# Analysis of variations in brain states and impact of TES during behavioral task

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(2024)

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A thesis submitted to the National University of Sciences and Technology, Islamabad,

in partial fulfillment of the requirements for the degree of

Master of Science in

Biomedical Engineering

Supervisor: Dr. Muhammad Nabeel Anwar

School of Mechanical and Manufacturing Engineering

National University of Sciences & Technology (NUST)

Islamabad, Pakistan

(2024)

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*Dedicated to my treasured family, whose unwavering support, encouragement and motivation have been the greatest source of strength and persistence throughout this journey. To my parents, Muhammad Arshad Bhatti and Asma Arshad, thank you so much for your constant prayers, endless love and sacrifices. To my siblings, Sadia, Arqam and Anas, thank you for your encouragement and motivation. This work is a testament to all the love, support, and inspiration you have all provided. Thank you!*

# ACKNOWLEDGEMENTS

First and foremost, “I would like to express my deepest gratitude to ALLAH Almighty, for giving me the courage, enthusiasm and perseverance, and granting me the triumph of this thesis”.

“I would like to express my sincere gratitude to my supervisor, Dr. Muhammad Nabeel Anwar, for his invaluable guidance and persistence, throughout my research. His expertise in this area has been the fundamental part in shaping this thesis”.

“I want to extend my heartiest gratitude to my beloved parents, whose constant prayers and unwavering support have been the bedrock upon which I was able to succeed in this research. Their continuous encouragement helped me conquer this challenging phase of my life”.

“I, also, want to thank my dear siblings for their relentless support through my tough times. My encouraging sister, whose words have always boosted my confidence, and my little brothers, whose energy and positivity have been a source of constant inspiration”.

“Lastly, I would like to thank my friend, Salwa Ali, who was also pursuing her thesis along with me, her support and help proved valuable throughout this journey”.

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| | |
|---|---|
| A | the letter A |
| CTT | Compensatory Tracking Task |
| DLPFC | Dorsolateral prefrontal cortex |
| ECG | Electrocardiography |
| EEG | Electroencephalography |
| EOG | Electrooculography |
| EPSP | Excitatory post synaptic potentials |
| HD-tACS | High definition transcranial alternating current stimulation |
| ICA | Independent component analysis |
| iTBS | Intermittent theta burst stimulation |
| M1 | Primary motor cortex |
| MEPs | Motor evoked potentials |
| PLV | Phase locking value |
| tACS | Transcranial alternating current stimulation |
| tES | Transcranial electrical stimulation |
| tDCS | Transcranial direct current stimulation |
| tPCS | Transcranial pulsed current stimulation |
| tRNS | Transcranial random-noise stimulation |
| TMS | Transcranial magnetic stimulation |
| β | Beta |
| γ | Gamma |
| θ | Theta |

# ABSTRACT

This research targeted to investigate the effects of low-gamma High Definition transcranial alternating current (HD-tACS) at the left DLPFC and primary motor cortex in healthy individuals performing continuous attention task. We selected an openly accessible dataset from openneuro.org. Dataset includes within participant implementation of High-Definition tACS (HD-tACS), stimulating two cephalic regions (frontal & motor) with biphasic stimulation waveform (30 Hz) with a total 600 stimulation trials in 30 sessions. The physiological data i.e. EOG, ECG, along with EEG and behavioral data were being recorded over the course of two 70 and 70.5 minutes' sessions. The demographic data were acquired before and after each session together with the wellness questionnaires. The participants were given two stimulation doses separately, with 20 stimulation trials per session. The within-subject results showed significant differences between the pre- and post-stimulation data (p-value <0.05) in the F30, in each frequency band. While in M30 session, there was a significant increase in alpha and beta oscillations (p-value < .05). The gamma oscillations were not altered by low-gamma tACS at M1, whereas the theta oscillations showed a significant decrease. The phase-locking values (PLV) of frontal channels decreased in theta, alpha, beta & gamma bands, suggesting a drop in the attention of participants with the onset of stimulation. Hence, the results indicate, that low-gamma HD-tACS over left DLPFC has the potential to inhibit attention and information processing. And the low-gamma HD-tACS can improve motor function over the left primary motor cortex.

**Keywords:** HD-tACS, DLPFC, behavioral task, EEG, BIDS EEG data.

# CHAPTER 1: INTRODUCTION

In the progressing field of neuroscience and rehabilitation engineering, the use of transcranial electrical stimulation (tES) has arisen as a hopeful approach for augmenting cognitive and motor performance of the human brain. This thesis explores the effects of High-Definition Transcranial Alternating Current Stimulation (HD-tACS) on the left dorsolateral prefrontal cortex (DLPFC) F5 location and on the primary motor cortex C5 location, of the brain, by visualizing the electroencephalography (EEG) collected from the 32 electrodes placed on the scalp using 10-10 electrode placement system.

## 1.1 Electroencephalography (EEG)

Electroencephalography (EEG) provides a non-invasive framework of brain activity by recording the neuronal activities in the form of electrical signals. These neuronal oscillations depict various frequency bands, reflecting the brain's functional states and connectivity. Understanding EEG patterns is crucial for straightening out the secrecies of cognition, perception, and behavior in mankind.

## 1.2 HD-tES

High-Definition transcranial electrical stimulation (HD-tES) is a neuro-stimulation technique that implicates the application of low-intensity electrical currents to particular parts of the brain.

Unlike conventional tES, HD-tES uses an array of smaller electrodes arranged in a ring configuration on the scalp, a center electrode and surrounding ring electrodes. The smaller electrode configuration in HD-tES enhances spatial resolution. The configurations are known as **montages**, which can vary with the number of surrounding electrodes. The primary advantage of HD-tES lies in its ability to target smaller and more defined regions of the brain. It can deliver electrical currents at various frequencies (e.g., gamma, beta, alpha, theta, delta) and with varying current intensities.

HD-tES has applications in various areas, including cognitive enhancement, motor learning, and therapeutic interventions for neurological and psychiatric conditions. Its potential in areas such as rehabilitation, neuroplasticity, and the modulation of cortical excitability is under exploration.

### 1.3 HD-tACS

HD-tACS is a type of tES, which uses sinusoidal current for stimulation. tACS sends alternating current at different frequencies to entrain or modulate neural oscillations. HD-tACS also employs the same configuration as explained in HD-tES. The electrodes are arranged in high density array for precise targeting of brain areas, achieving focality that was previously thought to be achievable only with high-power magnetic or implanted electrodes. [29]

The technical parameters of HD-tACS, typically followed are; current intensity varying from 0.5 to 2 mA, frequencies depend on the specific band of brain signal under research. There is also a fade in and fade out of 5-20 seconds before and after the stimulation. [29]

Transcranial alternating current stimulation (tACS) is a technique to stimulate the brain without causing adverse changes to it. This is done through applying low-depth alternating electrical currents to the cortex, which changes the pattern in the cortical activity. From the last two decades, this technique has been getting more and more attention because of its ability to modify neural oscillations and cortical excitability, that may affect many cognitive and motor processes of the brain.

Our study targets to add more to what's already known about non-invasive transcranial alternating current stimulation (tACS), and its possible uses in cognitive enhancement and motor rehabilitation. To further explore how tACS can be used in different areas of neuroscience and rehabilitation, this study aims to analyze the effects of low gamma short stimulation tACS at the DLPFC (F5) and M1 (C5) location in 15 neurologically healthy individuals performing continuous vigilance/alertness task for 70 minutes, the dataset provided by Gebodh, N. et al. (2021), an open access data available at openneuro.org [29].

This study intends to distinguish the plausible effects of 30Hz tACS application on two cephalic locations, mainly analyzing the variations befalling in the frequency bands i.e. theta, alpha, beta

and gamma, at different channel locations. The previous study done by Zaghi et al. (2010) found that 15 Hz tACS at C3 and C4 location causes reduction in intra-cortical facilitation (ICF) [24].

A. Giustiniani, et al. (2019) discovered in their research that 40 Hz tACS application at left primary motor cortex (M1) of the brain slowed down the response times of the participants performing a serial reaction time task (SRTT). Furthermore, the also found, by looking at the amplitudes of the motor evoked potentials (MEPs), inhibited the cortical reactivity [23].

### 1.4 Background

The dorsolateral prefrontal cortex (DLPFC) is a vital part of the brain that helps with enhanced problem-solving and memory, as well as making decisions and handling different situations. Previous studies have proven that application of tACS to the DLPFC may be able to alter these mental practices by fluctuating the currents in the neuronal circuits, responsible for them. Nonetheless, we still don't fully comprehend how tACS affects DLPFC and its neural mechanisms responsible for these mental processes. [2]

On the alternative hand, the primary motor cortex (M1) is very critical for executing and controlling voluntary movements. More and advanced knowledge suggests that tACS carried out over M1 can either enhance or reduce the excitability of the cortex as suggested by Zaghi et al. (2010) and Antal, A. et al. (2008). [24, 30] Nonetheless, it is still ongoing research as to how tACS modifies the mechanism of M1 or how it stimulates different motor regions of the brain.

In 2019, Gebodh, N. et al., investigated about the inborn physiological artifacts appearing during tDCS in EEG signals, the glitches that these artifacts pose, when EEG and High Definition-tDCS (HD-tDCS) data is acquired and interpreted. They identified physiological and non-physiological artifacts that arise from the body, like cardiac and ocular motor distortion, and those that arise from the hardware or power-line interference. They suggested several strategies to mitigate these artifacts such as improved experimental design, high-level signal processing. [19]

Electroencephalography (EEG), is a way to manuscript brain’s electrical activity without hurting the person. However, the EEG signals are frequently tousled through glitches, that make the recorded data an awful lot less beneficial and leads to incorrect conclusions to be drawn. Artifacts can hide or reduce the true features of cortical activity, which can result in wrong

conclusions from the research studies. So, getting rid of artifacts from EEG signals is specifically dynamic for accurate acquisition. Many unique methods, from simple filtering to complex signal filtering techniques like regression-based methods, wavelet transform, Independent Component Analysis (ICA) and blind source separation (BSS), have been fashioned to get rid of these artifact. Urigüen, J. A. et al. (2015) gave a thorough review of the latest EEG artifact elimination techniques. They provided practical guidelines to select appropriate techniques depending on the type of artifacts and computational efficiency. For example, ICA is best for handling artifacts like ocular and muscle artifacts, while wavelet transform is best for dealing with non-stationary signals. [20]

In our research, by looking at the connectivity patterns and channel plots of EEG signals, there were visible eye blink and muscle artifacts, therefore ICA ('runica') was opted for the removal of these artifacts.

Transcranial alternating current stimulation (tACS) modifies the natural rhythms of the cortex by means of applying external sinusoidal biphasic current. However, the study on tACS manifestations are still under research. Online tACS over the primary motor cortex (M1) had robust outcomes on the excitability of the cortex that rely upon frequency as concluded by Pozdniakov et al. (2021). However, the offline results of tACS were not prominent and lasted only for short duration. They used 10, 20 Hz and sham stimulation protocol. Their results showed a significant improvement in motor function with 20 Hz tACS. [22]

Zaghi et al. (2010) conducted a study, a 15 Hz transcranial alternating current stimulation (tACS) application at C3 and C4 location for 20 minutes. They found out that tACS at this frequency leads to diminished amplitude of MEPs and decreased intra-cortical facilitation (ICF). They proposed that this was achieved with using a significantly higher current of 0.8mA for a considerable longer duration of 20 minutes. The authors suggested that the inhibitory effects could be associated with frequency-specific modulation on the cortical activity. [24]

In transcranial alternating modern stimulation (tACS), a painless way to have an effect on the brain, low-intensity alternating electric currents are sent to the head. The purpose of this technique is to influence the activity inside the cortex by way of varying the neural oscillations which are linked to diverse cognitive and motor capabilities. Helfrich et al. (2014) explore the

functional connectivity in the interhemispheric gamma-band coherence in motor cortex. They used in-phase and antiphase stimulations. The in-phase oscillations synchronized with the on-going gamma activity in the brain. Thus, resulting in entrainment which lead to enhanced synchronization between the hemispheres. However, their results also revealed a decreased oscillatory alpha power. [2]

In our case, the opposite of findings of Helfrich et al. (2014) is happening. Our experiment is using low-gamma frequency waves to stimulate the motor cortex which is resulting in decrease in the oscillating gamma-band power and enhanced alpha power. [2]

Transcranial direct current stimulation (tDCS) or TMS are other ways to stimulate the brain. tDCS uses a constant direct current pulse while, TMS uses of magnetic pulses. tACS is more specific as it lets you alter the cortical activity by synchronizing with the natural neural oscillations, using different frequencies. tACS makes use of a sinusoidal biphasic signal that may be able to synchronize or entrain the evidently occurring neural oscillations within the stimulated brain vicinity. According to research by Pollok, B. et al. (2015), tACS may synchronize endogenous brain oscillations while stimulating the brain and may potentially be linked to long-lasting neuro-plastic alterations [26].

Entrainment is a plausible theory to explain the nature of tACS effects on neuronal oscillations. The technique of syncing an external AC current with internal rhythmic brain activity is known as entrainment. It is suggested that if entrainment intervenes to modify modulatory tACS effects, these effects would only last while tACS is turned "ON" and would drastically diminish once tACS is switched off. Up until now, this hypothesis has only had secondary proof from earlier study. For instance, many studies demonstrated the bulbous effects of 20 Hz stimulation on M1 during the tACS application by MEPs [26].

In our study, we are discerning similar effects of tACS entrainment with the brain. The effects of tACS are more visible at the start and mid of the stimulation, while by the end the effects are diminishing. This observation is supported by the work of Pollok, B. et al. (2015).

Barbey, A et al. (2013) found through their research, tACS is likely a rehabilitative technique for a whole lot of specific situations, consisting of improving cognitive processing, rehabilitate

motor competencies, and treating neurological and psychiatric disorders. tACS may also assist the brain's performance better with the aid of altering cortical excitability and synchronizing neural rhythms. This ought to cause better cognitive overall performance, motor skills, and may facilitate in rehabilitation [3].

tACS has been studied in a number of ways as to how it impacts the cognitive progressions like attention, working memory, and making assortments. Polania et al., (2012) demonstrated that tDCS can significantly modulate the brain's functional connectivity, with a current intensity of 1mA. Their results suggest that anodal tDCS over the left dorsolateral prefrontal cortex (DLPFC) enhanced cognitive processing. They utilized healthy participants who underwent both anodal and cathodal tDCS sessions on separate days. However, their study clearly suggests that the cortical excitability and inhibition depends on the polarity of tDCS [1].

Ruairidh M. Battleday et al. (2014) discussed in their paper that tACS is a technique involving weak electrical currents to stimulate brain regions. It proposes a unique way of modulating the neural networks of the brain resulting in cognitive enhancement. They also mentioned that frequencies like alpha (8-12) Hz may inhibit task irrelevant processing [4].

tACS has been under research as a promising technique to help with motor skills attainment and rehabilitation in the different areas of the brain. Using tACS inside the beta frequency range on the primary motor cortex (M1) has been proven to make significant alterations in the cortical activity. Wang et al. (2021) used different frequencies in tACS on left M1 location for 10 minutes. They observed the effects of 1 mA tACS with the frequencies of 10 Hz and 20 Hz over M1 on cortical activity during observation of action and execution. They found that their results were frequency-dependent and that 20 Hz significantly reduced motor cortex excitability of healthy adults performing button-tapping task. Compared to 10 Hz tACS which did not produce any significant results [5].

To absolutely distinguish the approach of tACS, numerous feasible experimental protocols have been worked on, such as, synchronizing the stimulation frequency with neural oscillations, changing the plasticity of synapses. Yet, more research is required to determine precisely, what parameters to select to make the stimulation best for producing optimal results in the cognitive or motor improvement.

## 1.5 Significance of the study

The dorsolateral prefrontal cortex (DLPFC) and the primary motor cortex (M1) are vital brain regions that are gaining plentiful attention in tACS research because of their crucial roles in attention, cognitive processing and motor learning.

The DLPFC is a critical part for performing higher-order brain functions like cognitive control, working memory, and making decisions. It is highly possible that changing the neural oscillations inside the DLPFC using tACS may enhance or reduce those cognitive abilities. This could lead to better cognitive overall performance in many regions, including training, work productivity, and cognitive refurbishment. Using tACS to improve working memory and executive capabilities could help humans analyze and resolve issues faster, and do a couple of element straight away. This ought to help students, people, and people with cognitive impairments [6].

Furthermore, reviewing the tACS effects on intricate neural networking of the brain, aids in comprehending the neural dogmas that are responsible for controlling and maintaining the brain functions such as thought process, motor performance. These findings could assist us analyze the neural networks working and could potentially lead to the progress of sophisticated and advanced protocols of non-invasive stimulation techniques.

tACS has several potential applications in the cognitive and motor domains, ranging from performance enhancement and medical interventions to research and healthcare sector. The purpose of this study is to shed light on the effects of tACS at the DLPFC and M1. This should result in the release of new treatment strategies and methods to optimize human potential in numerous areas such as rehabilitation, athletics, improved cognitive or motor learning.

This research is primarily focused on identifying the effects of low-gamma tACS applied over the left DLPFC and primary motor cortex in healthy adults performing a behavioral task. The effects are being investigated separately for both areas. For the DLPFC, the neuronal connectivity of alpha, beta, gamma and theta bands is being analyzed in the frontal cortex. Similarly, the primary motor cortex connectivity is being analyzed in the fronto-central, central, and centro-parietal regions.

The analysis is being done using the phase synchronization analysis, using the electrode channels within the frontal and motor cortices. The channel selection was done by visually inspecting the connectivity plots. Then the frontal 8 channels and their combinations were selected, for analysis of neural connectivity as a consequence of tACS at DLPFC. And for primary motor cortex, the central 13 channels were selected for examining differences in neural connectivity with the tACS application at C5 location.

Since, larger research has been conducted on the application of tACS using high-gamma frequencies i.e. 60-100 Hz, as compared to low-gamma tACS. This research is focused on utilizing this gap and delve into the modifications in neural connectivity occurring as a manifestation of low-gamma tACS over DLPFC and primary motor cortex.

The significance of this research is to investigate, how low intensity gamma frequency (30 Hz) tACS effects the neural connectivity of the brain in the frontal and motor region. Secondly, the aim is to analyze whether the tACS in this frequency lessened or enhanced the underlying neural processes, such as cognitive performance, attention, motor planning and execution.

### *1.5.1 Aims & Objectives*

- This research is aimed to investigate the effects of tACS applied to F5 and C5 location of the brain and to identify how the neuronal excitability of the cortex is altered in those and other regions of the brain.
- To discover how tACS applied to the F5 location affected cognitive processing, especially in the experimental setup that required continuous attention, decision-making, and working memory.
- To discover how tACS effects overall motor performance, when applied at primary motor cortex (C5) with regards to movement execution.

### *1.5.2 Hypothesis*

- Low gamma HD-tACS at the F5 and C5 locations modulates theta, alpha, beta, and gamma oscillatory activities, leading to enhanced attention and cognitive processing in healthy individuals performing a continuous attention task.

- Low gamma HD-tACS at F5 location inhibits vigilance and information processing in healthy individuals performing continuous vigilance task.
- Low gamma HD-tACS at C5 location enhances motor function in healthy individuals performing continuous vigilance task.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 DLPFC

A very critical a part of the prefrontal cortex is the dorsolateral prefrontal cortex (DLPFC). It is typically active in higher-order cognitive functions like working reminiscence, planning and judgement, and executive control (Barbey et al., 2013). The DLPFC helps us use and trade information that comes from many regions of the body, which lets us act in a manner that allows us execute our bodily functions in a regulated and balanced way. [3]

Alekseichuk et al. (2016) discovered that applying tACS at theta and gamma frequencies led to boosted working memory and amplified neocortical connectivity. Specifically, their results exhibited improvements in the information processing and memory formation. These outcomes highpoint that DLPFC plays vital role in cognitive processes and advocate that tACS at explicit frequencies may bring concrete implications for memory augmentation. [32]

Analyzed the effects of tDCS and tACS at 6 Hz, on the working memory performance. They discovered that tDCS exhibited lower neural connectivity online, while tACS brought about larger effects in neural connectivity during stimulation. Vice versa for the resting state-functional connectivity (rs-FC). [33]

A recent study done by Maiella, M. et al. (2022) showed that applying simultaneous iTBS with γ-tACS at high-gamma frequency (70 Hz) over the both DLPFCs results in enhanced spectral gamma power and an escalation of gamma band local connectivity. They utilized phase synchronization analysis and selected wavelet-PLV values for the frontal (F3 vs. F5; F3 vs. F2) electrodes. Using repeated measures ANOVA, they calculated the difference in the oscillations of gamma band [38].

