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Examining the Effects of Intermittent Theta Burst Stimulation on Accuracy and Reaction Time in N Back Tasks

Dipmala Salunke
JSPM's Rajarshi Shahu College of Engineering, Pune, India
dipmala.salunke@gmail.com
https://orcid.org/0000-0002-2921-5342

Yash Bandal
JSPM's Rajarshi Shahu College of Engineering, Pune, India.
yashbandal25@gmail.com
https://orcid.org/0009-0003-1944-2829

Alexander Zakharov
Neuroscience Research Institute, Samara State Medical University, Russia.
a.v.zaharov@samsmu.ru
https://orcid.org/0000-0003-1709-6195

Natalia Romanchuk
Neuroscience Research Institute, Samara State Medical University, Russia.
n.p.romanchuk@samsmu.ru
https://orcid.org/0000-0003-3522-6803

Maria Sergeeva
Neuroscience Research Institute, Samara State Medical University, Russia.
m.s.sergeeva@samsmu.ru
https://orcid.org/0000-0002-0926-8551

Yuliya Komarova
Neuroscience Research Institute, Samara State Medical University, Russia.
ju.s.komarova@samsmu.ru
https://orcid.org/0000-0003-3435-1477

Igor Shirolapov
Neuroscience Research Institute, Samara State Medical University, Russia.
i.v.shirolapov@samsmu.ru
https://orcid.org/0000-0002-7670-6566

D. Jude Hemanth
Karunya University, Coimbatore, India.
jude_hemanth@rediffmail.com
https://orcid.org/0000-0002-6091-1880

Abstract: *Transcranial magnetic stimulation (TMS), especially intermittent theta burst stimulation (iTBS), has been extensively studied to improve brain function. Its ability to enhance cognitive functions, particularly working memory, has been widely investigated. This study aims to analyse the effects of a single session of iTBS applied to the left dorsolateral prefrontal cortex (DLPFC) on cognitive test performance, electroencephalography (EEG) correlates of working memory in healthy young subjects, and to assess accuracy and reaction time. A group of 14 healthy young adults aged between 19 and 23 years participated in the study. The subjects were arbitrarily distributed into two groups: with active (N=7) and sham (N=7) iTBS. The active 3-back test showed a positive impact on working memory performance with an increasing accuracy and with reduced average reaction time after 30 minutes of TMS session. It is concluded that iTBS over the DLPFC was responsible for enhancing working memory performance in healthy young adults, based on an observation that depicted improved accuracy and faster response times, especially within the Active 3 back test.*

Keywords: *intermittent theta burst stimulation (iTBS); transcranial magnetic stimulation (TMS); dorsolateral prefrontal cortex (DLPFC); working memory; electroencephalography (EEG); N back test, accuracy; reaction time.*

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1. Introduction

The human brain is a complex organ. Billions of neurons, the brain cells are responsible for t generating electrical impulses that orchestrate neural activity. These rhythmic patterns, termed brain waves, can be measured using the non-invasive technique called electroencephalography (EEG).

A broad variety of cognitive operations, ranging from learning and reasoning to decision-making, are supported by working memory (WM) (Hu et al., 2024).

Impairments within it are typically found in many neuropsychiatric conditions such as schizophrenia, depression, and attention deficit/hyperactivity disorder (ADHD) (Baddeley, 2012). As such, interventions aimed at modulating working memory performance have become a major area of research in cognitive neuroscience. TMS is a brain stimulation method which is responsible for the transformation of cortical excitability and neuroplasticity (Kaneko et al., 2024). Among various TMS protocols, intermittent theta burst stimulation (iTBS) has attracted interest because it can induce long-term potentiation (LTP)-like effects in the human cortex (Huang et al., 2005). The DLPFC is a frequent target of iTBS, as it plays an important role in working memory. Various studies indicate that modulating DLPFC activity can influence cognitive task performance, especially in tasks involving sustained attention and memory updating (Fitzgerald et al., 2006). Although several studies have investigated the effects of iTBS in clinical populations, whether iTBS has a prolonged positive effect on cognitive performance and working memory remains unclear. Knowledge of how iTBS affects working memory and related neural correlates in a healthy sample may offer insight into the action mechanisms of this neurostimulation technique and its potential as a cognitive enhancing strategy.

