo et al. 2007). They showed that the MEPs in resting left abductor pollicis brevis muscle were significantly higher after PAS in the evening (8 P.M). In the morning (8 A.M), when cortisol levels were high, there was no significant MEP amplitude increase. More about this topic is to find in our cortisol paper (Zulkifly et al. 2021b).

Regarding alertness, no correlations were found between alertness and cortical excitability in any other sessions except a significant positive correlation in the placebo 1.0 mA tACS sessions (See figure 13). This positive correlation indicates at least an association between alertness and plasticity aftereffects of tACS. A hypothesis for this is that fatigue may increase the alpha power. Sauseng et al. (2009) showed that MEPs were higher when alpha amplitudes were low. Nevertheless, we measured the vigilance with pupillometry and not EEG. Thus, specific information about oscillatory brain activity is missing in this study.

Finally, tACS remains a promising method, because it has some advantages compared to other NIBS methods. Firstly, tACS at 140Hz goes along unnoticed by the subjects as already mentioned by Moliadze (2010). Secondly, tACS may interfere with ongoing neuronal oscillations and has a great potential in treating neurological disorders with abnormal oscillatory pattern (Antal et al. 2013), specifically Parkinson's disease or schizophrenia (Gonzalez-Burgos and Lewis, 2008; Burns et al. 2011), recurrent gliobastoma (Kirson et al. 2007) or optic nerve injury in human (Gall et al. 2010; Sabel et al. 2011). However, these advantages and disadvantages do not indicate that tACS is generally better than the other NIBS methods. The final purpose of the intervention needs to be taken into account (Antal et al. 2022).

4.5 Limitations of the study

There are several limitations.

First, the study design needed to be limited to four variables in this study: stimulation (Actual and Sham tACS), time of day, alertness and caffeine. This led to 8 sessions, which made finding participants already difficult. On the other hand, even more experimental conditions might have been helpful.

Second, light deprivation and the long duration of experiment design may have all influenced the results of this study, which makes the interpretation of the data more difficult as compared to “normal” results under daylight conditions.

Third, we used pupillometry to measure the level of alertness, a very elegant method. However, other methods, like EEG, would have given different information about the

brain states. With this information, we could have compared our results better with e.g. the study of Sauseng et al. (2009). Another methodological limitation of this study is the lack of a neuro-navigation system. This TMS coil positioning system provides precision in targeting the M1.

And finally, other parameters, such as genetic polymorphism and activity of specific metabolizing enzymes, may also have influenced the results. However, it is very difficult to control these parameters. The main liver enzyme responsible for caffeine metabolism, specifically CYP1A2 is worth mentioning here (dePaula and Farah 2019). Different CYP1A2 SNPs can therefore affect the rate of caffeine metabolism (Jacobson et al. 2022).

5 Summary

Transcranial alternating current simulation (tACS) at a "ripple" frequency of 140 Hz may interfere with ongoing brain oscillations and thus causes changes and modulates cortical excitability. Response variability further hinders transcranial electric stimulation (tES) techniques to be used in clinical practice. Therefore, this study aimed to better understand the factors that might cause a response variability in plasticity induction studies. We concretely examined the effects of time of day, caffeine and alertness on plasticity aftereffects of tACS (140Hz, 1mA) on the motor cortex (M1) in moderate caffeine consumers (200-400 mg/day).

This study (n = 30, Male : Female = 1 : 1) was a randomized, double-blind, cross-over study and consisted of 8 sessions. An experimental session lasted 2,5 hours. First, the motor threshold was determined, which was followed by the oral admission of caffeine (200mg) or placebo tablet and a 45-minute waiting period. After that, tACS or Sham tACS was applied for 10 minutes over the M1 in a randomized order: Actual tACS stimulation, morning; Sham stimulation, morning; Actual tACS stimulation, afternoon; Sham stimulation, morning. At the same time, the level of alertness was monitored by pupillometry for 11 minutes as the spontaneous oscillations in pupillary size reflect the level of central nervous activation. The aftereffects of stimulation were recorded every 5 minutes till 30 minutes post stimulation. At the end of experiment, the participant filled in a questionnaire regarding short term adverse effects or stimulation-related sensations.

The data analysis showed a consistent reduction in cortical excitability (except tACS placebo morning) probably due to the light deprivated situation. tACS induced excitatory effects compared with Sham, particularly in the placebo morning sessions. Furthermore, we observed that caffeine increased alertness and that there was no significant difference between Actual or Sham tACS in caffeine sessions. Finally, our analysis found non-significant correlations between vigilance and motor cortex excitability in the Sham or Actual stimulation sessions except a significant positive correlation in the 1.0 mA tACS placebo sessions.

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Curriculum vitae

I was born on the 17th of August, 1996 in Vlore, a beautiful city along the Albanian Adriatic and Ionian Sea Coasts, as the youngest daughter of two lovely parents.

Good knowledge in science, art and foreign languages were seen as an important part of education from my parents. Therefore, foreseeable, I attended a musical school, specializing in piano, till the 5th Class followed by a general gymnasium in Albania. After high school graduation, in September 2014, I moved to Germany. In the first year in Hannover/Germany, I attended a preparatory course for academic studies at the university. During this year, I improved my German and met many international students.

In Winter semester 2015/16, I started to study Medicine at Georg August University Göttingen. Studying medicine required time, dedication and focus from me. Nevertheless, in lecture-free time, I had the opportunity to gain medical experience in different medical fields, such as paediatrics, endocrinology, neurology, internal medicine in different cities, like Göttingen, Hannover, Aachen, Wien, Berlin and Munich. I graduated in Medicine on the 1st of June, 2022.

After the 1st State Examination, I had the chance to work for a short time as a student assistant at Clinic for Clinical Neurophysiology, University Medical Center Göttingen. This Dissertation expanded our collaboration, as in March 2019 Prof. Paulus trusted me this thesis. Our "caffeine" project with the main author, Dr. Zulkifly, consisted of 3 publications[1].

In almost 8 years in Germany, I tried different part-time jobs from babysitting, to nursing to student assistant. Also, I surprisingly, found the whole world in the small intellectual city of Göttingen, as I met a lot of international students by participating in InDiGU program. All of these experiences made me more tolerant and a better person. I speak German, English and Albanian.

[1] Zulkifly MFM, Merkohitaj O, Paulus W (2020): Transcranial alternating current stimulation induced excitatory aftereffects are abolished by decaffeinated espresso and reversed into inhibition by espresso with caffeine. Clin Neurophysiol 131, 778-779

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