Marchesotti, S., et al. (2020) revealed in their study, low-gamma (30 Hz) tACS over left auditory cortex considerably improved linguistic and semantic processing and reading accurateness, meted out instantly after tACS, in individuals with dyslexia. They used two frequencies 30 and 60 Hz tACS, with the total duration of 20 minutes, on non-dyslexia and dyslexia patients. The results of 30 Hz tACS on phonemic awareness showed a significant improvement [36].

A study by Xiao et al. (2023), employed a double-blind, randomized crossover design to examine the effects of fronto-parietal theta transcranial alternating current stimulation (θ-tACS) on working memory and postural control in twenty healthy individuals. Participants were given tACS over the left (LDLPFC) frontal and parietal cortex, for 20 minutes at 2mA. The outcomes revealed that θ-tACS significantly improved working memory performance and increased EEG theta power, indicating that tACS can indeed, modulate on-going neural progressions. Phase synchronization analysis showed alterations in theta-band connectivity between P3 & F3 [42].

As Palm, U. et al. (2022) demonstrated in their study that healthy individuals received low-gamma tACS (40 Hz) over both DLPFCs and found decreased cognitive performance in them. The current intensity of tACS was 2 mA for both healthy participants and patients with major depressive disorder (MDD) in the single session. Moreover, there wasn't any significant difference observed in cognition of the MDD participants [40].

Using tACS at the DLPFC has been shown in numerous research to change neural rhythms and improve cognitive performance. Working memory; Polania et al. (2012) determined that healthy humans may get an improvement in their working memory through tACS within the theta frequency (4–8 Hz) over the DLPFC. Theta rhythms are accepted to play a pivotal role in working reminiscence approaches, are notion to drive this impact [1].

Shiga et al. (2024) utilized the beta-range (20 Hz) β-tACS over the DLPFC and fronto-parietal network, in their study. They administered the effects of β-tACS during the early short periods of the implicit motor task and discovered that tACS of the specified frequency, produced no change during implicit motor task on microscale learning in healthy individuals [41].

Multiple research has proven that the DLPFC is activated in working memory tasks, where it acts like a RAM, to help preserve information and communicate it to other regions of the brain (Curtis and D'Esposito, 2003) [9]. Distinctively, the DLPFC is the area to play a role in actively reading, processing and updating data in working memory, in addition to picking out essential data from distracting ones (D'Esposito et al. (1999) [10]. The DLPFC is more occupied in constructing perceptual complex decisions, whilst performing many other functions like retaining memory and resolving cognitive conflicts (Heekeren et al., 2004) [11].

As the frontal lobe is fundamental for carrying out executive duties like information updating and monitoring, inhibitory control, and shifting between functions. This part of the brain enables you to switch between exclusive conducts of wondering, preventing attention to pre-potent reactions, and concentrating attention at the important tasks (Miyake et al., 2000) [12]. The DLPFC is also apprehensive for planning, higher-order questioning, and solving dilemmas. It is involved in inductive and deductive reasoning (Goel and Dolan, 2004) [13].

Haller et al. (2020) employed the use of low-gamma (40 Hz) tACS on six patients of major depressive disorder, over the left and right DLPFCs. They formulated two sessions of tACS; one group received 10 min tACS application twice daily, while the second group received 20 min single tACS session, for 10 days. They found that the depression symptoms in all patients improved in general. Moreover, they found that the group 1 (2 times 10 min stimulation) showed better performance in the objective and subjective rating scales as compared to group 2. Hence, they suggested that the twice-daily 10 min stimulation exerted a potentiation effect compared to once-daily 20 min stimulation [43].

A unique configuration of tACS adapted by Saito et al. (2023), over the left DLPFC caused enhancement in the tactile perceptual discrimination performance of the index finger of the right hand of neurologically healthy individuals. They applied two different stimulation configurations, one is the random-noise tRNS which comprised of sinusoidal current of 0.7 mA (p-p) applied at random frequencies between 0.1 to 640 Hz, second is the anodal tPCS comprised of a unidirectional pulsating and rectangular current of 0.7 mA with a pulse duration of 50 ms. The former tACS configuration improved the tactile perceptual discrimination performance immediately after stimulation. Whereas the later did not affect the tactile spatial discrimination performance [44].

In a behavioral task, on the appearance of a stimulus (red circle) on screen, participants had to press a button as fast as possible. The alpha (10 Hz) and theta (6 Hz) tACS was given over the right DLPFC (F4) of the brain for 25 min. Martínez-Pérez et al. (2022) found in their results that the arousal component of vigilance improved in participants with application of alpha tACS [28].

Gebodh et al. (2024) utilized the similar dataset as this research [29]. They found that the HD-tACS of 30 Hz applied at the frontal cortex can produce vigilance enhancement without

associated cardiovascular (HRV) or sustained subjective sleepiness (KSS) changes, as compared to HD-tACS at motor cortex. The EEG outcomes were calculated from the electrodes; P8, P4, O2, P7, P3, and O1. The results showed an increase in alpha versus decrease in theta activity from the pre- to post-stimulation, in F30 as compared to motor cortex M30 [54].

Overall, the DLPFC could be very essential for many intellectual responsibilities, consisting of running memory, making choices, executive manage, wondering, problem-solving, and controlling feelings. Its role in these better-order features indicates how important it's far for goal-directed behavior, cognitive flexibility, and placing together knowledge from specific assets.

### 2.2 Primary motor cortex (M1)

The M1 region, may be very important for executing and controlling voluntary movements. By applying tACS to M1, you probably are able to change the excitability of the cortex, which can assist human beings who have trouble with executing movements or neurological disorders of motor function. This could bring about large aids in helping human beings with problems like stroke, tremor related disorders, or any motor disabilities, to improve their motor capabilities. Additionally, tACS brought on motor learning facilitation may assist athletes, artists, and other folks that want to enhance their motor skills and performance [7].

A. Giustiniani, et al. (2019) discovered in their research that 40 Hz tACS application at left primary motor cortex (M1) of the brain slowed down the response times of the participants performing a serial reaction time task (SRTT) using their right hand. The assignment involved doing the same set of moves again and again in response to the visual stimuli. Furthermore, the also found, by looking at the amplitudes of the MEPs, inhibited the cortical reactivity [23].

The primary motor cortex (M1) is a key area for executing and regulating voluntary movements (Schieber, 2001; Sharma and Baron, 2013). Applying tACS on M1 has proven to vary the excitability of the motor cortex. This may benefit in improving motor planning and balancing of movements.

Miyaguchi, S. et al. (2019) demonstrated in their study that the effects of gamma (70 Hz) tACS over the left M1 improved motor performance and increased the functional connectivity between

M1 and cereberal hemisphere. They applied tACS with a phase difference of 180 between both applications and found that tACS has phase specificity. Their results showed that the task error in participants reduced significantly [39] (b).

tACS within the beta frequency range (20 Hz) applied over M1 has shown in to elevate cortical excitability in the beta range and slow down motor activity in healthy humans, as suggested by Pogosyan et al. (2009). They discovered that beta activity increases when suppressing a movement voluntarily. [34]

Wach et al., (2013) also backed the study of Pogosyan et al. (2009). They used two frequencies; 10 and 20 Hz, over the left M1 for 10 minutes respectively. They also discovered similar effects of 20 Hz tACS over left M1, that the beta frequency slows down the movements of healthy individuals performing a continuous behavioral task i.e. finger tapping. The results of 10 Hz tACS, however, showed a significant increase in the movement variability after 30 min of stimulation cessation [35].

Another study, Marchesotti, S., et al. (2020) revealed in their study, low-gamma (30 Hz) tACS at left auditory cortex significantly improved phonological processing and reading accuracy in individuals with dyslexia [36].

In 2016, Cappon et al., found in their study that 20 Hz tACS over the supplementary motor area and primary motor cortex (SMA-M1), reduced the amplitude of motor evoked potentials MEP at the T3 location. They concluded that tACS can modulate the automatic motor inhibition with 20 Hz applied at motor area during a visuo-motor task. They observed from the results that the negative compatibility effect increased with tACS [37].

During a motor learning task, Bologna et al. (2019) employed two stimulation frequencies i.e. beta (20 Hz) and gamma (70 Hz), in order to assess the effects caused by tACS over M1 region. They concluded that tACS in the 20 Hz range reduced motor performance while, the gamma (70 Hz) tACS accelerated motor learning but caused a detrimental effect on motor retention [17].

Helfrich, R. F. et al. (2014) investigated the effects of 10 Hz transcranial alternating current stimulation (tACS) over the parieto-occipital cortex using a combination of tACS with simultaneous electroencephalogram (EEG) recordings. This method allowed for the separation of

stimulation artifacts from ongoing and event-related cortical activity. Their findings demonstrated that 10 Hz tACS increased parieto-occipital alpha activity and caused entrainment with cortical oscillations. Moreover, tACS altered the target recognition in a phase-dependent manner, highlighting the pivotal role of alpha oscillations in visual perception [16].

Primary motor cortex (M1) is a central region of planning, executing and balancing movements. It additionally works with the somatosensory cortex which integrates sensory input to improve and adapt actions. The perceptual changes occurring along with motor learning engage other areas like the somatosensory cortex, supplementary motor cortex and ventral premotor cortex. These messages assist in planning and getting ready for movements execution. Motor learning actually happens when a part of a larger motor network that consists of the cerebellar cortex, primary motor cortex, and dorsal premotor cortex, are all involved (Vahdat et al., 2011) [14].

Another study, Miyaguchi et al. (2019) demonstrated the effects of high-gamma (70 Hz) over the left M1 and right cerebellar hemisphere. The motor performance of 20 healthy participants improved significantly. There were two current intensities; 1 and 2 mA employed in the experimental protocol. The results showed that the effects of gamma tACS over the chosen locations does not depend on current intensity, as both intensities showed improvement in performance of visuomotor task [27] (a).

Guerra et al. (2021) used γ-tACS (70 Hz) in three combinations in separate sessions; first with the intermittent theta-burst stimulation (iTBS) over the first dorsal interosseous (FDI) hotspot, second with sham-tACS and third γ-tACS alone over the M1. The current intensity was set at 1mA then increased up to 2mA. They also found that current intensity does not affect alterations in brain oscillations. They discovered that the effects of γ-tACS are weaker in producing alterations such as LTP in flexibility of M1 as compared to γ-tACS-iTBS in older adults as compared to young adults [15].

During a motor learning task, Bologna et al. (2019) employed two stimulation frequencies i.e. beta (20 Hz) and gamma (70 Hz), in order to assess the effects caused by tACS over M1 region. They concluded that tACS in the 20 Hz range reduced motor performance while, the gamma (70 Hz) tACS accelerated motor learning but caused a detrimental effect on motor retention [17].

A study presented by Guerra et al. (2018), tACS was delivered at two different frequencies: 20 Hz (β) and 70 Hz (γ) over the right FDI hotspot in two stimulation types; one (iTBS-γ tACS) and the other (iTBS-β tACS), also γ tACS was given alone as well, to check the LTP-like plasticity. The results showed that gamma tACS-iTBS can enhance plasticity in primary motor cortex. However, iTBS-β tACS did not produce any modifications in M1 [45].

Neurons function within networks where they influence each other through excitatory and inhibitory synaptic connections. These neuronal modulations are measured via local field potentials (LFP) and EEG. Oscillations of varying frequencies can co-occur in the same brain regions, and phase synchronization between different brain regions is fundamental for communication and plasticity. Phase synchronization aids in aligning the firing of action potentials, assisting in effective neural communication and supporting cognitive processes like working memory (Fell, J. et al. 2011) [18].

Neural synchronization helps link different neural modifications in response to a stimulus, while warranting they are considered as a single occurring. Gamma oscillations, for instance, enhance information conduction between brain regions by facilitating synchronized EPSPs to trigger action potentials. Neural synchronization is an essential mechanism for communication between neural networks and plasticity. Understanding its role in processes like memory, maintaining attentional focus, can provide insights into how neural synchronization takes place in different brain regions [18].

Miyaguchi et al. (2018) examined the effects of 70 & 20 Hz tACS over the left M1, at 1 mA current intensity. They discovered that gamma tACS improved the motor performance in healthy adults who underwent visuomotor task. However, the beta tACS did not cause any improvement. Their results indicate that motor improvement is frequency dependent [46].

In general, the primary motor cortex (M1) is a vital region for controlling and generating voluntary actions, and using sensory inputs it can improve and adapt actions according to the stimulus. The structure it has and how it connects to different motor areas, it is a principal region for developing and coordinating neural alerts that control voluntary muscle motion enhance our motor skills.

This literature overview outlines the research done on how tACS impacts the DLPFC and M1, as well as how this viable method brings about significant variations in brain states. Building on this, the purpose of our study, is to find out about how tACS affects attention of a healthy brain, its cognitive performance and motor capabilities.

Transcranial alternating current stimulation (tACS) is a non-invasive brain treatment method that alters the excitability of the cortex using susceptible biphasic currents. Using electroencephalography (EEG), we can measure brain's neural activity as a consequence of stimulation. Researchers have employed biological source-specific variations in body impedance, ECG monitoring and EEG to visualize modulations in brain. tACS effects produce high voltage artifacts, using conventional experimental designs and may be insufficient to acquire proper data. Signal integrity may deteriorate with physiological artifacts which have to be eliminated with applying filters and algorithms. These artifact removal techniques are often employed for EEG are efficient during data acquisition.

# CHAPTER 3: METHODOLOGY

The dataset can be retrieved openly at: doi.org/10.18112/openneuro.ds003670.v1.1.0 . This is Brain Imaging Data Structure (BIDS) [13]. A BIDS dataset is a well-organized data which has a raw data directory that includes subfolders of the experimental data folder, and '.tsv', '.json' files. In addition, there are two directories containing the 'sourcedata' for the non-formatted data as well as a 'stimuli' directory. The 'json' file which describes the data includes the general information concerning the experiment as well as the authors.

In the experimental data folder there is a subject folder of each subject. In those subject folder is the 'eeg' subdirectory holding the EEG and its metadata. There are several json and tsv files as well as a set and an fdt file. The 'set' file is the file containing the recorded EEG signals.

## 3.1 Experimental Protocol

The experimental protocol of this data is explained below.

### *3.1.1 Participants*

Ten neurologically healthy persons (six males and six females) between the ages of 19 and 43 (mean ages: 29, 10±6. 75; median age: 30) were hired for this research from New York [29].

Participants completed the tES eligibility assessment exercise before enrollment. Their medical and mental health histories e.g. depression, fits, surgeries, pain, ear trauma, cardiac problems, allergies related to skin, metallic or non-metallic device implantations, liquor dependence, brain injuries, transient unconsciousness, patterns of sleep, collapsing at the view of blood, and history of drug abuse (licit or illicit) were all questioned about. Finally, after going through the assortment procedure, informed agreement was obtained from all the subjects.

For both experimental sessions, applicants were instructed to follow their regular sleep/wake schedule, avoid alcohol and excessive caffeine in foods and beverages, and avoid eating at least 4 h before the session. To further expedite the EEG modus operandi, applicants were also urged not to apply thick make-up or lotion, and hair products or scalp treatments [29,47].

Participant ‘17’ had to be disqualified from the experimentation owing to failure in following directions in the completion of the behavioral task. Four participants (participant number: Patients (IDs 12, 15, 21, 22) were asked to return, to perform both their test sessions. These returning participants were given new participant identification numbers in a numerical sequence of the participant number task (that is the numeral ‘1’ was assigned to the first participant registered, ‘2’ to the second participant registered, and so on). Participant 12 came back once and he was allotted a new participant number: ‘19’; the participant ‘15’ came back once and he was given new participant number that is ‘18’; the participant ‘21’ came back twice and she was assigned new participant number twice that is ‘25’ and ‘26’; the participant ‘22’ came back twice and he was given new participant number twice that is 23 and 24. [29]

### *3.1.2 Questionnaires.*

Participants completed a demographic survey at the conclusion of registration to provide information about their age, sex, height, weight, education, dominant hand, English proficiency, average weekly workout time, experience with electrical stimulation, and Pittsburgh Sleep Quality Index (PSQI78)-ascertained sleep quality. Once participants were enrolled in the first session, these questionnaires were given out. [29]

Prior to the commencement of every session, attendees were asked to respond to a pre-questionnaire on their activities over the previous 24 hours. Additionally, they completed the evaluation forms just afore and afterwards each session (Fig. 1c–h). The following assessments were included in these scales: mood (from Saddest=1 to Happiest=9), anxiety (from Most relaxed=1 to Tensest=9), pain (numerical pain scale; from No pain=0 to Worst pain=10), and sleep quality (from extremely attentive=1 to Very sleepy=9) [29].

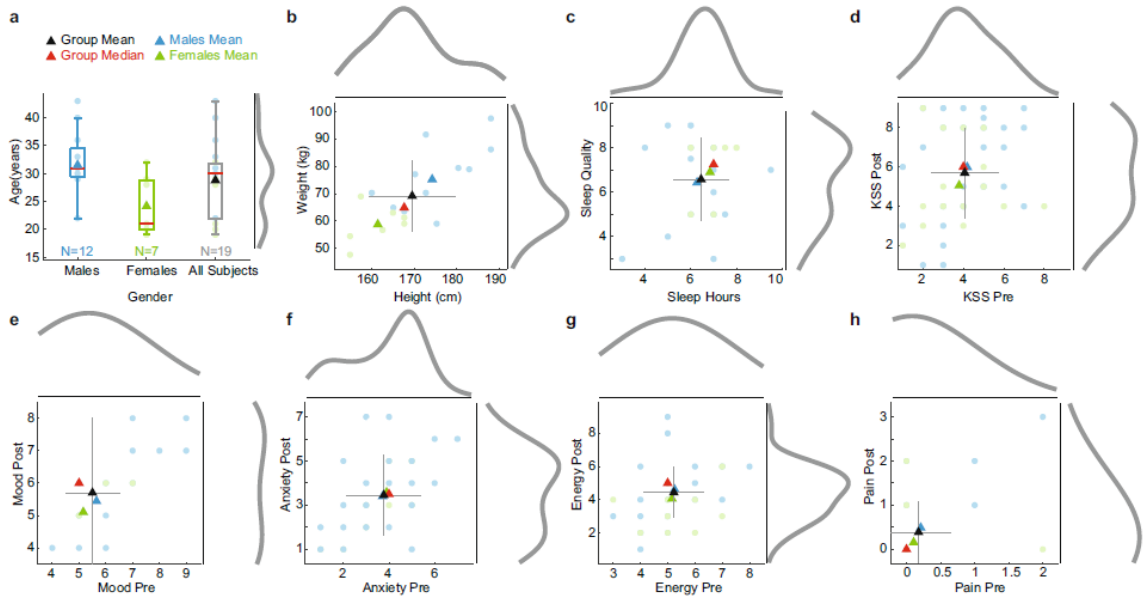



***Fig. 3.1*** *a demographic summary and questionnaire. (a) The age range of the participants was 19–43, with a mean age of 28.79 years. (b) The average weight and height of the participants were 69.11 kg and 169.50 cm, in that order. (c) 6.45 hours was the mean number of hours slept, while 6.57 hours (5-Normal Quality) was the average grade for sleep quality. (d) Beforehand (KSS 'Pre') and afterwards (KSS 'Post') each session, the mean sleepiness ratings (KSS) were 4.01 and 5.70, correspondingly. (e) The average mood scores for each session were 5.50 (5-Usual self) before and after (Mood Pre and Post), respectively. (f) The average anxiety scores were 3.77 (5-Usual self) before each session and 3.45 (5-Anxiety Post) thereafter. (g) The average energy ratings were 3.77 and 3.45 (5-Usual self) previously (Energy 'Pre') and afterward (Energy 'Post') each session. (h) The average pain ratings were 0.18 and 0.39 (0-No discomfort, 10-Worst possible pain) before and after each session, respectively. Squares (c–h) provide data for the total number of sessions (including those that individuals attended more than once), whereas panels (b–h) show one standard deviation represented by gray lines extending from each group mean.*

### *3.1.3 Compensatory tracking task*

The participants were invited to sit in a room with around 10 lux of lighting. To aid with sound reduction, foam earplugs were supplied to a few of them. A 17 inches LCD screen having a refresh rate of 60 Hz, was positioned about 57 cm away from the participants, and they were probed to complete a Compensatory Tracking Task (CTT) continuously for the duration of each session. Participants saw the task guidelines shown on a screen, which stated: *Using a mouse or any other pointing device, the objective is to retain the ball as nearby to the target as you can. Although the ball moves on its own, the mouse's actions have an impact. Try to retain the ball in the middle of the circle or as near to the inner ring as you can, if at all feasible.*

The two dimensional task's goal was to maintain a moving circle's constant constraint in the screen's center (Fig. 2a). The circle had damping and oscillating forces, among other kinematic

characteristics [48]. The experimental psychology task application, PEBL 2.1, which is based on C++, was used to source and administer the CTT [49].