Globally, cognitive impairments are estimated to occur in more than 5 to 15% of the general population, and early preventive interventions or preventive measures might lower the risk of developing cognitive decline later in life (World Health Organization, 2023). Thus, examining how a single session of iTBS can increase cognitive performance in healthy individuals is of scientific and translational importance.

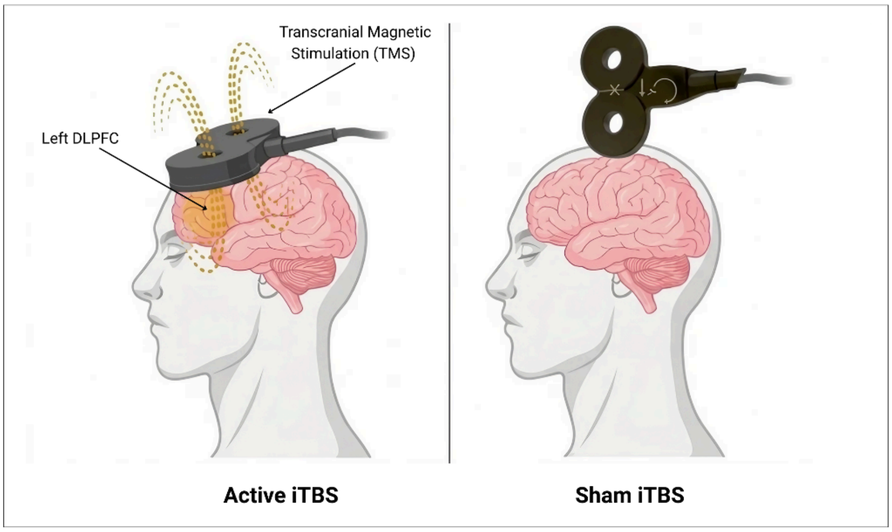

Figure 1. Active and Sham iTBS Stimulation in the DLPFC

This paper explores the effect of a single session of iTBS over the left DLPFC on the performance of working memory and EEG patterns in healthy young adults. In particular, we aim to evaluate behavioural measures (accuracy and reaction time) in 2-back and 3-back tasks, as well as EEG correlates, pre- and post- stimulation. We further intend to ascertain whether there are any substantial differences between active and sham iTBS groups as shown in figure 1.

2. Literature Review

Hu et al. (2024) directly compared single sessions of high-frequency rTMS and iTBS over the left DLPFC in healthy adults as part of a study. They proved that even a single session of iTBS is important enough to enhance the N2 ERP component during a 2-back task, suggesting an increased early cognitive resource allocation, and improved 2-back accuracy without affecting other executive functions. This finding confirms that single-session iTBS can induce immediate neurophysiological changes in working memory networks, which we examine further by combining time-frequency EEG markers and machine learning.

During iTBS applied over the left DLPFC in treatment-resistant patients with schizophrenia, visuospatial working memory performance improved.This addresses the promise of iTBS to the treatment of cognition in schizophrenia and related vsWM deficits in TRS (Wang et al., 2022).

A study comparing tDCS and iTBS suggested that both these intervention techniques were responsible for improving the working memory of individuals undergoing it. Also, this research went a step further to investigate the combined effect of tDCS and iTBS, thus suggesting that there may be more certain benefits from multimodal stimulation techniques for enhancing cognition (Razza et al., 2023).

An investigation has been performed (Sun et al., 2022) on the influence of iTBS on visual working memory in patients with methamphetamine use disorder (MUD). In this study, the participants underwent five daily sessions of iTBS on the left prefrontal cortical region or sham stimulation. Therefore, it can be inferred that active iTBS was responsible for enhancing the working memory capacity as compared to the sham group, and there were progressive benefits seen across multiple sessions, thereby remedying the cognitive deficits that are related to MUD.

The cognitive and neurophysiologic impacts of repeated iTBS applied to the left prefrontal cortical region and cerebellum of healthy subjects (Xu et al., 2024). According to the research, there was a major cognitive improvement induced by prolonged iTBS sessions that would indicate an enhancement of efficacy for repeated stimulation protocols in enhancing cognitive functions.

The impact of iTBS on resting-state neural activity with the use of the Brain Engagement Network (BEN) as a biomarker was investigated (Liu et al., 2025). It was found that BEN is sensitive to iTBS, and its varying levels of stimulation induce changing neural reactions. This signifies that iTBS has the ability to modulate resting-state networks, which could affect cognitive functions related to these networks.