Through the use of a mouse participants provided input for the experiment using their dominant hand. They were not to be stopped during the job execution (even if they were obviously sleepy; inconsistent with previous CTT designs). Participants engaged in a quick practice session lasting one to three minutes before each sitting [29].

The work was designed to last 70 minutes, with an extra 0.5 minutes added for the experiment. For the duration of the experiment, the CTT operated continuously. The research was single-blind, meaning that neither the stimulation type nor the block design of the experiment was known to the participants [29].

#### *3.1.4 EEG data acquisition*

EEG data was collected using a wired cap equipped with 29 interwoven plastic HD-holders and 32 Ag/AgCl record electrodes. HD-holders (see HD-tES) held the stimulation tools, which comprised conductive gel and stimulation electrodes. The conductive gel was filled using blunt-tipped injects between the scalp and electrodes for both stimulation and recording. The 10-10 electrode placement methodology (Fig. 2b) was followed exactly while placing the electrodes for the EEG recording [29].

The signals were online grounded at AFz, sampled at 2 kHz, and denoted as CPz. An amplifier provided the amplification of signals. It was set up with a bandwidth of 0 to 520 Hz. The acquisition's peak-to-peak voltage range was kept at 1 V. Scalp impedances were measured before to each recording in order to guarantee that the impedance was less than 20 kΩ. A trigger would also be delivered to the amplifier at the beginning of the CTT in order to time-lock it with the contemporaneous EEG. Additionally, data were exported to.cnt files [29].

### 3.2 HD-tES

At two distinct cephalic regions, HD-tES was delivered for 30 seconds per trial, with an extra 5-second ramp-up/down, using a biphasic stimulation waveform. The 2 stimulation types were a blend of (stimulation location: 2; frequency: 1; duration: 1).

The stimulations, divided into two categories: frontal and motor, and they were applied at 30 Hz. The Arabic number representing the frequency and the first letter of the stimulation site were used to represent each blend of stimulation location and frequency. The 2 dosage combinations were as follows: frontal 30 Hz (F30), motor 30 Hz (M30). [29]

| Sub# | Session | File Num | F30 | M30 | Stim Type | Stim Amplitude (mA) |
|---|---|---|---|---|---|---|
| 11 | 01 | 1101 | 7 | | F30 | 1 |
| | 02 | 1102 | | 8 | M30 | 1 |
| 12■ | 01 | 1201 | | 8 | M30 | 1 |
| | 02 | 1202 | 7 | | F30 | 1 |
| 13 | 01 | 1301 | 7 | | F30 | 1 |
| | 02 | 1302 | | 8 | M30 | 1 |
| 14 | 01 | 1401 | | 8 | M30 | 1 |
| | 02 | 1402 | 7 | | F30 | 1 |
| 15▲ | 01 | 1501 | 7 | | F30 | 1 |
| | 02 | 1502 | | 8 | M30 | 1 |
| 16 | 01 | 1601 | | 8 | M30 | 1 |
| | 02 | 1602 | 7 | | F30 | 1 |
| 17 | 01 | 1701 | 7 | | F30 | 1 |
| 18▲ | 01 | 1801 | | 8 | M30 | 1 |
| | 02 | 1802 | 7 | | F30 | 1 |
| 19■ | 01 | 1901 | 7 | | F30 | 1 |
| | 02 | 1902 | | 8 | M30 | 1 |
| 20 | 01 | 2001 | 7 | | F30 | 1 |
| | 02 | 2002 | | 8 | M30 | 1 |
| 21♦ | 01 | 2101 | 7 | | F30 | 1 |
| | 02 | 2102 | | 8 | M30 | 1 |
| 22● | 01 | 2201 | | 8 | M30 | 1 |
| | 02 | 2202 | 7 | | F30 | 1 |
| 23● | 01 | 2301 | 7 | | F30 | 1 |
| | 02 | 2302 | | 8 | M30 | 1 |
| 24● | 01 | 2401 | | 8 | M30 | 1 |
| | 02 | 2402 | 7 | | F30 | 1 |
| 25♦ | 01 | 2501 | | 8 | M30 | 1 |
| | 02 | 2502 | 7 | | F30 | 1 |
| 26♦ | 01 | 2601 | 7 | | F30 | 1 |
| | 02 | 2602 | | 8 | M30 | 1 |

***Table 3.1.*** *an account of each participant's session in the experiment, including the conditions and level of stimulation. Columns contain information about each participant (Sub #), each session (Session), each file number (File Num) for each session, each detailed description of the stimulation conditions used in each session in all stimulation trials (Stim Type), and the correspondent amplitude of stimulation (Stim amplitude) used for each stimulation type. If the identical symbols show up along with a participant's number, it means that they are the same*

*person who came back for more sessions and was allotted a new subject number. After making one retune, participant 12 received a new number, 19 (■). After coming back, participant number 15 was given a new number, 18 (▲). After making two returns, participant 21 was given new numbers, 25 and 26 (♦). After making two returns, participant 22 received new numbers: 23 and 24 (·).*

Seven sintered ring stimulation electrodes were allocated to standard EEG 10/10 electrode system. The electrode placement [50] resulted in the creation of two possible Nx1 HD-tES, with N = 3 and 4 for the frontal and motor areas, correspondingly [29].

For each montage, "N" electrodes were carefully chosen for the outside (surround) electrode whereas one electrode was designated as the central electrode. It should be mentioned that montages with a combination of "ring" and "center" tasks shared a number of electrode locations. This is how a single 7 electrode place HD-tACS was configured for both study sessions. For frontal cortex stimulation, the surrounding electrodes (N = 3) were positioned at AF3, FT7, and FC3, whereas the central electrode were positioned at F5 (Fig. 2b, c). The central electrode was positioned at C5, and the outside or surrounding electrodes (N = 4) were positioned at FT7, FC3, CP3, and TP7 for motor stimulation (Fig. 2b, c).

Using an MRI-derived head model and the ROAST toolkit [51] [52] in MATLAB stimulation electrode placement was shown in realistic 3D space. The two stimulation montages were not administered simultaneously, and each montage shared stimulation locations [29]. A biphasic sinusoidal waveform (30 Hz) was applied for stimulation, where all electrodes interchanged between serving as an anode and cathode at the frequency of stimulation [29].

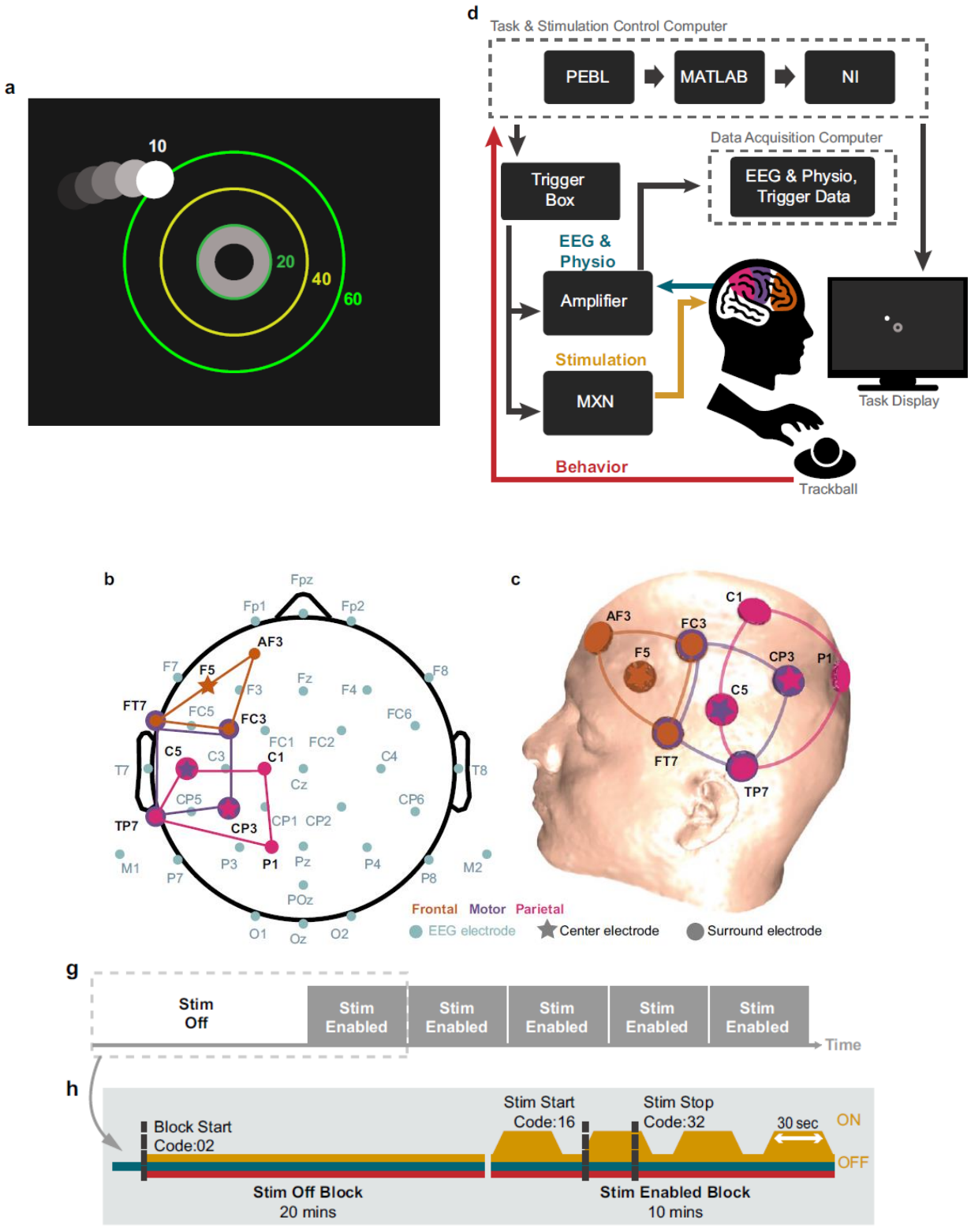



***Fig. 3.2*** *Hardware configuration, CTT, EEG and stimulation montage, and review of the experiment. (a) Completing the CTT, in which the goal was to keep the moving ring (~10 pixels) close to the central annulus's center*

*(~20 pixels) throughout the duration of the trial (70 minutes). The ball has intrinsic motion as well as damping and oscillating forces. Note that participants could only see the white-colored ball and gray hoop; other rings and distances in the panel are only for illustration. (b) Frontal (orange), motor (purple), and parietal (magenta) (for another experiment), stimulation sites are interspersed with EEG locations (light blue). The central electrodes for both stimulation montages are indicated by a star, and the surround electrodes by spheres. It should be noted that some electrode positions may be intended to function as the outside electrodes of one montage and the center electrode of another. (c) A 3D head model created from MRI data, where the electrode placement is seen by placing the stimulation sites on the scalp. (d) Hardware configuration for data collection and stimulation with respect to participant input and output. (g) The block design for Experiment, as programmed to be carried out within the hardware and software configuration shown in pane (d). The study was split into two groups: stimulation enabled blocks (Stim Enabled), where the chosen tACS stimulation type was delivered four times, and stimulation off blocks (Stim Off), which did not include tES. The detailed block design for Experiment (h) include the relevant triggers (dashed vertical line) and trigger codes. It should be noted that the block design is not scaled. Concurrent Stimulation was used to capture simultaneously the EEG and Physiology (EEG, EOG, and ECG denoted by teal colour); and Behavior (CTT denoted by red) and (stimulation is denoted by gold). The EEG and Physiology acquisition began many seconds before to the delivery of any trigger. The purpose of (g,h) is to show how the experimental design was intended to be carried out.*

Conventionally [53], stimulation electrodes were inserted side by side with EEG electrodes in the EEG cap and grounded in plastic HD-holders. At each stimulation location, the hair of participant was separated using a blunt Q-tip or a blunt tip syringe to expose the plastic HD-holder annulus. The HD-holders were then carefully filled with about 15 mL of gel. After that, the stimulation electrodes were fully immersed in electrolytic gel and inserted into the HD-holders. To securely attach the electrode, the extra plastic cover was placed back over the plastic holder. The frontal and motor regions of the brain were stimulated using the MxN 7-channel high-definition transcranial electrical stimulator. With the use of the multi-channel stimulation device's current-controlled current source, each stimulation channel's current amplitude could be changed individually [29]. Before stimulation, the impedance values of these channels were measured in relation to F5. In every session, the DAQmx device received a pulse created in MATLAB as the trigger. To synchronize the stimulation with the EEG amplifier, a different trigger would be used (Fig. 2d). Table 3 provides information on the particular stimulation parameters for each kind of stimulation [29].

The respondent completed a pre-stimulation questionnaire before to each stimulation session in order to gauge each participant's comfort level and tolerance with each montage. About 20 s (5-s rise/fall duration) of stimulation were given at a current of 1 mA (peak-to-peak). After then, participants were probed to rank their intensity of pain or discomfort on a scale of 0 to 10, with 10 being the worst pain they had ever felt and 0 representing no pain at all. In order to make sure the stimulation was well tolerated by the body, the adjusted stimulation level was comparable to 0. 5 mA at the very least. A current of 1 mA was given to each participant (Table 2) [29].

### 3.3 Experimental overview

The participants were occupied in two 70.5 minutes' sessions. Earlier and after the experimental session, participants finished a sequence of pre- and post-questionnaires. For the session participants completed the behavioral task uninterruptedly for 70 minutes (or 70.5 minutes) meanwhile the recording of EEG and physiology (ECG, EOG) was also taking place [29].

Designed for the experiment, two modified stimulation conditions were used (F30, M30). Both of the two sessions, F30 and M30, included one stimulation 'OFF' block (20 min) followed by five stimulations 'ON' blocks of 10 min each (Fig. 2g, h). During each session, one of the two stimulation types, either F30 or M30, was arbitrarily allotted to be supervised throughout the five stimulation 'ON' blocks. The allotted stimulation type was observed as four successive stimulation trials within each stimulation 'ON' periods. Therefore, it was possible to conduct a total of 20 trials of stimulation in each session during the experiment, with each trial lasting 30 seconds and including 5 seconds for ramp up and 5 seconds for ramp down [29].

The detailed experimental categorization with EEG reference channel 'Cz', behavior time-series, and related triggers is shown in Fig. 3 for Experiment with the best participant identified as 24. This shows a subject-wise fulfilment of the experimental scheme in Fig. 2e–h, from the data acquisition viewpoint. In both experimentations, participants uninterruptedly completed the task, and the connected competence, EEG, and physiology were acquired [29].

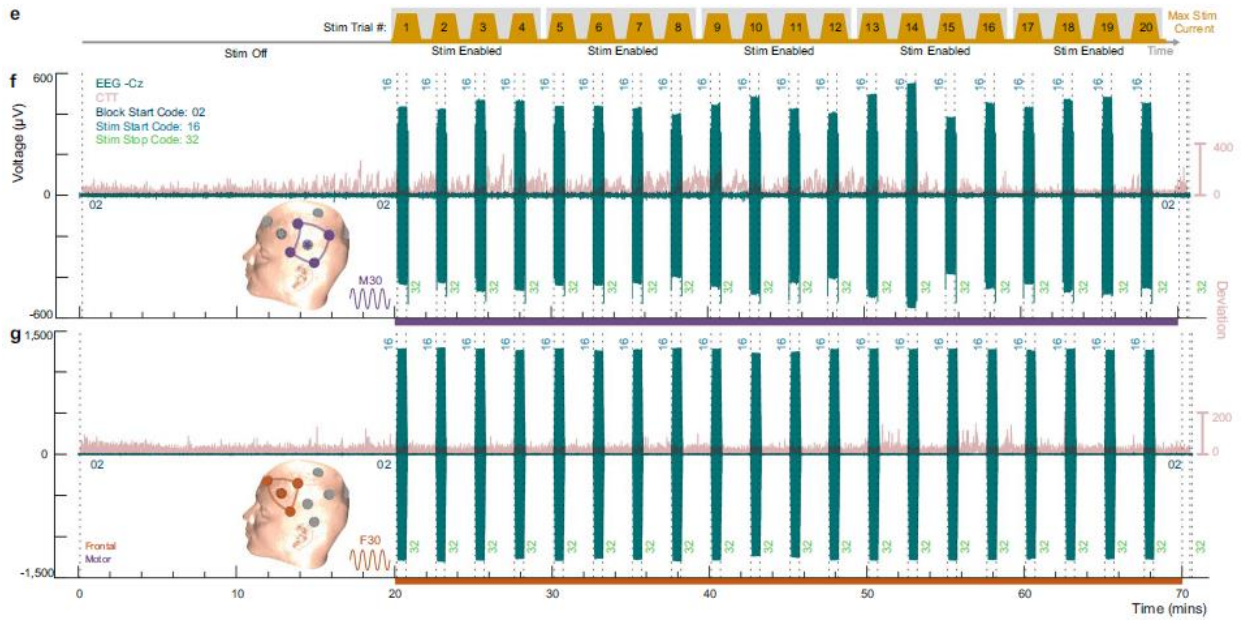



***Fig.3.3*** *Block execution for exemplary participants using time-series of a whole experimental series. (e) Experiment 2 session design. EEG and CTT time-series for the first and second sessions implemented by participant 24 (f and g, respectively). Throughout each session, the same stimulation type whichever M30 or F30; cartoon insets were administered twice. Experiment 2 had a 20-minute Stim Off phase, followed by a 50-minute Stim Enabled period with 20 stimulation sessions. Trigger codes are used to identify time-series triggers in EEG data (vertical dashed lines). For all trials, physiology (not displayed) and CTT output (pink) as well as EEG (teal) were constantly recorded during all sessions.*

The M30 stimulation type, indicated by insets, was administered to participant 24 during the first session of Experiment 2 (Fig. 3f). Twenty consecutive trials, each of which was further divided into five Stim Enabled blocks, were conducted in the M30 stimulation trial. During each trial, the current was progressively increased over for five seconds, maintained for thirty seconds at the maximum selected current intensity of either 0. 5/1 mA, max Stim-Current, and then decreased. Similarly, participant 24 sustained the F30 stimulation type throughout the *enabled* blocks of stimulation in the second session of Experiment (Fig. 3g) [29].

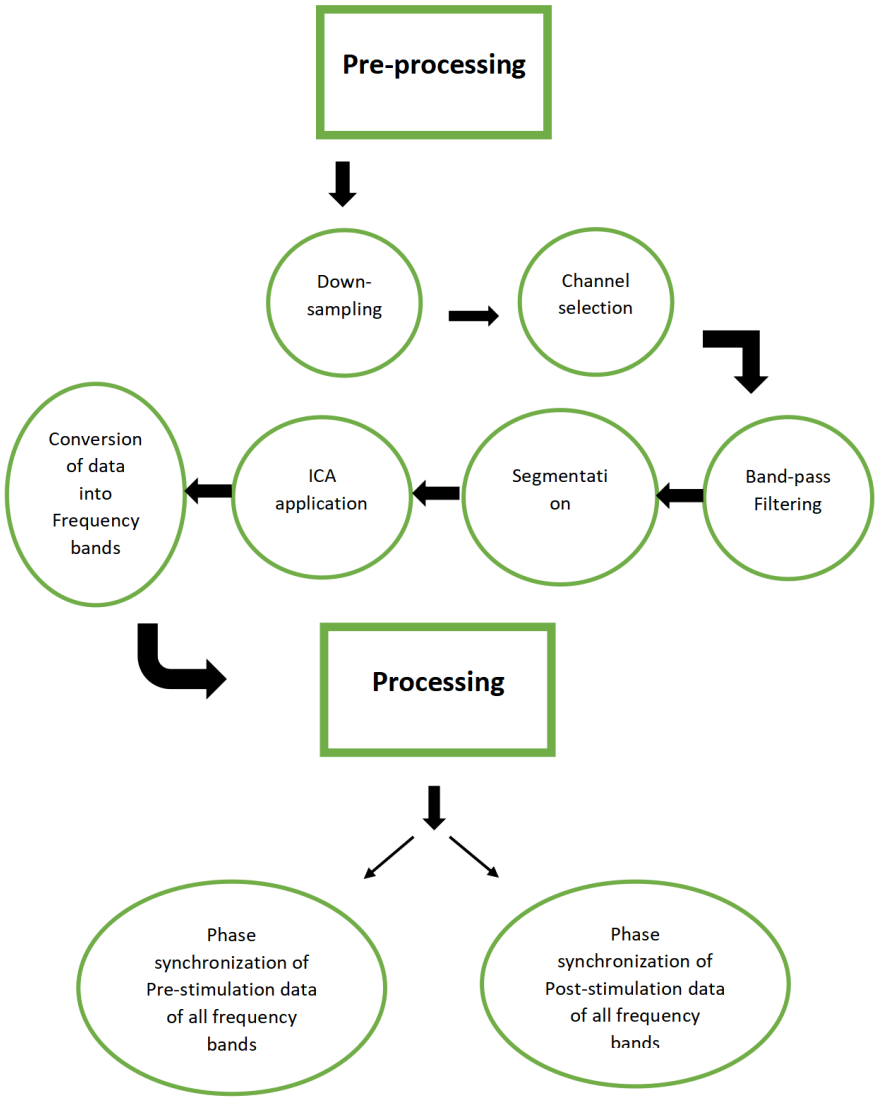
As stimulation creates huge voltage artifacts in the brain signals during ramp-up and ramp-down and produces DC voltage artifacts which are prominent in the unaltered EEG-stimulation data, the data was band pass filtered for illustration. [19] [29].

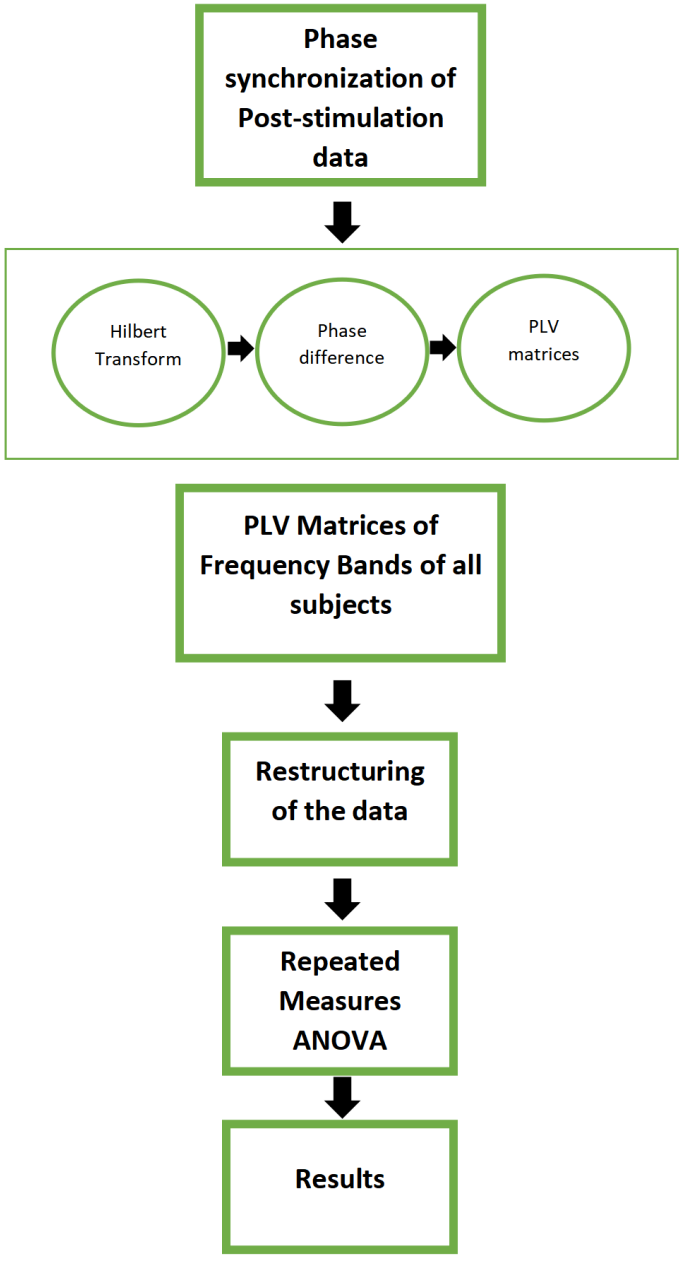
## 3.4 DATA PROCESSING

The data processing consists of three main phases: Preprocessing, processing, and statistical analysis. All these steps were conducted for both the pre and post-stimulation datasets. The

signal processing was done using MATLAB specifically developed toolbox for handling EEG data known as the **EEGLAB**.

Schematic diagram of the Processing of data is as under:




Phase synchronization of Post-stimulation data
Hilbert Transform
Phase difference
PLV matrices
PLV Matrices of Frequency Bands of all subjects
Restructuring of the data
Repeated Measures ANOVA
Results

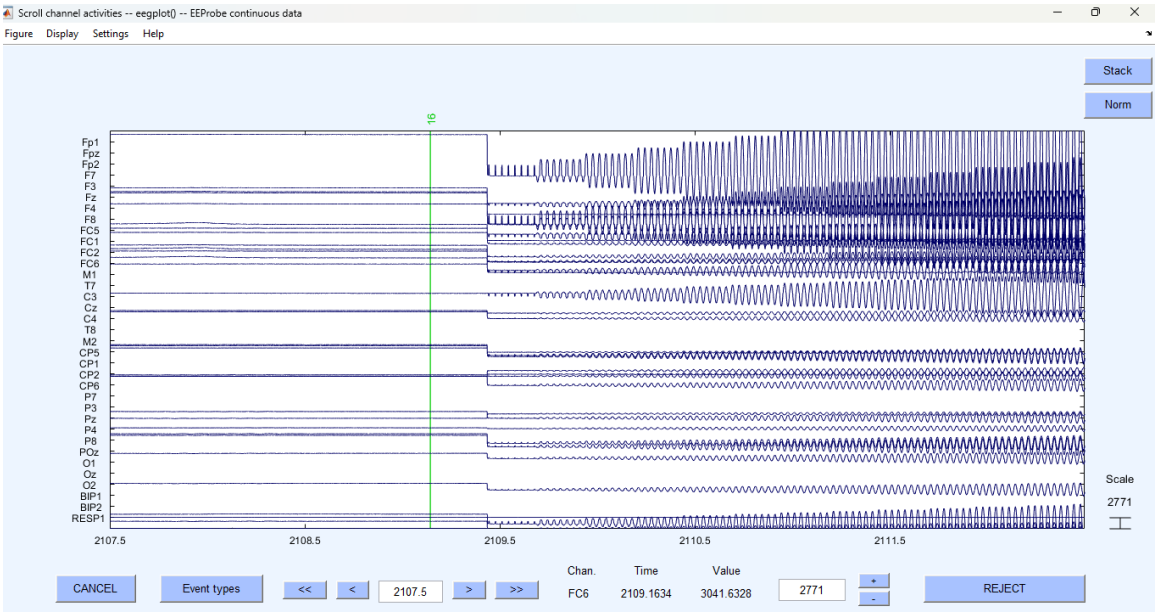



***Figure 3.4*** *Raw EEG data*

### 3.4.1 Pre-processing

#### *3.4.1.1 Down-sampling*

The EEG data were first down-sampled from 2 kHz to 512 Hz to reduce computational load and improve processing efficiency. Using EEGLAB's **pop_resample** command. This step is crucial to ensure efficient processing without compromising the signal quality.

#### *3.4.1.2 Filtering*

Next, the first 32 channels of the EEG data were selected, excluding the physiological data recording channels i.e. ECG, EOG & RESP.

A band-pass filter ranging from 0.5 to 45 Hz was applied to remove any noise outside the frequency range of interest. EEGLAB's **pop_eegfiltnew** function is utilized to apply the bandpass filter to the EEG data.

#### *3.4.1.3 Data segmentation*

The data collected were then split into pre-stimulation and post-stimulation files. From the continuous EEG data using the event markers, we found the indices of first occurrence of the **'2'**

**event**; one epoch of 92 seconds was considered. The epoch was then baseline-corrected using a time frame of -1 to 1 seconds relative to the event marker.

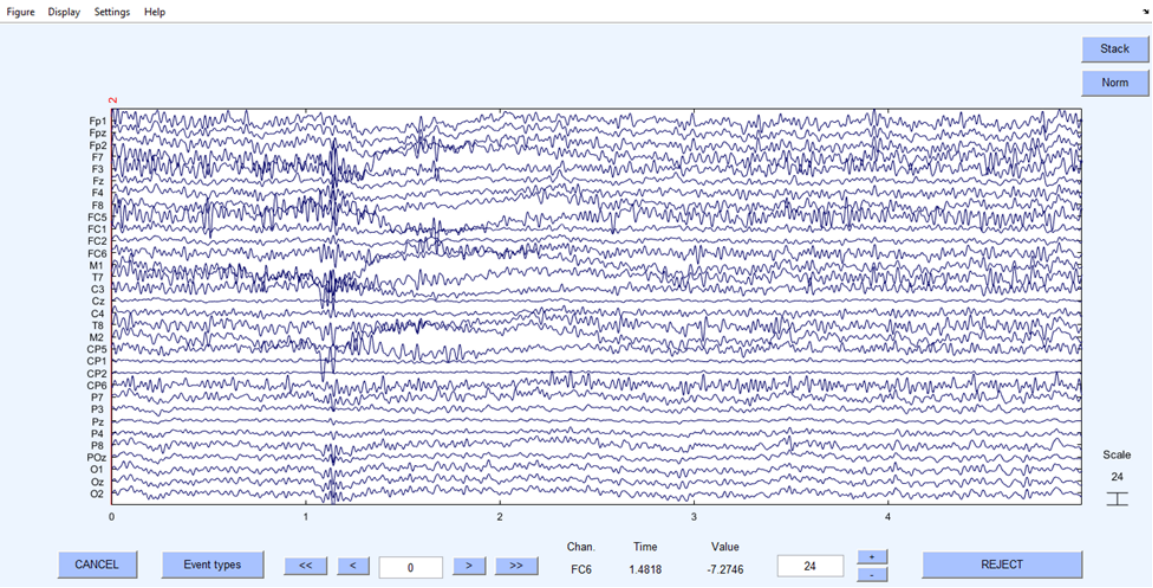



***Figure 3.5*** *Filtered and Epoched pre-stimulation data*

For the post-stimulation data, epochs were formed based on the stimulation paradigm which consisted of 20 cycles of 30s stimulation with additional 5s of ramp-up and ramp-down. Therefore, the post-stimulation continuous EEG data were subdivided into 20 segments each of 100 seconds in duration. In particular, we determined the **'16' event** indices, which signified the **stimulation 'ON'** state in the post-stimulation period. The epochs of post-stimulation data were also baseline- corrected using a time frame of -1 to 1 s relative to the event marker.

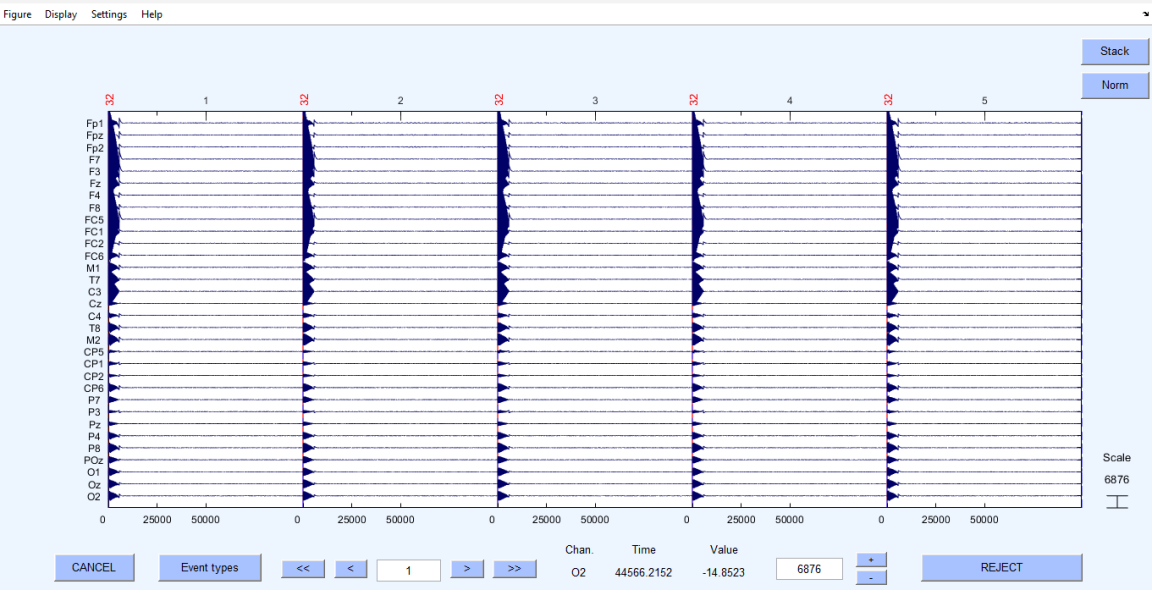



***Figure 3.6*** *filtered and epoched post-stimulation data*

To remove transient effects, the first 8 seconds of raw data were discarded from all epochs, resulting in epochs of 92 seconds each. The adjustment was necessary for consistency in both types of data for future comparisons.

### *3.4.1.5 Artifact removal*

Artifact removal is a vital process, especially when working with bio-potentials. For EEG data, many artifact removal techniques have been invented including regression-based methods, wavelet transform, Independent Component Analysis (ICA), and more often, a combination of two or more techniques gives the best justice.

ICA was applied to the pre- and post-stimulation data to extract artifact components such as eye blink, muscle activity, and other non-neural signals from the data. Both stimulation data were visually checked to eliminate segments containing artifacts before applying ICA. The ICA was then performed, in order to perform data cleaning.

In their article published in 2015, Urigüen, J. A. et al., provided an extensive review of the current state of knowledge regarding EEG artifact removal methods. They suggested that ICA is suitable for removal of data contaminated with ocular and muscle artifacts as well as for the purpose of maintaining the quality and information content in the EEG signals. They pointed out

that depending on a scenario, one of the ICA algorithms may be preferable to others in terms of efficiency.

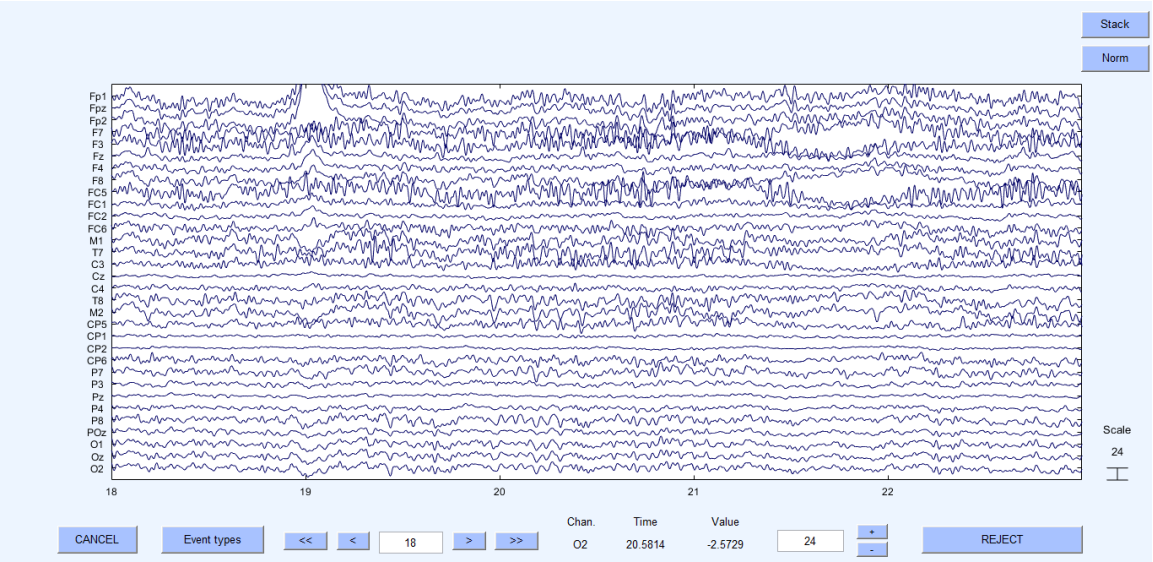



***Figure 3.7*** *ICA on pre-stimulation data*

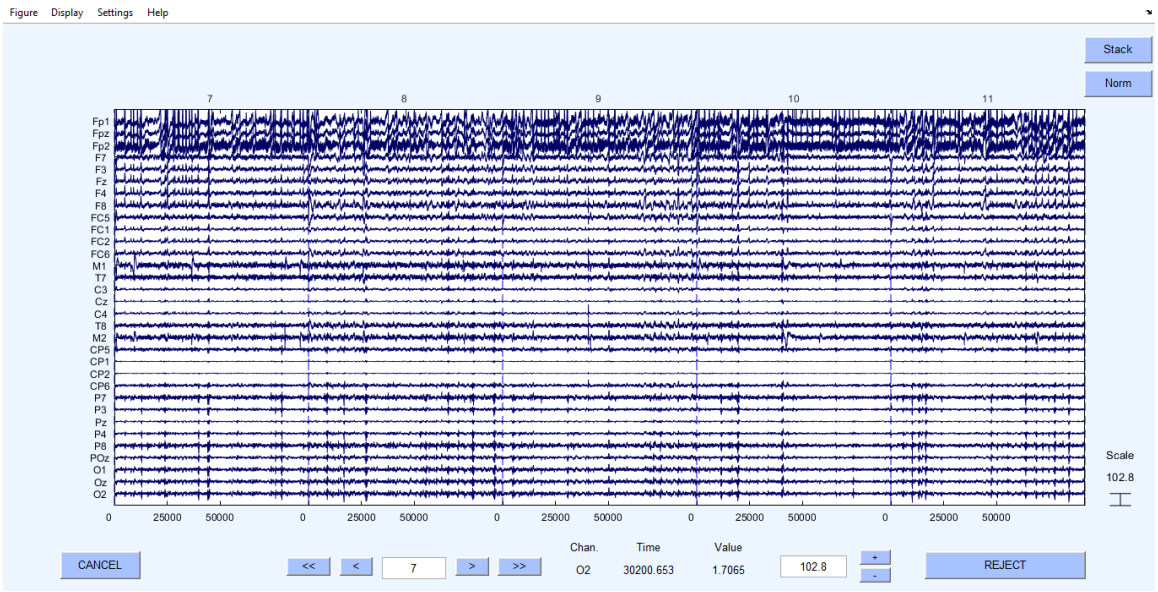



***Figure 3.8*** *ICA on post-stimulation data*

### *3.4.1.6 Frequency band selection*

After cleaning the data from artifacts, both pre- and post-stimulation data were subsequently divided into the following frequency bands for all 15 subjects.

a. Theta (4-8 Hz),
b. Alpha (8-12 Hz),
c. Beta (13-30 Hz),
d. Gamma (30-45 Hz)

Then both data of all frequency bands was saved for every subject in separate folders.

### 3.4.2 Processing

#### *3.4.2.1 Phase Synchronization*

To analyze the impact of low gamma HD-tACS on neural synchrony, phase synchronization analysis was performed in all the frequency bands. Using the Hilbert Transform, Phase-Locking Value (PLV) matrices were calculated for pre- and post-stimulation recordings. Then the phase difference between each pair of channels was computed and saved in the PLV matrices.