As per the guidelines laid down by Frontiers for having clear pipelines, Sun et al. (2022) created MBSincNex, a fast and transparent model which combines information about when, where, and at what frequency brain signals occur. They determined that it could generalise a new sample's working memory load with over 90% accuracy, even when tested on new people. Taken together, these works show that if you pick the right EEG features and use straightforward algorithms like Random Forests, you can reliably read out a person’s mental state from their brain waves (Zhang et al., 2025).

Another study examined the addition of iTBS to computerised working memory training in patients with aphasia. The findings stated that the combined intervention led to substantial gains in working memory functioning, thus indicating a synergistic effect that guides future rehabilitation techniques (Song et al., 2020; Economidou & Kambanaros, 2020).

EEG and eye-tracking data were combined during n-back tasks to classify mental workload in university student participants (Aksu et al., 2024). Using a Random Forest model with both physiological streams, they were successful in achieving over 92% accuracy in differentiating between low (1 back) versus high (3 back) load. This study determines how multimodal feature sets along with ensemble classifiers can robustly decode cognitive states, an approach we adapted by concentrating on time-frequency EEG features in isolation to selectively track the neural signatures of iTBS effects.

3. Materials and Methods

3.1. Participants

In this experiment, we involved 14 healthy female students of SamSMU aged 19 to 23 years (mean 20.07±2.83) as healthy subjects who had no contraindications undergoing TMS. All study members were right-handed as determined by the Edinburgh Handedness Questionnaire. Volunteers provided written informed consent to participate in the study. Participants were randomly assigned to two groups: active iTBS (EG, experimental group, N = 7) and sham iTBS (CG, control group, N = 7), as shown in Figure 2.

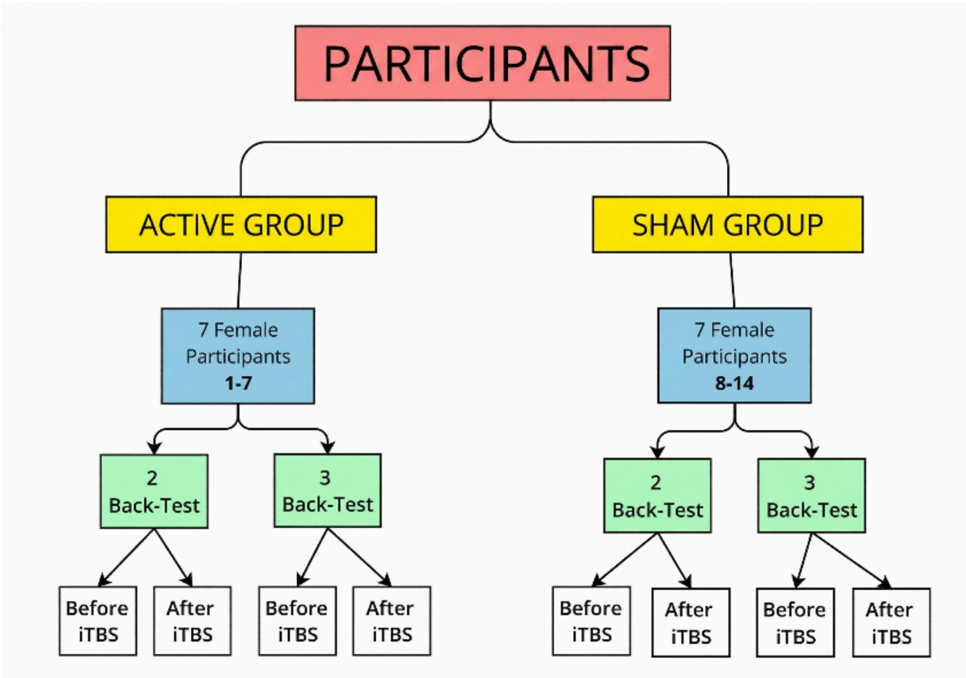

Figure 2. Participant Grouping and Experimental Design

3.2. Research Design

Double masked randomized placebo-controlled trial. The experiment had three such consecutive phases and was carried out on one experimental day.

The first and third phases were EEG recordings on the sequential performance of cognitive tests (2 back, 3 back) with a 5-minute interval between tests. In the first phase, EEG was recorded once during cognitive testing, while in the third phase, EEG on working memory tests was recorded twice: 10 and 30 min after iTBS.