Hilbert Transform

The Hilbert transform is used to transform a real-valued signal into a complex-valued analytical signal. It is one of the widely known signal processing techniques for the extraction of instantaneous phase and amplitude information from time domain signals. The Hilbert transform of a signal $x\ (t)$ where *x* is denoted as $\tilde{x}\ (t)$, and it is denoted in the following equation:

$$\tilde{x}(\mathrm{t}) = \mathcal{H}\{x(t)\} \ = \ {}^{1}\!/_{x} \ \int_{-\infty}^{\infty} \frac{x(\tau)}{t-\tau} d\tau$$

Where $\mathcal{H}$ denotes the Hilbert transform operator.

#### *3.4.2.1.2 Steps in Hilbert Transform*

i. Analytic Signal Construction:

Given a real-valued signal *x*(*t*), the Hilbert transform constructs an analytical signal $\tilde{x}(t)$ by convolving $x(t)$ with the Hilbert kernel $\frac{1}{\pi t}$.

ii. Frequency Domain Interpretation:

In the frequency domain, the Hilbert transform shifts the positive frequency components of $(t)$ by 90 degrees' phase shift, while the opposite happens to the negative frequency components, which are shifted by -90 degrees.

iii. Phase and Amplitude Extraction:

- The phase (φ) of $x(t)$ represents the instantaneous phase of the original signal $x(t)$ at each time point.
- The magnitude (||) of $x(t)$ represents the instantaneous amplitude of $(t)$.

To perform phase synchronization, the following steps were followed:

1. Using Hilbert Transform to calculate PLVs

The Hilbert transform was applied to the all the frequency bands of the pre- and post-stimulation EEG data, which resulted in a complex-valued analytical signals.

2. PLV Calculation
    - For pre-stimulation data, a single PLV matrix was calculated for each subject. As the pre-stimulation data did not contain epochs.
    - For post-stimulation data, a three dimensional PLV matrix was calculated per subject. It comprised of the PLV values between all 32 pairs of EEG channels, time, and 20 epochs. Hence, there were a total of 20 PLV matrices corresponding to the 20 epochs, per subject.

Mathematical Representation of PLV

The PLV between two EEG signals $(t)$ and $y(t)$ at frequency $f$ is computed as:

$$PLV_{xy}\ (f)\ =\ \frac{1}{N}\left|\sum_{n=1}^{N} e^{i\left(\phi_x(n)-\phi_y(n)\right)}\right|$$

Where:

- $N$ is the total number of samples.

- $\phi_x(n)\ and\ \phi_y(n)$are the instantaneous phases of signals $x(t)$ and $y(t)$ at sample $n$, respectively.
- $i$ is the imaginary unit.
- $|\cdot|$ denotes the absolute value.

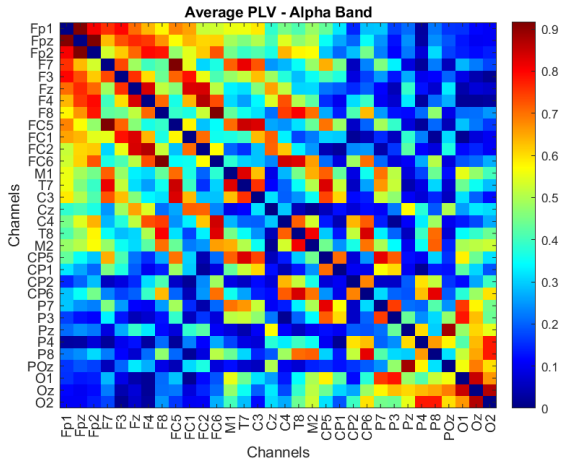
Phase synchrony tells the phase relationships of channel pairs within a specific frequency band. These phase relationships manifest the areas of the brain, where significant changes are visible, as an effect from an experiment or stimulation.

The resulting post-stimulation PLV matrices were divided into 3 categories; the first three, middle three, and last three epoch PLV matrices of the post-stimulation data were averaged to form three distinct post-PLV matrices, namely, Post_start, Post_mid and Post_end.

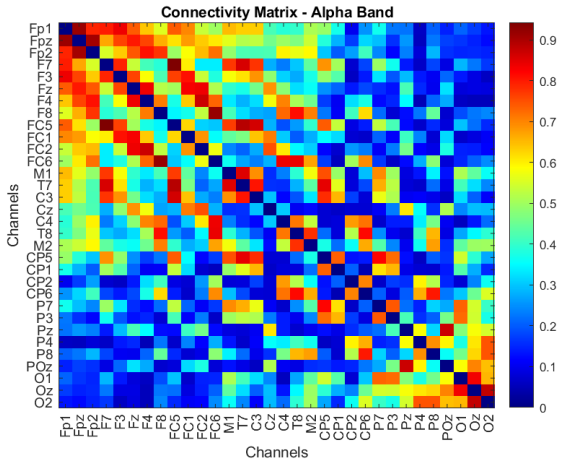
#### *3.4.2.2 Connectivity plots*

The data were visualized using connectivity matrices' plots created with '**imagesc**' command in MATLAB, in order to compare the neural connectivity between the frontal, motor, parietal, and occipital areas. These discoveries suggested visible changes in these areas in the post-stimulation data due to HD-tACS application. Hence from these averaged matrices of pre- and post-stimulation of all the subjects, frontal channels for F30 and fronto-central, central, centro-parietal channels were chosen for M30 responses.

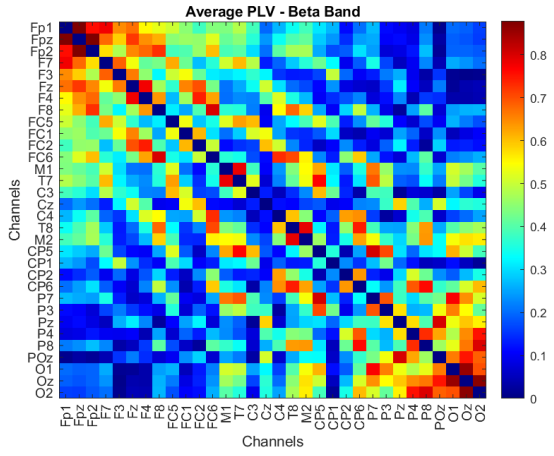
# F30



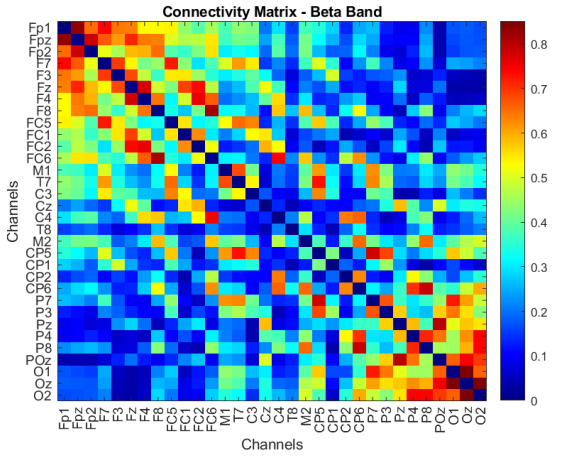

Alpha Pre-stimulation connectivity plot



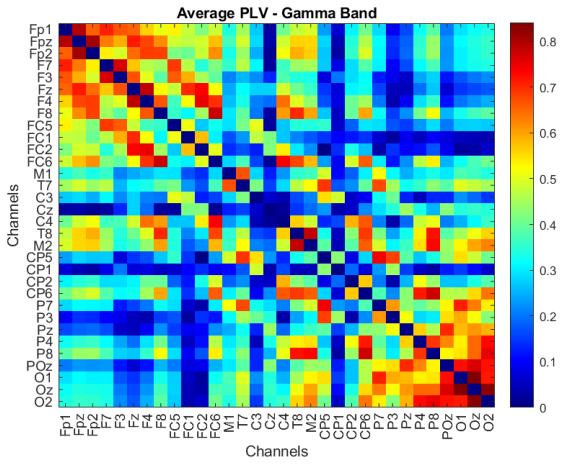

Alpha Post-stimulation connectivity plot



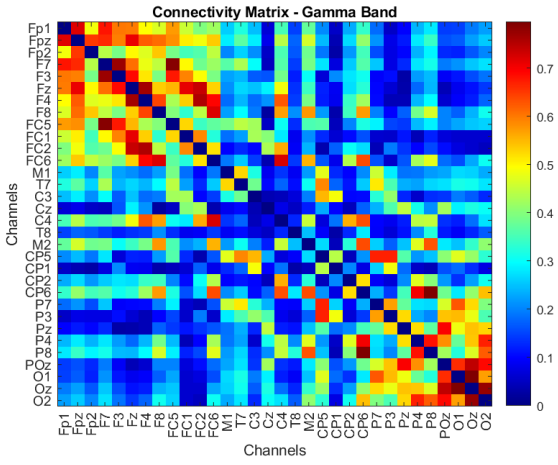

Beta Pre-stimulation connectivity plot



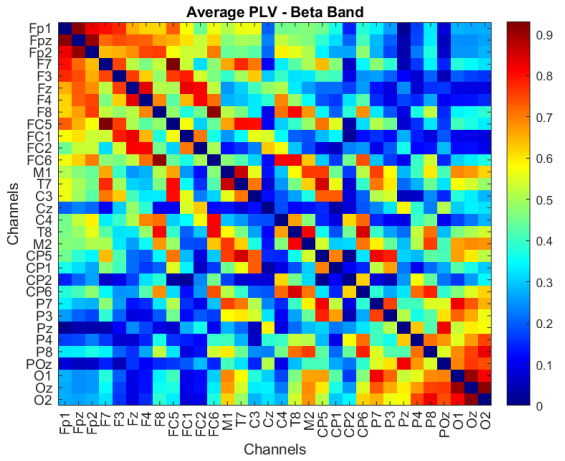

Beta Post-stimulation connectivity plot



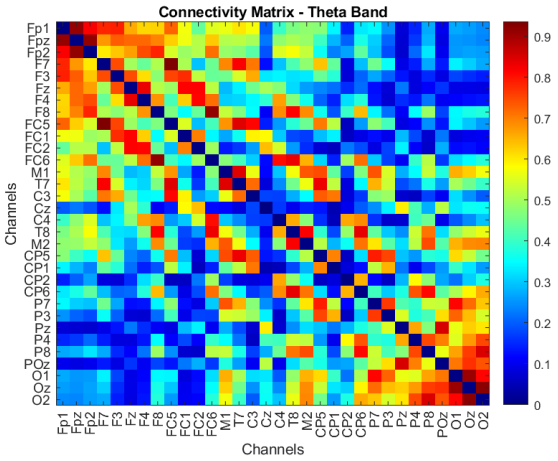

Gamma Pre-stimulation connectivity plot




Gamma Post-stimulation connectivity plot

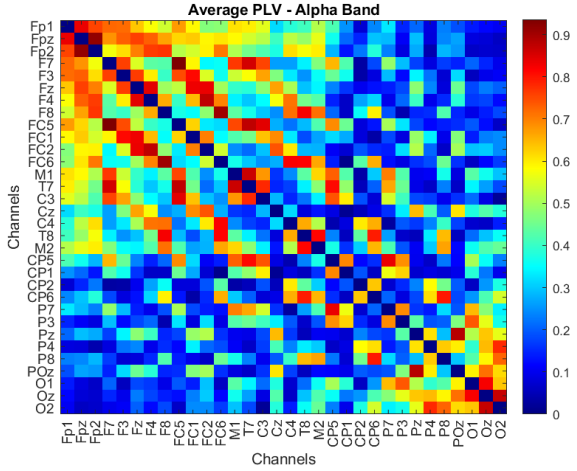



Theta Pre-stimulation connectivity plot

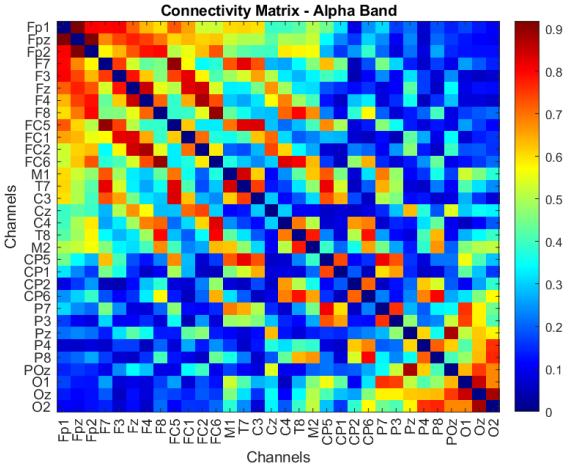



Theta Post-stimulation connectivity plot

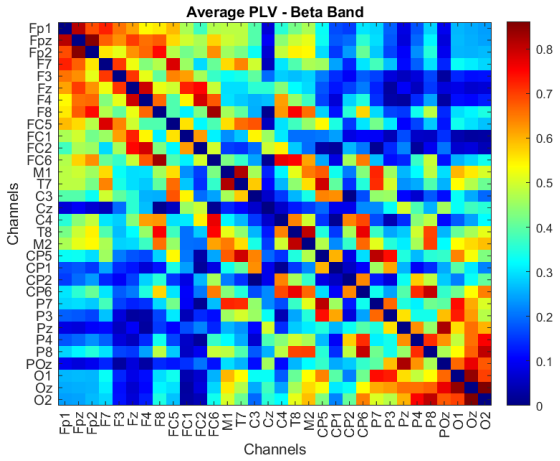
# M30



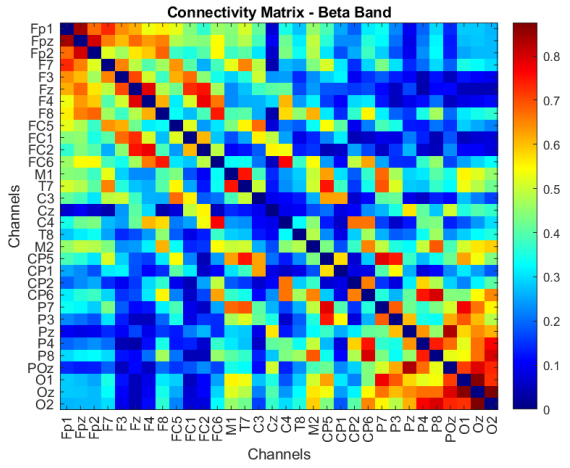

Alpha Pre-stimulation connectivity plot



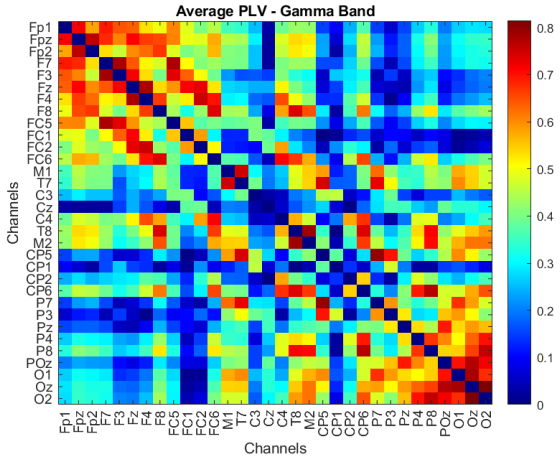

Alpha Post-stimulation connectivity plot



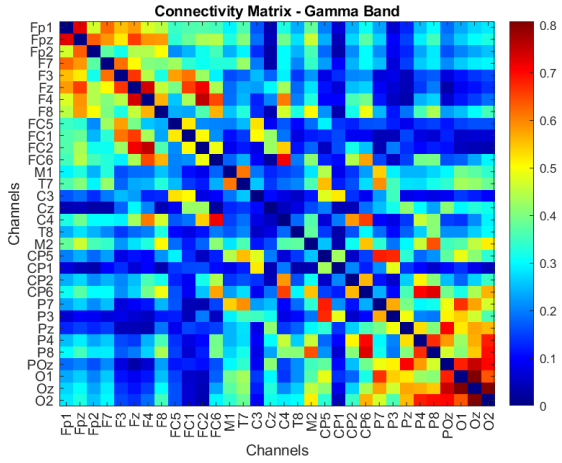

Beta Pre-stimulation connectivity plot



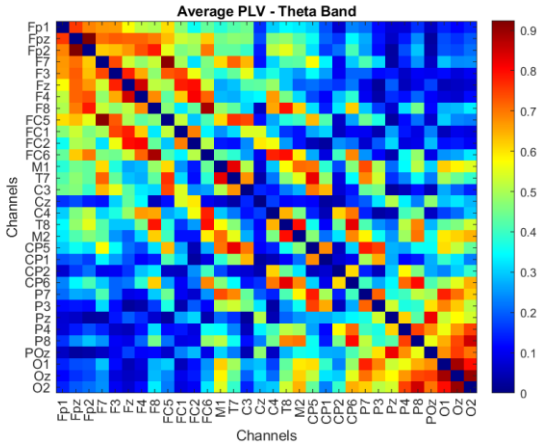

Beta Post-stimulation connectivity plot



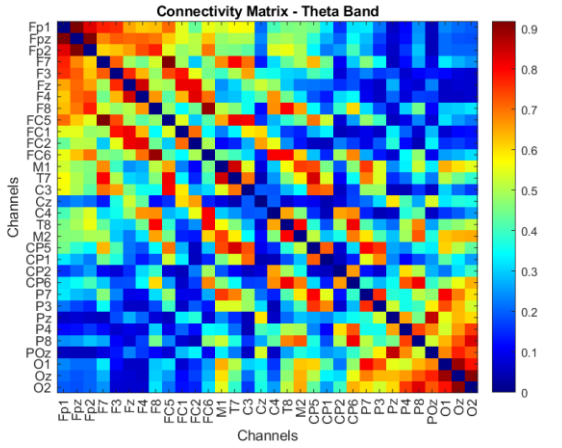

Gamma Pre-stimulation connectivity plot




Gamma Post-stimulation connectivity plot




Theta Pre-stimulation connectivity plot




Theta Post-stimulation connectivity plot

#### *3.4.2.3 Restructuring of PLV matrices*

Through the connectivity plots, it was observed that the differences were visible in some channels. Therefore, for frontal HD-tACS, we selected all 8 channels of the frontal cortex (FP1, FPz, FP2, F7, F3, Fz, F4, F8) for analyzing the results. For the HD-tACS application on primary motor cortex analysis, the number of channels selected were 13, from the frontal and fronto-central region i.e. FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, CP5, CP1, CP2, CP6.

To bring about statistical results of the data, these PLV matrices of 8 & 13 channels of F30 & M30, respectively, from the pre- and post-stimulation data were then converted to column vectors. The column vectors of the four PLV matrices, one from the pre-stimulation, three from the post-stimulation were arranged in tabular form. The arrangement of these vectors is represented as in the table below (Table 2).

We used a formula to accurately extract out the PLV values from the upper triangular part of the PLV matrix, in order to abstain duplicate values in the column vector. This step was performed for both types of HD-tACS.

For instance, the frontal channels PLV extraction is given as

$$\text{No. of channels} = 8$$

$$8 \times 8 = 64$$

In order to extract only the upper part of the PLV matrix, the diagonal was to be subtracted as well. So,

$$8 \times 7 = 56$$

$$\frac{56}{2} = 28$$

28 PLV values per subject. As there were 15 subjects, the table arranged those 28 values of each subject row-wise. So, the total number of rows in the final table, for the F30 stimulation type, became 420 and 4 columns. For M30 stimulation type, the total number of rows became 1170 for all subjects, with each subject corresponding to 78 rows, and four columns. The table was saved in a csv file to perform repeated measures ANOVA on it using a statistical software e.g. SPSS.

| **Pre** | **Post_start** | **Post_mid** | **Post_end** |
|---|---|---|---|
| **0.813931** | 0.787003 | 0.86072 | 0.836417 |
| **0.65973** | 0.645237 | 0.67243 | 0.689137 |

| | | | |
|---|---|---|---|
| **0.807344** | 0.707023 | 0.74205 | 0.74013 |
| **0.450965** | 0.595213 | 0.685033 | 0.65949 |
| **0.475143** | 0.585947 | 0.60834 | 0.600247 |
| **0.454653** | 0.42661 | 0.475793 | 0.47986 |
| **0.499846** | 0.518403 | 0.662203 | 0.59592 |
| **0.509921** | 0.614083 | 0.66357 | 0.630183 |
| **0.394906** | 0.3947 | 0.479753 | 0.453543 |
| **0.317802** | 0.638287 | 0.670503 | 0.636757 |
| **0.52348** | 0.48323 | 0.584513 | 0.553307 |
| **0.686184** | 0.650203 | 0.68584 | 0.67533 |
| **0.552281** | 0.417987 | 0.473187 | 0.469037 |
| **0.284612** | 0.409587 | 0.411993 | 0.396777 |
| **0.609653** | 0.711423 | 0.720707 | 0.699887 |
| **0.50254** | 0.408247 | 0.548917 | 0.48589 |
| **0.621435** | 0.549763 | 0.62379 | 0.59434 |
| **0.612508** | 0.350513 | 0.4676 | 0.438603 |
| **0.383561** | 0.426053 | 0.448203 | 0.403737 |
| **0.412742** | 0.539873 | 0.555233 | 0.518773 |
| **0.651037** | 0.66125 | 0.68437 | 0.653117 |

| | | | |
|---|---|---|---|
| **0.503687** | 0.51966 | 0.58294 | 0.565417 |
| **0.556191** | 0.586177 | 0.600547 | 0.596047 |
| **0.611776** | 0.571443 | 0.61831 | 0.633827 |
| **0.480032** | 0.548317 | 0.58739 | 0.546747 |
| **0.260447** | 0.394483 | 0.426273 | 0.386307 |
| **0.320354** | 0.344933 | 0.358783 | 0.34785 |
| **0.56754** | 0.58545 | 0.59418 | 0.56377 |

***Table 3.2*** *Structured dataset*

### *3.4.2 Statistical Analysis*

The structured data, comprising PLV values for pre- and post-stimulation conditions across all 8 and 12 channels for each frequency band, were exported to csv files, separately. These csv files were then imported into SPSS software for statistical analysis.

**Repeated Measures ANOVA**

A repeated measures ANOVA was performed for each frequency band, to determine the significance of differences between pre-stimulation (baseline) data and post-stimulation conditions. It is particularly advantageous when there are multiple measurements being applied on the same participants under different conditions.

As this particular study involves multiple PLV values of same subjects in three different conditions and the data was collected over time. Then within-subject measurements were quite suitable for this data. As each subject has multiple values so repeated measures ANOVA helps control those individual differences, allowing more sensitive results of the effects of the conditions.

Repeated measures ANOVA has increased statistical power which makes it easier to detect the within-subject differences compared to between-subject differences. It is also highly capable of testing the hypothesis. As in this study, ANOVA will check the effects of low gamma HD-tACS at DLPFC reduces cognitive processing in healthy individuals. The results are discussed in the "Results" chapter in detail.

# CHAPTER 4: RESULTS

The purpose of this research was to examine how healthy persons' attention, cognitive processing, and motor performance were affected by low-gamma HD-tACS applied to the left DLPFC and primary motor cortex during a continuous attention task. Participants performed a continuous attention task over a period of 70 minutes, with the first 20 minutes reserved as a pre-stimulation baseline. The stimulation was applied 20 times, within the subsequent 50 minutes. We analyzed the connectivity matrices' plots of different EEG bands (theta, alpha, beta, and gamma) of pre- and post-stimulation to weigh the impact of the stimulation on brain activity.

Data were collected from 15 subjects across four conditions: pre-stimulation (Pre), immediately post-stimulation (Post_start), mid-stimulation (Post_mid), and at the end of the stimulation period (Post_end). The selected channels for analysis were based on those most relevant to vigilance, cognitive processing and motor function.

## 4.1 F30 results

### *4.1.1 Theta Band Analysis*

In order to compare the PLV values in the theta band among the four conditions, a repeated measures ANOVA was performed. Greenhouse-Geisser estimations of sphericity ($\varepsilon$ =.396) were used to adjust degrees of freedom after Mauchly's test revealed that the assumption of sphericity was broken, $\chi^2$ (5) =.045, $p < 0.00$. The results showed a significant effect of time on theta band power, F-statistic = 6964.3, $p < 0.001$.

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Pre | .6523947 | .15508005 | 420 |
| Post_start | .6347301 | .16574584 | 420 |
| Post_mid | .6289803 | .16332207 | 420 |
| Post_end | .6320537 | .16328419 | 420 |

***Table 4.1*** *Descriptive Statistics of theta F30*

Pairwise comparisons revealed significant differences between the pre-stimulation and post-stimulation conditions:

| (I) stimulation | (J) stimulation | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 (Pre) | 2 (Post_start) | .018* | .005 | .001 | .005 | .030 |
| | 3 (Post_mid) | .023* | .004 | .000 | .011 | .035 |
| | 4 (Post_end) | .020* | .005 | .000 | .008 | .032 |

***Table 4.2*** *Pairwise comparisons of theta F30*

The significance of mean change is kept at 0.05 level. Bonferroni correction was applied for adjustment.

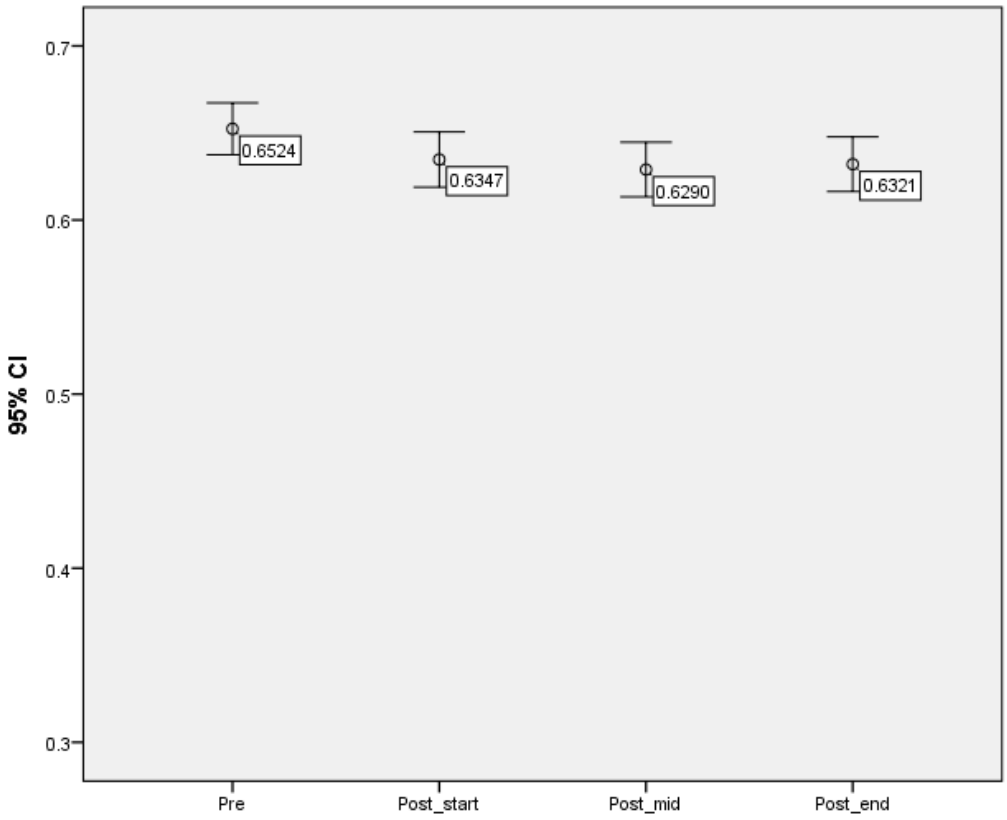



***Fig 4.1*** *Confidence Interval graph of theta F30*

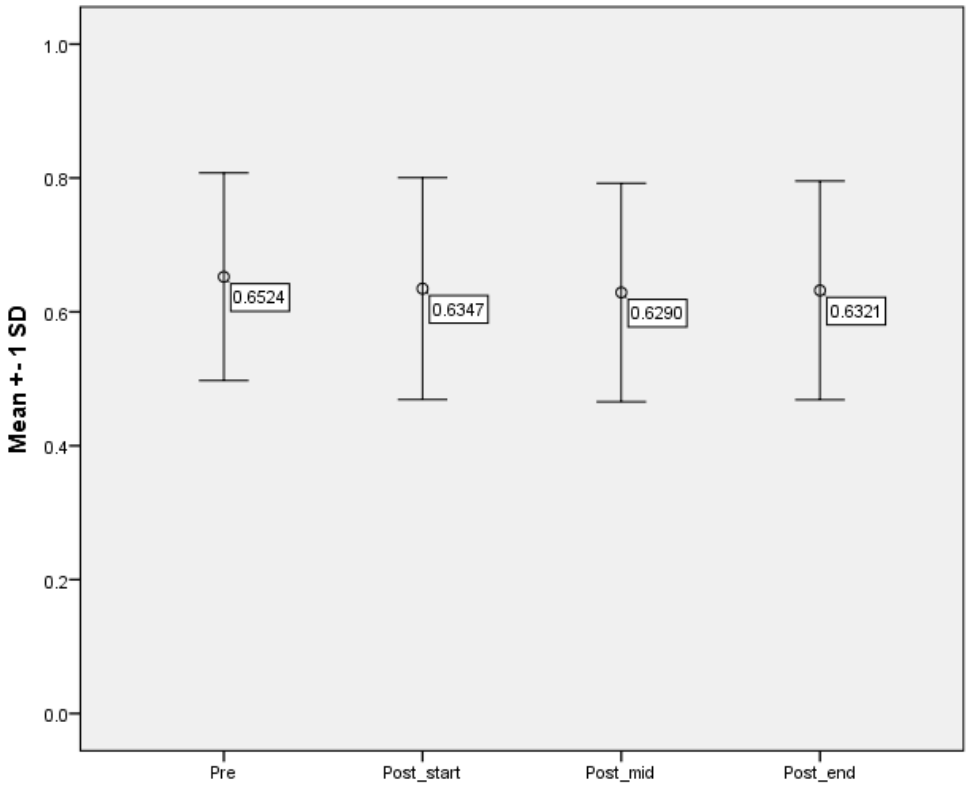



***Fig 4.2*** *Standard deviation error graph of theta F30*

These results indicate a significant decrease in theta band power following stimulation, with the most pronounced changes occurring in the middle and end of the stimulation.

### *4.1.2 Alpha Band Analysis*

For the alpha band, Mauchly's test also indicated a violation of sphericity, $\chi^2(5) = .040$, $p < 0.001$. Greenhouse-Geisser correction was applied ($\varepsilon = 0.396$). The ANOVA results showed a significant effect of time on alpha band power, F-statistic = 8241.1, p = 0.001.

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Pre | .6725665 | .14943396 | 420 |
| Post_start | .6521796 | .16332547 | 420 |
| Post_mid | .6594031 | .16164789 | 420 |
| Post_end | .6473590 | .16217431 | 420 |

***Table 4.3*** *Descriptive Statistics of alpha F30*

Pairwise comparisons indicated insignificant differences between:

| (I) Stimulation | (J) Stimulation | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 (Pre) | 2 (Post_start) | .020* | .006 | .007 | .004 | .037 |
| | 3 (Post_mid) | .013 | .006 | .241 | -.004 | .030 |
| | 4 (Post_end) | .025* | .006 | .000 | .009 | .042 |

***Table 4.4*** *Pairwise comparisons of alpha F30*

The significance of mean change is kept at 0.05 level. Bonferroni correction was applied for adjustment.

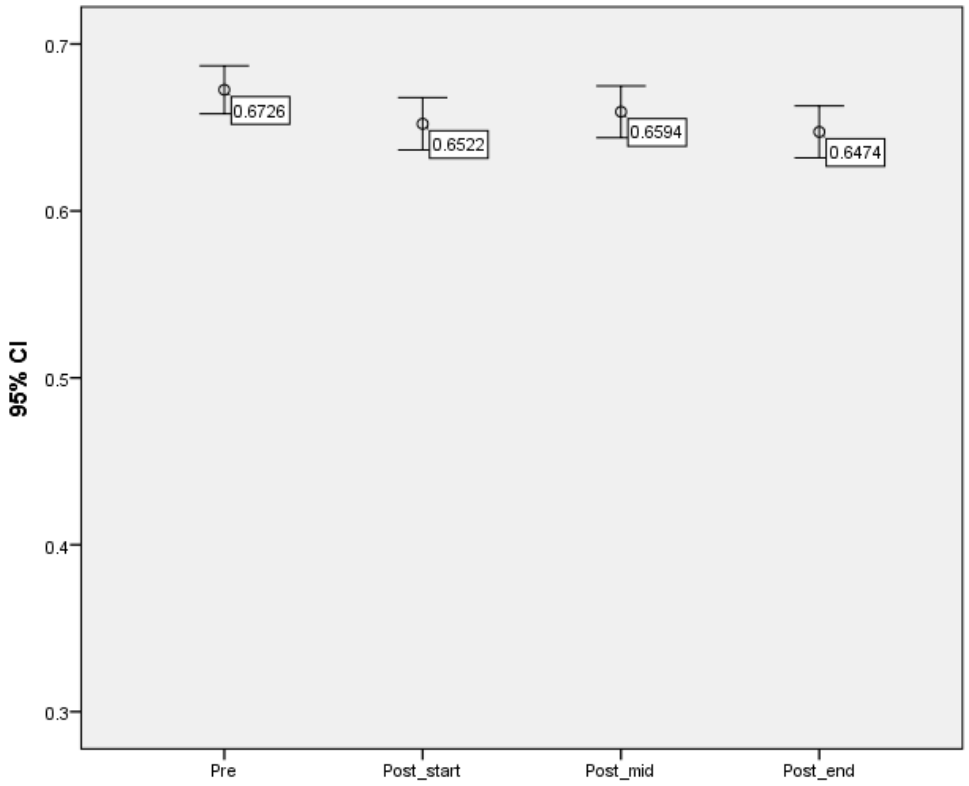



***Fig 4.3*** *Confidence Interval graph of alpha F30*

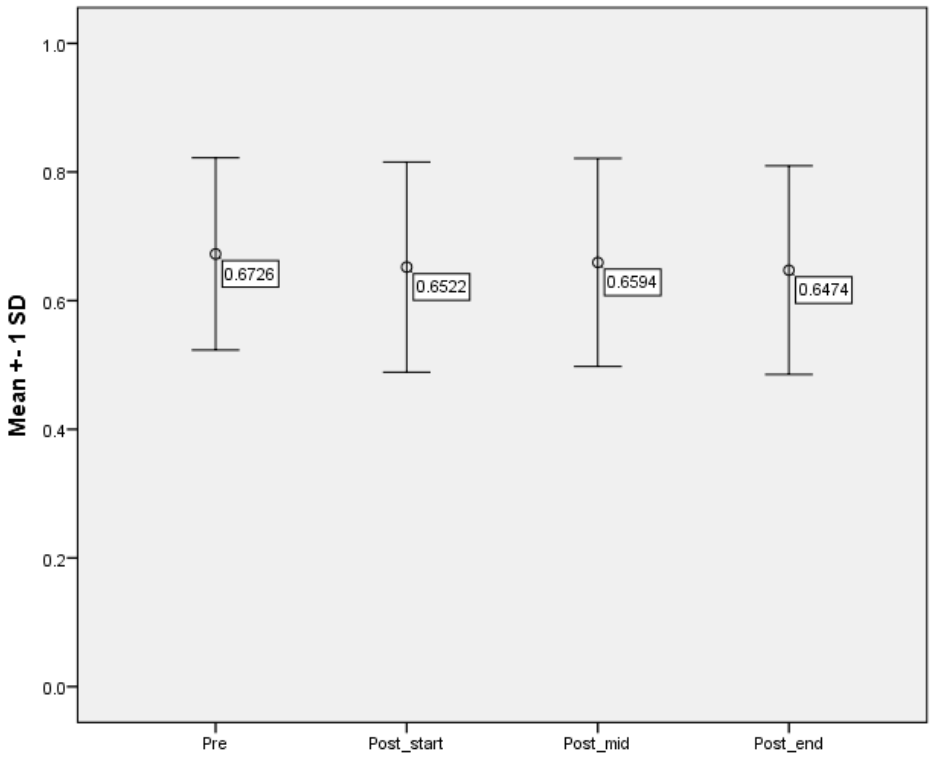



***Fig 4.4*** *Standard deviation error graph of alpha F30*

The alpha band results suggest a trend towards decreased connectivity during the stimulation period. The results show an insignificant difference between pre- and post-stimulation.

### *4.1.3 Beta Band Analysis*

Analysis of the beta band also required correction for sphericity, $\chi^2(5) = 0.073$, $p < 0.001$, with Greenhouse-Geisser correction applied ($\varepsilon = 0.421$). The ANOVA showed a significant effect of time on beta band, F-statistic = 2441.9, $p < 0.001$.

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Pre | .5118313 | .16938179 | 420 |
| Post_start | .4103231 | .20868433 | 420 |
| Post_mid | .4188880 | .20172469 | 420 |
| Post_end | .4142895 | .20353749 | 420 |

***Table 4.5*** *Descriptive Statistics of beta F30*

Pairwise comparisons demonstrated significant differences between:

| (I) Stimulation | (J) Stimulation | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 (Pre) | 2 (Post_start) | .102* | .008 | .000 | .080 | .123 |
| | 3 (Post_mid) | .093* | .008 | .000 | .072 | .114 |
| | 4 (Post_end) | .098* | .008 | .000 | .076 | .119 |

***Table 4.6*** *Pairwise comparisons of beta F30*

The significance of mean change is kept at 0.05 level. Bonferroni correction was applied for adjustment.

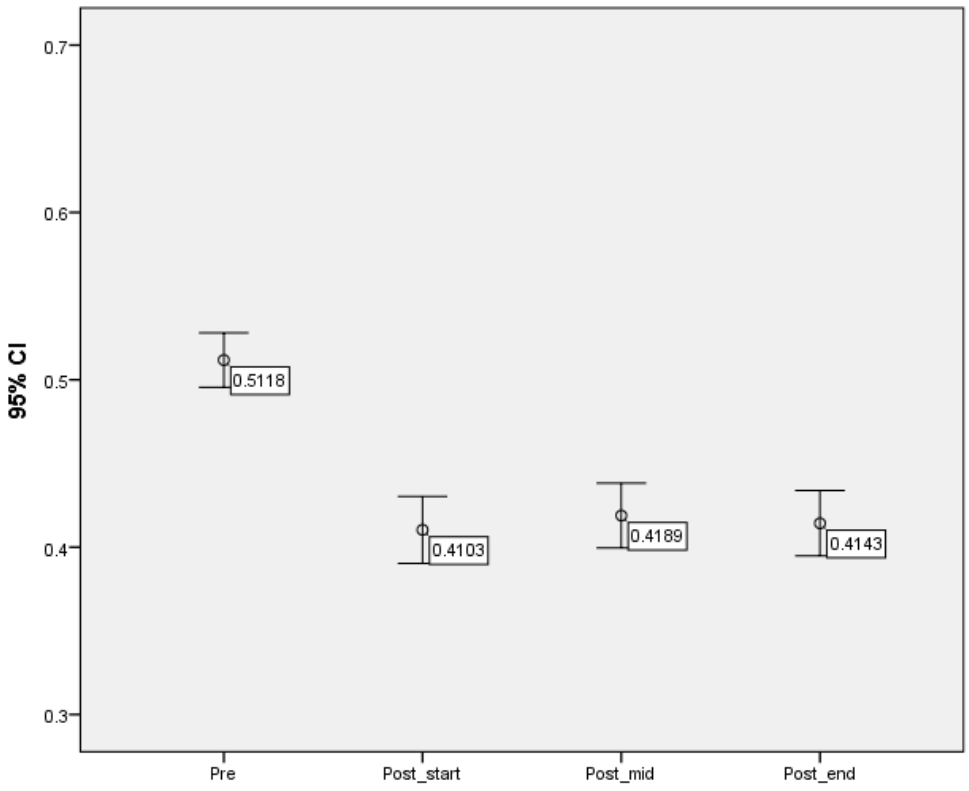



***Fig 4.5*** *Confidence Interval graph of beta F30*

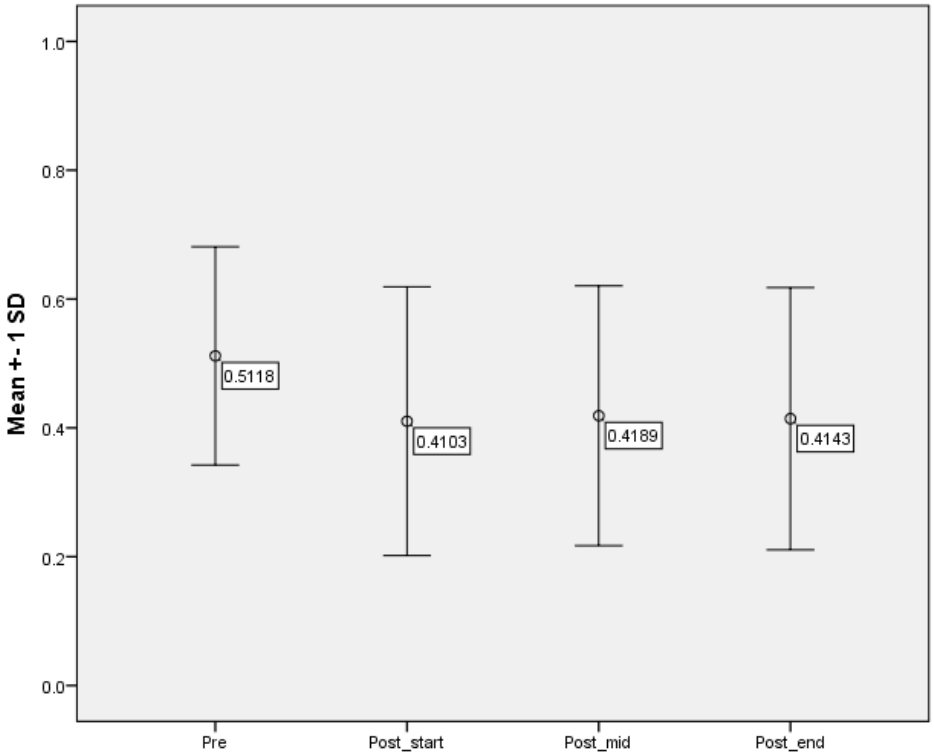



***Fig 4.6*** *Standard deviation error graph of beta F30*

The beta band exhibited a significant decrease in frontal cortex cortical activity following stimulation, which narrates reduced attention and cognition.