The second phase of the study consisted of iTBS (active or sham) of the left prefrontal cortical region with individually chosen stimulation magnitude.

At the end of each phase of the study, the subjects' discomfort and wakefulness were measured. The discomfort was subjectively graded by the subject on a numerical discomfort scale with scores from 0 (absence of pain) to 10 (intense pain), Wakefulness was also rated on a 10-point numerical rating scale (0 very awake, 10 very sleepy, very tired).

Experiments were carried out during the daytime (09:00 to 16:00).

3.3. EEG Recording

EEG recording was carried out with participants seated in an EEG chair, wearing a 126-electrode R Net MR cap (Brain Products GmbH, Germany), following the 10-10 system (Styles et al., 2021). The R Net MR cap consists of electrolyte (KCl) soaked sponges and passive Ag/AgCl electrodes covered in a hard and flexible silicone casing. CPz is the reference electrode, and FPz is the ground. In order to capture and then remove eye movement artefacts on the

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electroencephalogram, 2 electrodes function as electrooculographic (EOG) electrodes (Ghosh, 2025).

On electrode mounting,

$contact\ resistance \leq 20\ kOhm$

$sampling\ rate = 5\ kHz/ch$

$input\ noise \leq 1\ \mu Vpp,$

$suppression\ in\ phase\ (CMR) \geq 110\ dB$

was realised.

3.4. TMS Session

Transcranial magnetic stimulation (TMS) was carried out with the subject sitting in a treatment chair (MagVenture, Denmark), with a vacuum cushion used to fix the head (Li et al., 2024). Before iTBS, the resting motor threshold was established with a C B60 coil on a MagPro X100 stimulator and MagOption (MagVenture, Denmark) and single biphasic pulses. The site for the "hotspot" location was the region of the potential cortical activation area corresponding to the APB (abductor pollicis brevis) muscle. The location with the largest amplitude of the evoked motor response of the relaxed right hand's APB was designated as the "hotspot." A 4x4 grid with 5x5 mm point to point spacing was created over the "hotspot" within the motor area with TMS Navigator SW Version 3.3 software (LOCALITE). Subsequently, three single pulses were delivered to each point on the grid with a navigation system. Resting motor threshold was defined in the site of maximum amplitude of the evoked motor response, i.e., the stimulus magnitude necessary to invoke five of ten consecutive motor evoked potentials with a peak value ≥ 50 µV within the relaxed APB of the right hand. Surface electromyography (EMG) recordings were carried out with self adhesive surface bipolar electrodes. Theta burst stimulation was delivered with an intermittent protocol (iTBS) to the left DLPFC at an amplitude of 75% of the resting motor threshold. The iTBS mode was set on the stimulator based on the Huang et al. (2005) with a 2 second burst of pulses repeated every 10 seconds for a total duration of 180 seconds. During the 2 second series, trains of 3 biphasic stimulation pulses of 50 Hz frequency were applied, and they were reapplied 10 times during the series with a frequency of 5 Hz. iTBS lasted for 3 minutes with 600 pulses. A/P CoolB65 (Cool B65 Active/Placebo MagVenture) and MagPro X100 stimulator with MagOption (MagVenture, Denmark) were employed in the iTBS session. The A/P CoolB65 coil has two same sides that transmit either sham or active stimulation, which is chosen based on a random code entered into the MagPro X100 stimulator with MagOption by the operator. Localisation of the stimulation point in iTBS mode (left dorsolateral prefrontal cortex) was established by the Fitzgerald et al. (2006) using Montreal Neurological Institute (MNI) coordinates on the TMS Navigator SW Version 3.3 stereotaxic neuro navigation system (Li et al., 2024).

3.5. Working Memory Testing

Working memory testing was performed using the 2-back and 3-back tests.The cognitive tasks were programmed in the PsychoPy software. The visual stimuli of the n-back tests consisted of the Russian alphabet letters (from A to Я), which appeared individually in a pseudorandomised order on a 15-inch monitor screen located 80 cm in front of the participant seated in the EEG chair. Each letter was presented in white on a black display for 500 ms followed by an interstimulus interval of 1500 ms. Each n-back task contained 150 letters, of which were 25% target stimuli. The volunteers were asked to respond by pressing a key when the letter shown was the same as the letter shown 2 positions before (2-back test) or 3 positions before (3-back test). This phase of the research was carried out in a dark room. A block of n-back tests (2-back and 3-back) was administered prior to iTBS, and two test blocks were administered subsequent to stimulation (10 and 30 min following iTBS). EEG was recorded in parallel with working memory testing. A brief training session having 20 stimuli was administered immediately before the initial n-back sessions (Bae & Chen, 2024).