### *4.1.4 Gamma Band Analysis*

The gamma band analysis also showed a significant effect of time, with Mauchly's test indicating sphericity violation, $\chi^2(5) = .139$, $p < 0.001$, and Greenhouse-Geisser correction ($\varepsilon = .461$). The ANOVA results revealed a significant effect of time on gamma band power, F-statistic = 2061, $p < 0.001$.

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Pre | .4620124 | .18874211 | 420 |
| Post_start | .3632855 | .19079161 | 420 |
| Post_mid | .3668609 | .18849547 | 420 |
| Post_end | .3671803 | .18809545 | 420 |

***Table 4.7*** *Descriptive Statistics of gamma F30*

Pairwise comparisons indicated significant differences between:

| (I) Stimulation | (J) Stimulation | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 (Pre) | 2 (Post_start) | .099* | .007 | .000 | .080 | .118 |
| | 3 (Post_mid) | .095* | .007 | .000 | .076 | .114 |
| | 4 (Post_end) | .095* | .007 | .000 | .075 | .115 |

***Table 4.8*** *Pairwise comparisons of gamma F30*

The significance of mean change is kept at 0.05 level. Bonferroni correction was applied for adjustment.

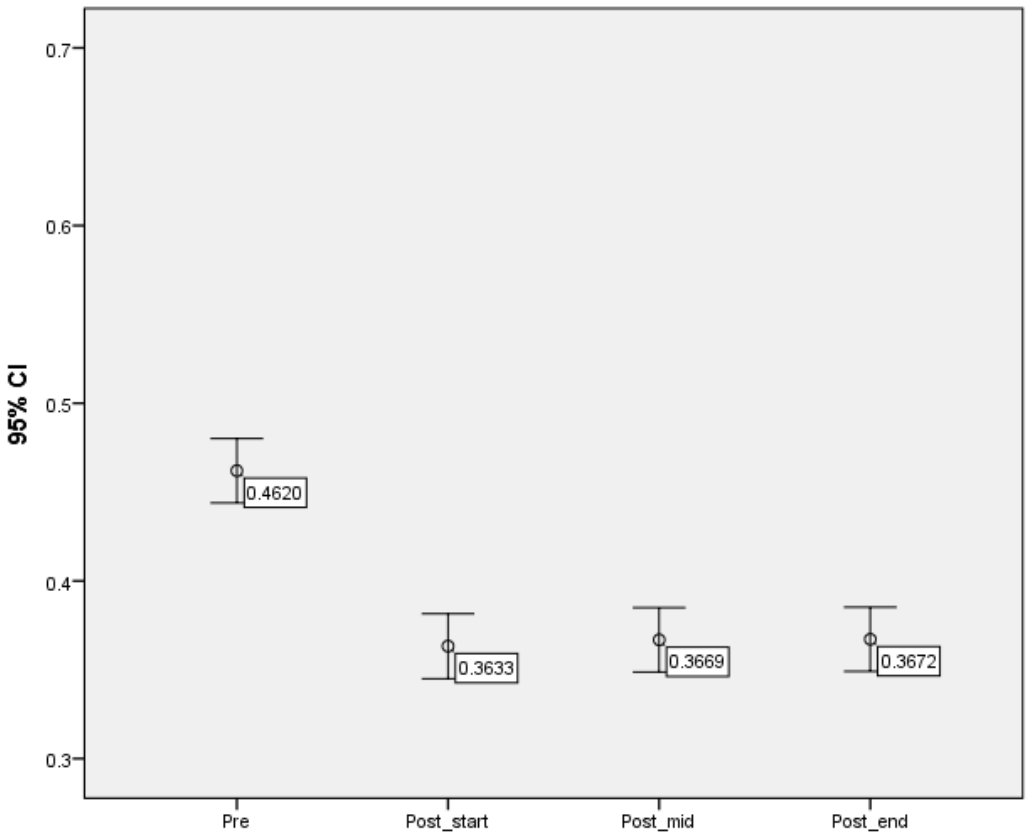



***Fig 4.7*** *Confidence Interval graph of gamma F30*

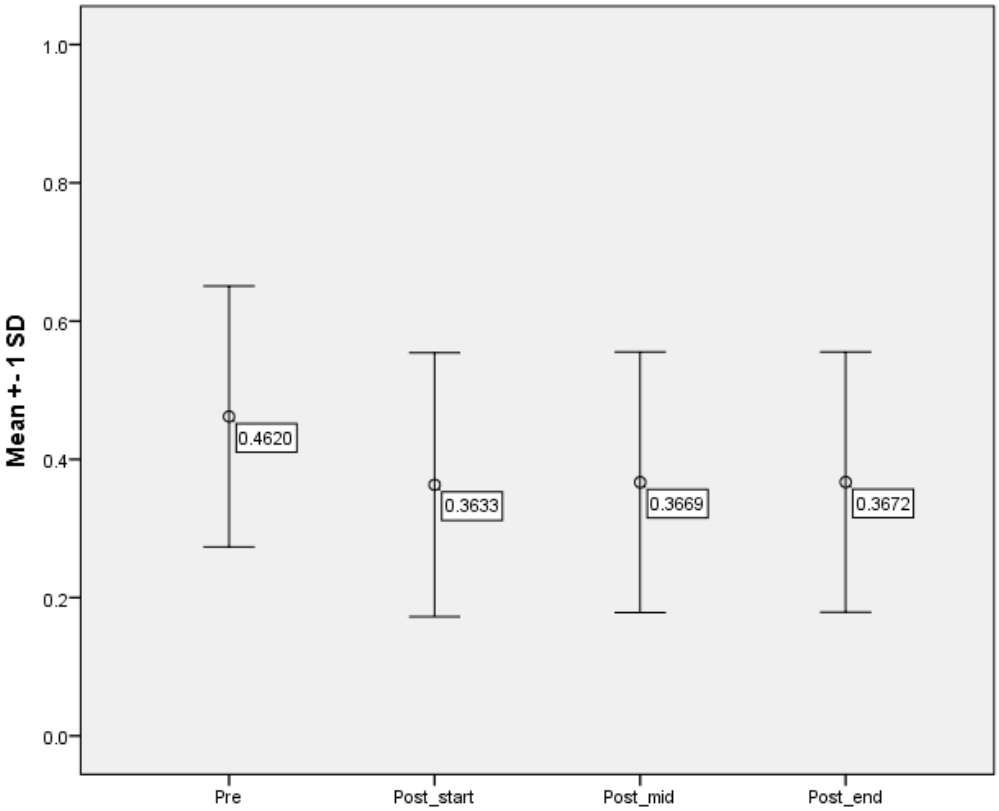



***Fig 4.8*** *Standard deviation error graph of gamma F30*

These findings suggest a substantial decrease in gamma band connectivity in frontal cortex, consistent with reduced neural synchronization and information processing capabilities following stimulation.

## 4.2 M30 results

For analyzing the results of the motor cortex stimulation, the central, fronto-central and centro-parietal channels (FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, CP5, CP1, CP2, CP6) were selected. The selections were made by visualizing the connectivity matrices' plots of pre- and post-stimulation. The plots displayed changes following stimulation in these channels.

### *4.2.1 Theta Band Analysis*

A repeated measures ANOVA was conducted to compare the PLV values in the theta band across the four conditions. Mauchly's test indicated that the assumption of sphericity was

violated, $\chi^2(5) = .612$, $p < 0.00$; therefore, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .739$).

The results showed a significant effect of time on theta band power, F-statistic = 2546.4, $p < 0.001$.

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Pre | .3438082 | .23094095 | 1170 |
| Post_start | .3393247 | .23216246 | 1170 |
| Post_mid | .3402425 | .23390515 | 1170 |
| Post_end | .3442183 | .23451483 | 1170 |

***Table 4.9*** *Descriptive Statistics of theta M30*

Pairwise comparisons indicated a bit significance between pre ad post-stimulation:

| (I) Stimulation | (J) Stimulation | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 (Pre) | 2 (Post_start) | .004* | .001 | .004 | .001 | .008 |
| | 3 (Post_mid) | .004* | .001 | .036 | .000 | .007 |
| | 4 (Post_end) | .000 | .001 | 1.000 | -.004 | .003 |

***Table 4.10*** *Pairwise comparisons of theta M30*

The significance of mean change is kept at 0.05 level. Bonferroni correction was applied for adjustment.

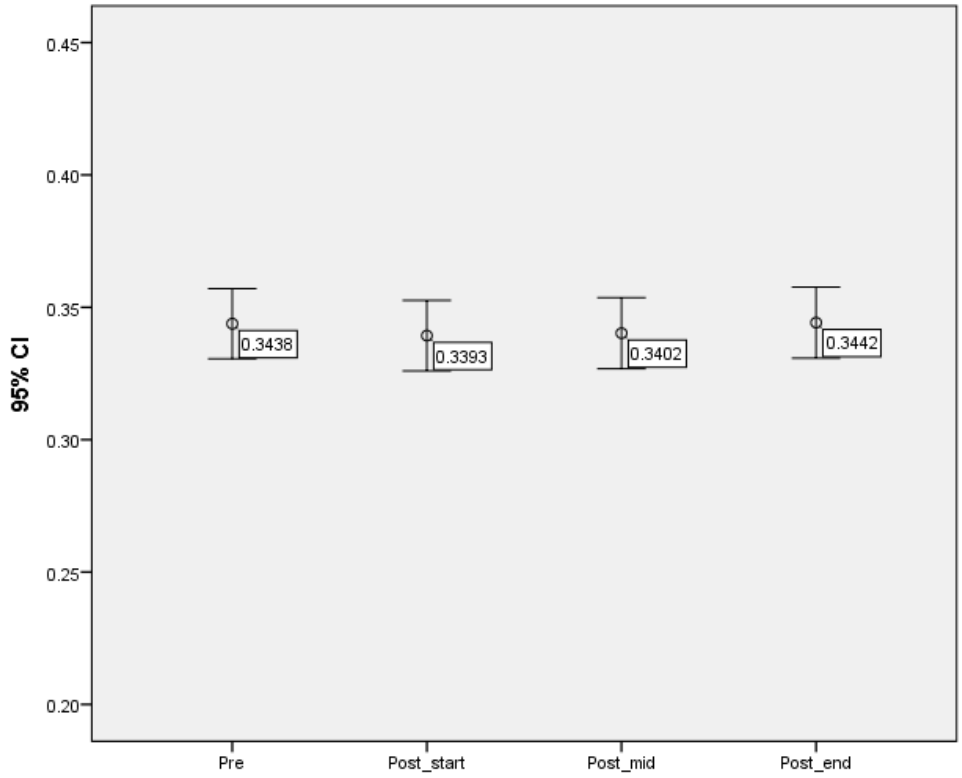



***Fig 4.9*** *Confidence Interval graph of theta M30*

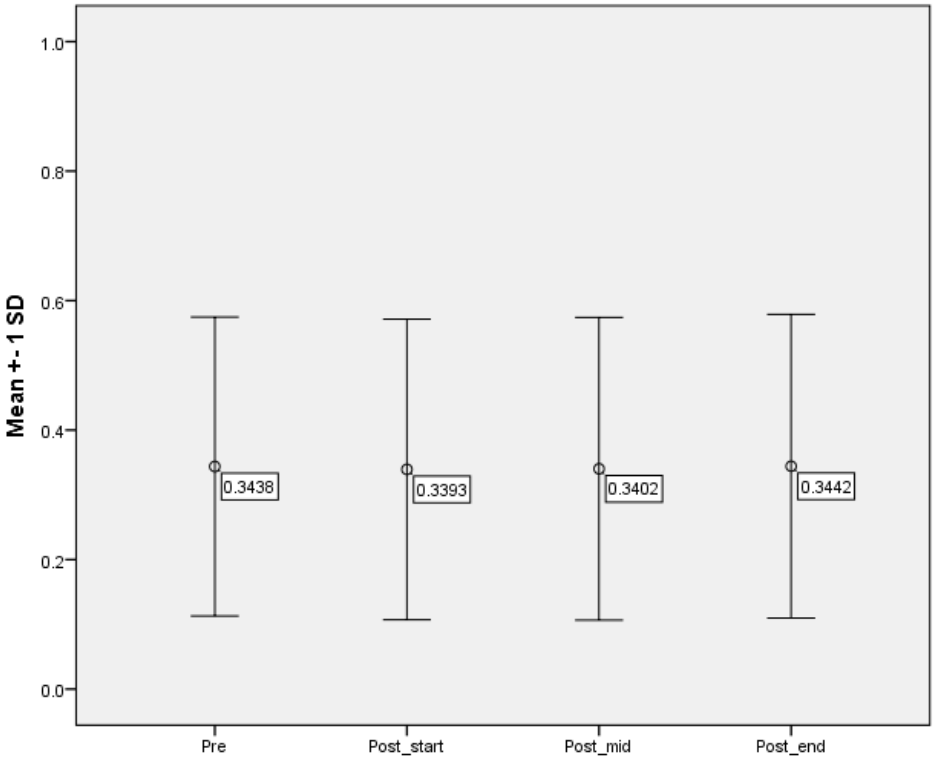



***Fig 4.10*** *Standard deviation error graph of theta M30*

These results indicate a significant decline in theta band connectivity in the frontal and fronto-central region of the brain, following stimulation.

### *4.2.2 Alpha Band Analysis*

For the alpha band, Mauchly's test also indicated a violation of sphericity, $\chi^2(5) = .602$, $p < 0.001$. Greenhouse-Geisser correction was applied ($\varepsilon = 0.731$).

The ANOVA results showed a significant effect of time on alpha band power, F-statistic = 3291.8, p = 0.001.

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Pre | .3762758 | .23353324 | 1170 |
| Post_start | .4004966 | .23525224 | 1170 |
| Post_mid | .3896511 | .23627710 | 1170 |
| Post_end | .3969812 | .23529218 | 1170 |

***Table 4.11*** *Descriptive Statistics of alpha M30*

The pairwise comparisons revealed a significance:

| (I) Stimulation | (J) Stimulation | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 (Pre) | 2 (Post_start) | -.024* | .002 | .000 | -.029 | -.020 |
| | 3 (Post_mid) | -.013* | .002 | .000 | -.018 | -.008 |
| | 4 (Post_end) | -.021* | .002 | .000 | -.025 | -.016 |

***Table 4.12*** *Pairwise comparisons of alpha M30*

The significance of mean change is kept at 0.05 level. Bonferroni correction was applied for adjustment.

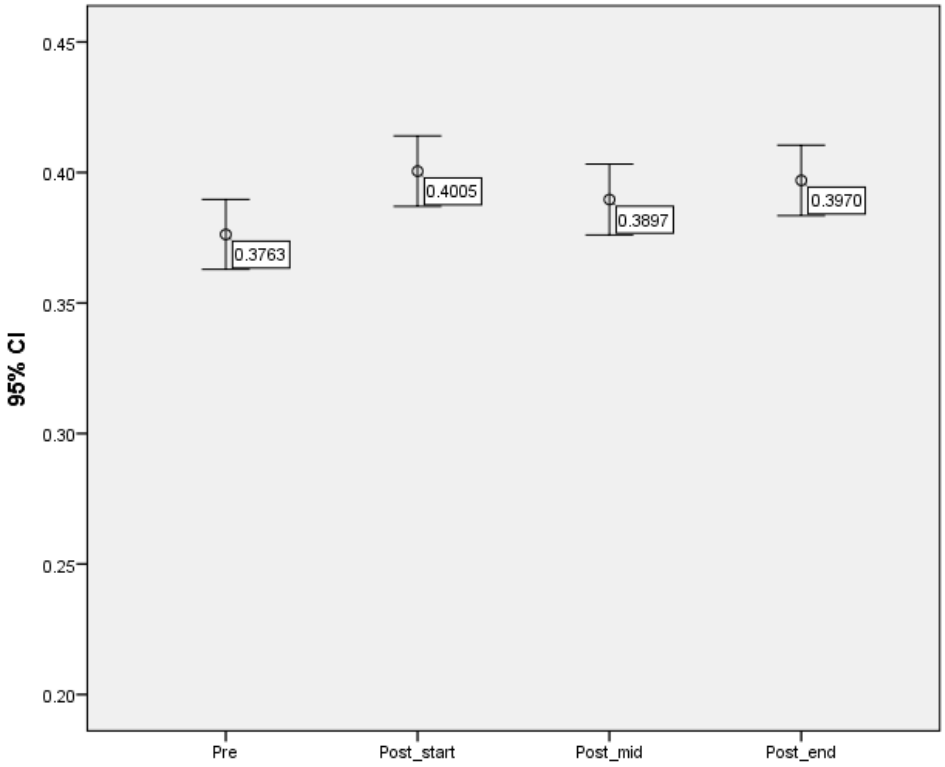



***Fig 4.11*** *Confidence Interval graph of alpha M30*

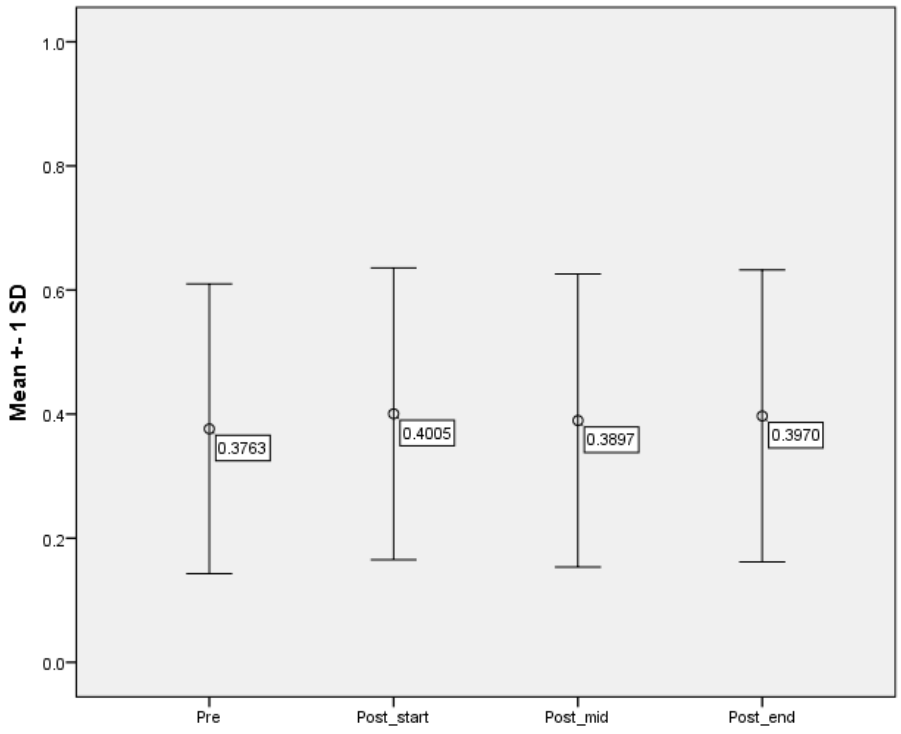



***Fig 4.12*** *Standard deviation error graph of alpha M30*

### *4.2.3 Beta band Analysis*

Analysis of the beta band also required correction for sphericity, $\chi^2(5) = 0.308$, $p < 0.001$, with Greenhouse-Geisser correction applied ($\varepsilon = 0.585$). The ANOVA showed a significant effect of time on beta band, F-statistic = 2262, $p < 0.001$.

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Pre | .2642581 | .19493426 | 1170 |
| Post_start | .2724038 | .19793039 | 1170 |
| Post_mid | .2718340 | .19984113 | 1170 |
| Post_end | .2723020 | .19868955 | 1170 |

***Table 4.13*** *Descriptive Statistics of beta M30*

The pairwise comparisons are as under:

| (I) Stimulation | (J) Stimulation | Mean Difference (I-J) | Std. Error | Sig.[b] | 95% Confidence Interval for Difference[b] | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 (Pre) | 2 (Post_start) | -.008* | .002 | .000 | -.013 | -.003 |
| | 3 (Post_start) | -.008* | .002 | .011 | -.014 | -.001 |
| | 4 (Post_start) | -.008* | .002 | .002 | -.014 | -.002 |

***Table 4.14*** *Pairwise comparisons of beta M30*

The significance of mean change is kept at 0.05 level. Bonferroni correction was applied for adjustment.

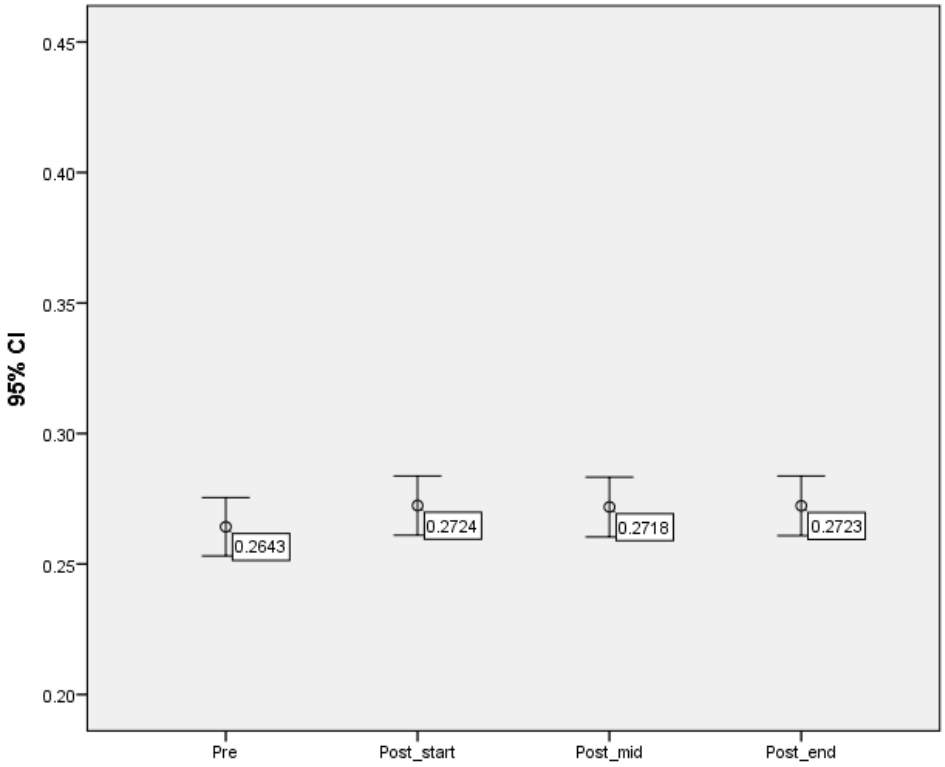



***Fig 4.13*** *Confidence Interval graph of beta M30*

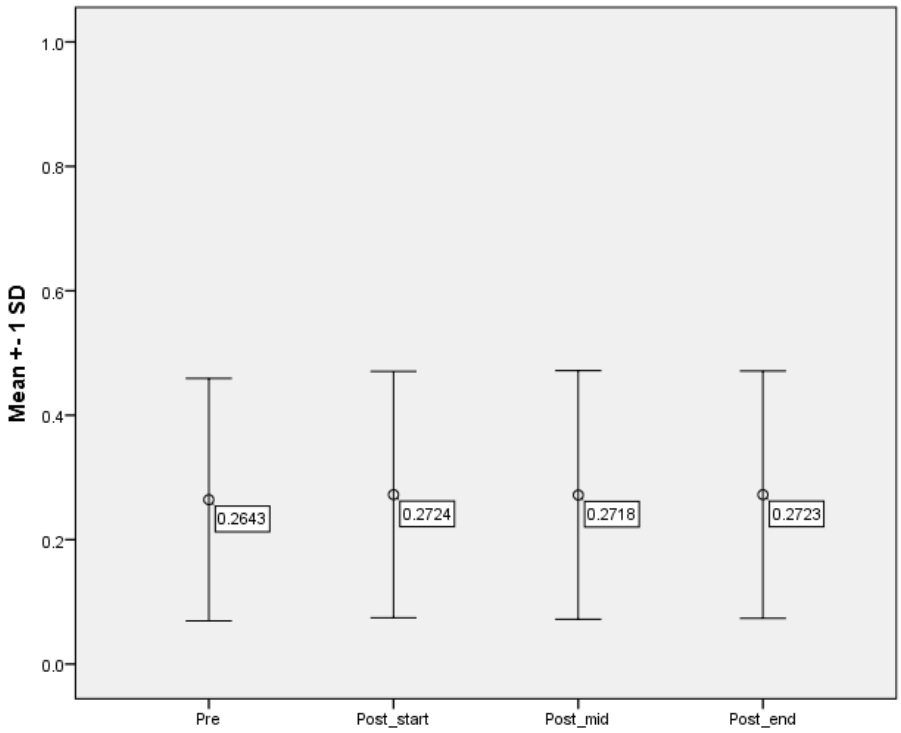



***Fig 4.14*** *Standard deviation error graph of beta M30*

### *4.2.4 Gamma Band Analysis*

The gamma band analysis also showed a significant effect of time, with Mauchly's test indicating sphericity violation, $\chi^2(5) = .417$, $p < 0.001$, and Greenhouse-Geisser correction ($\varepsilon = .627$). The ANOVA results revealed a significant effect of time on gamma band power, F-statistic = 2101, $p < 0.001$

| | Mean | Std. Deviation | N |
|---|---|---|---|
| Pre | .2285008 | .17984066 | 1170 |
| Post_start | .2308533 | .17735977 | 1170 |
| Post_mid | .2340514 | .17928046 | 1170 |
| Post_end | .2338156 | .17698720 | 1170 |

***Table 4.15*** *Descriptive Statistics of gamma M30*

The pairwise comparisons are as under:

| (I) Stimulation | (J) Stimulation | Mean Difference (I-J) | Std. Error | Sig.[a] | 95% Confidence Interval for Difference[a] | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| 1 (Pre) | 2 (Post_start) | -.002 | .002 | 1.000 | -.009 | .004 |
| | 3 (Post_mid) | -.006 | .003 | .256 | -.013 | .002 |
| | 4 (Post_end) | -.005 | .003 | .237 | -.012 | .001 |

***Table 4.16*** *Pairwise comparisons of gamma M30*

The significance of mean change is kept at 0.05 level. Bonferroni correction was applied for adjustment.

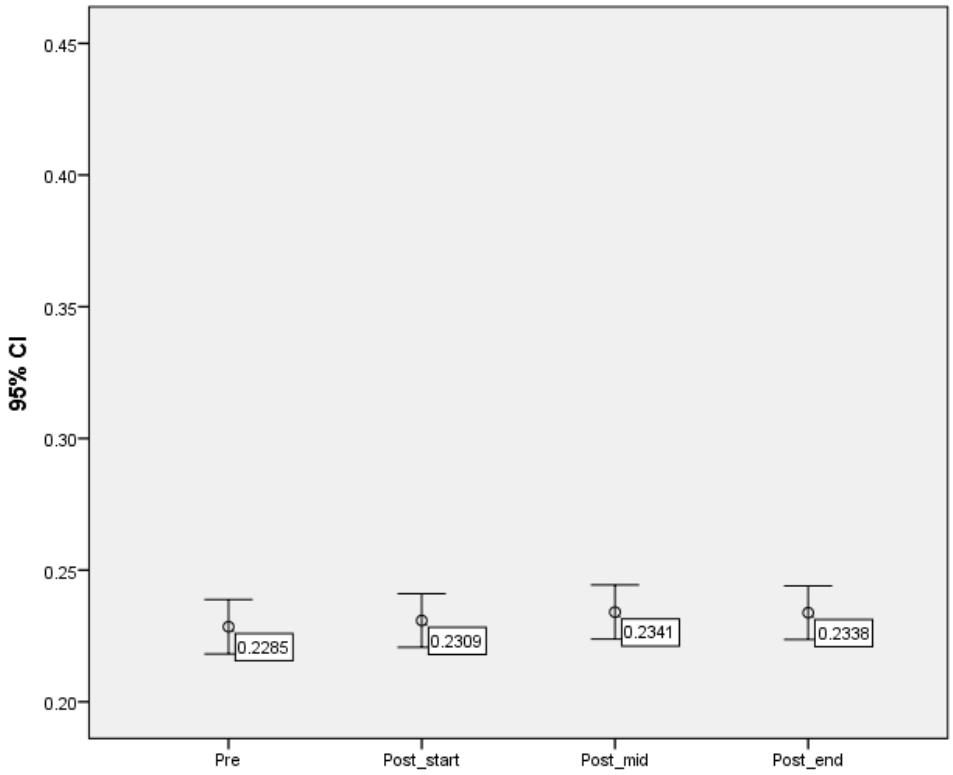



***Fig 4.15*** *Confidence Interval graph of gamma M30*

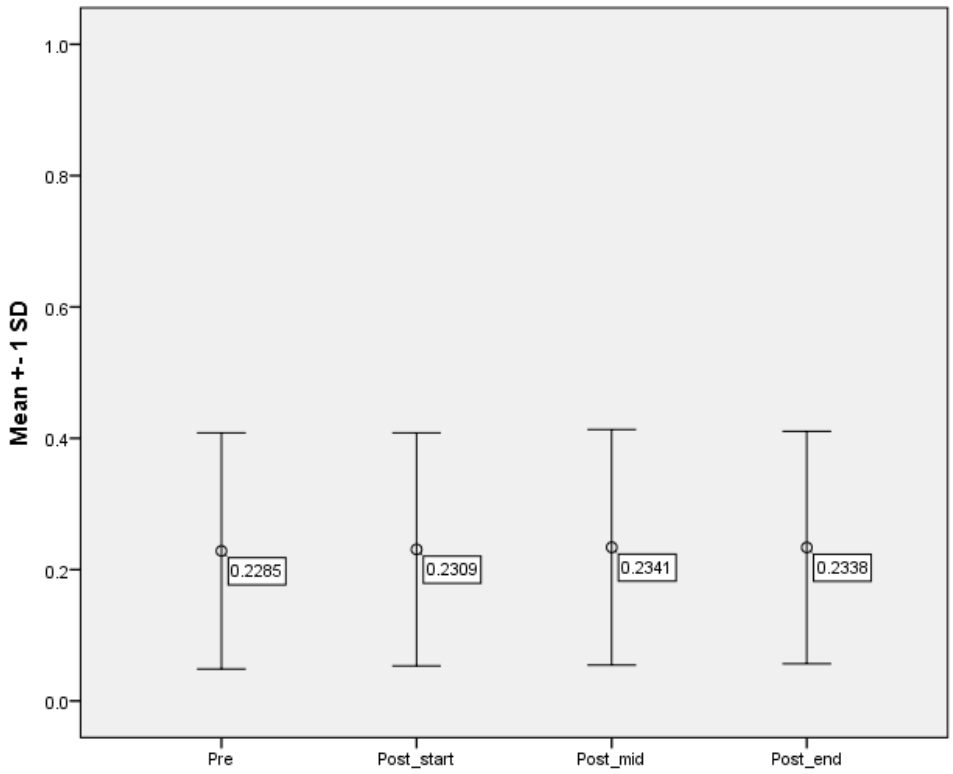



***Fig 4.16*** *Standard deviation error graph of gamma M30*

## 4.3 Interpretation of Results

The outcomes of this study demonstrate that HD-tACS at 30 Hz applied over the F5 and C5 locations, significantly affects the frequency bands i.e. alpha, beta, gamma and theta, during a continuous attention task. Specifically, these variations in these frequency bands are associated with various neural processes such as attention, memory, motor functions and information processing. The results for both locations greatly differ from each other, as the roles of both cortices are distinct in the brain.

The significant reduction observed in the theta, beta and gamma bands' oscillations in the frontal cortex, suggests altered attention, information processing and neural synchronization in the frontal cortex. While, the alpha band activity also reduced significantly at the start and end of the stimulation, might suggest cortical inhibition. However, there was no significant change observed in alpha oscillations, in the middle of stimulation. These findings suggest that low gamma HD-tACS at the F5 location might reduce attention and information processing in healthy individuals performing a continuous vigilance task.

On the other hand, by looking at the statistical results of low-gamma HD-tACS application at primary motor cortex C5 location, there is no significant difference observed in the gamma oscillations in the primary motor cortex, following stimulation. This suggests that gamma oscillations are not affected by tACS of low-gamma range at the primary motor cortex.

However, a significant increase in the alpha and beta oscillations in the primary motor cortex, might indicate attainment of a relaxed mind and enhanced working memory or motor function, respectively. On the contrary, a significant decrease in the theta band oscillations at the start and mid, suggest an impact of low-gamma tACS at this location in terms of attentional focus and working memory. Nonetheless, this exploration warrants further in-depth investigation.

# CHAPTER 5: DISCUSSION

This research intended to explore the effects of low-gamma HD-tACS over the left dorsolateral prefrontal cortex (DLPFC) and primary motor cortex in healthy individuals involved in a continuous attention task. It was hypothesized that tACS at low-gamma frequency would reduce attention and neural synchronization. Adapting phase synchronization analysis, the study inspected the connectivity of the frontal and motor cortices during tACS application. For statistical analysis, repeated measures ANOVA was employed. The data was sourced from Nature Scientific Reports, with all the raw, experimental, EEG, physiological datasets openly available on openneuro.org.

The main discoveries have revealed that the application of tACS over the DLPFC exhibited a significant reduction in theta, beta, and gamma connectivity in the frontal cortex, whereas alpha connectivity decreased at the beginning and end, however, did not change during the middle period of stimulation.

First, let's dive into the significant decline in the beta and gamma oscillations in the frontal cortex, which suggests that there might be a decrease in the performance of higher order functions i.e. attention, information processing etc. As investigated by Palm, U. et al. (2022) which supports our findings, they observed reduction in cognitive performance following gamma (70 Hz) tACS over the DLPFCs. There was no improvement in reaction time and working memory.

Subsequent to the previous bands come the theta oscillations in frontal cortex, they were also significantly dropped. As the theta oscillations are involved in memory encoding and retrieval, their reduction implies modulated attention and neural connectivity. Gebodh et al. (2024) investigated the same EEG data as in our study. They explored the tACS effects on parieto-occipital cortex and discovered that there was a significant decline in the beta activity [54].

Following this previous mentioned research by Gebodh et al. (2024), they also found a significant increase in the alpha activity in the parieto-occipital cortex. However, our results of the alpha activity in the frontal cortex exhibit a unique pattern. The alpha activity was decreased

significantly at the start and end of the stimulation but not during the mid-stimulation phase. This suggests a dynamic response of alpha that might be influenced by the duration and timing of tACS employment. These results suggest further exploration.

In contrast to the findings of the F30, the application of tACS over the primary motor cortex exhibited an increase in beta and alpha connectivity in the motor area. Pogosyan et al. (2009) explored the impact of tACS over M1 and discovered that beta frequency increases when trying to stop a movement voluntarily. Our results show a similar pattern in the beta range. While the increase in alpha range may be suggesting an attention-related enhancement.

With gamma connectivity remaining unaffected with HD-tACS, it is also backed by the existing literature, as discovered by A. Giustiniani, et al. (2019). They applied 40 Hz over M1, and found no improvement in the reaction time of the participants. The lack of change in gamma oscillations suggests that they are less susceptible to modulation by low-gamma tACS.

Subsequently, the theta activity reducing significantly at the start and mid-period but not at the end of stimulation. These results underscore the need to further investigate into the temporal effects of HD-tACS on neural oscillations. Indulging in the modulations of theta oscillations could provide valuable insights into boosting tACS protocols.

The clinical implications of this research are considerable for rehabilitative purposes, suggesting that the experimental protocol may prove to be useful for treating neurological disorders e.g. anxiety and stress or improving motor functions. Nonetheless, the study's limitations include a small sample size of only ten participants, limiting the confidence in the findings.

Additionally, the frequency bands were not comprehensively explored, and the findings were not linked to behavioral outcomes. By further limiting channel combinations for calculating phase relationships between channels, the results would validate the interpretations more accurately.

# CHAPTER 6: CONCLUSIONS AND FUTURE RECOMENDATIONS

From this study, we investigated the effects of low γ-tACS applied at the left DLPFC and primary motor cortex. Our findings suggest a nuanced impact on attentional focus and cognitive processing, with diverse effects witnessed in the frontal and motor cortices.

Scrutinizing the statistical results, in the frontal cortex (F30), with p-values < .05, it is clear that there is significant reduction in the neural connectivity in all the frequency bands; theta, alpha, beta and gamma, in this area. However, no significant difference is observed during the mid-stimulation period in alpha band, this proposes further investigation.

Specifically, visualizing the confidence interval plots of gamma and beta band in the F30 stimulation, and verifying through demographical data of Karolinska Sleep Index, it is evident that the participants were experiencing hypnogogic states which advises reduced attention and information processing.

In case of the primary motor cortex, no significant difference is observed in the gamma oscillations with the HD-tACS application at C5 location, with the p-value > .05. Whereas, alpha and beta oscillations exhibited an increase in the neural connectivity in this area. Conversely, the theta band oscillations exhibited a decrease in its connectivity. These discoveries highlight an intricate interaction between HD-tACS and neural activity in the primary motor cortex, indicating that low-gamma stimulation may have fluctuating effects depending on the cortical region being targeted.

The results of both stimulation conditions i.e. F30 and M30, vary greatly with respect to their functions. The reduction in the connectivity of the frequency bands in the F30 stimulation condition might suggest lessened attention. However, the differing results in the neural connectivity of the frequency bands in M30 stimulation condition at the primary motor cortex, warrants further investigation.

Overall, this study provides valuable insights into the regional effects of low-gamma tACS on neural synchronization and on the connectivity of frequency bands.

Future research should aim to include a larger and more diverse participant pool to boost the confidence in the findings. Moreover, it is needed to explore the effects of low-gamma HD-tACS on other frequency bands, such as alpha, theta, and delta, across all cortical regions. Evaluating more precise channel pair phase-locking value (PLV) measurements will provide deeper insights into the neural mechanisms underlying low-gamma tACS effects. Furthermore, linking neural changes to behavioral outcomes will help in comprehending the clinical implications of neural modulations induced by HD-tACS. Such comprehensive studies will aid in developing the application of HD-tACS in clinical settings for cognitive enhancement, potentially improving treatments for anxiety, stress disorders, and motor function enhancement.

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