4. Proposed Method

A parallel framework was designed to perform comparative analysis between the active and sham groups, along with time-series analysis of the signals.

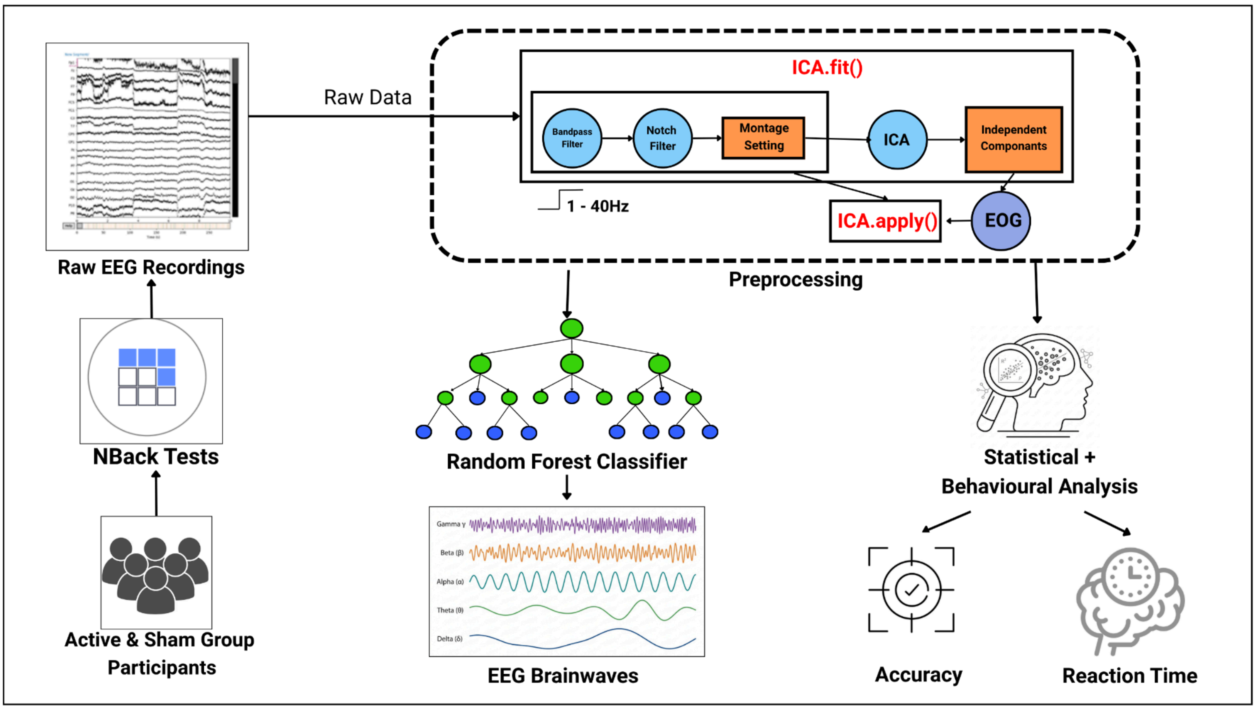

Figure 3. Enabled EEG Processing Framework for Proposed Behavioural and Time series analysis

Figure 3 depicts an overview of the system architecture and methodology. The system is trained and implemented using the MNE Python library and BrainVision with the help of Google Colab.

4.1. Data Acquisition

Following the TMS session, raw EEG data were recorded and stored in BrainVision file formats (.vhdr, .eeg, .vmrk). A total of 3.06 GB of data was acquired. During the acquisition, several sessions were interrupted, which resulted in partial file records. Data from the 14 participants were categorised into four groups: Active 2-back, Active 3-back, Sham 2-back, and Sham 3-back. This data were then loaded using the MNE BrainVision, and partial recordings were merged into single file records to ensure continuity and consistency (Gramfort et al., 2013).

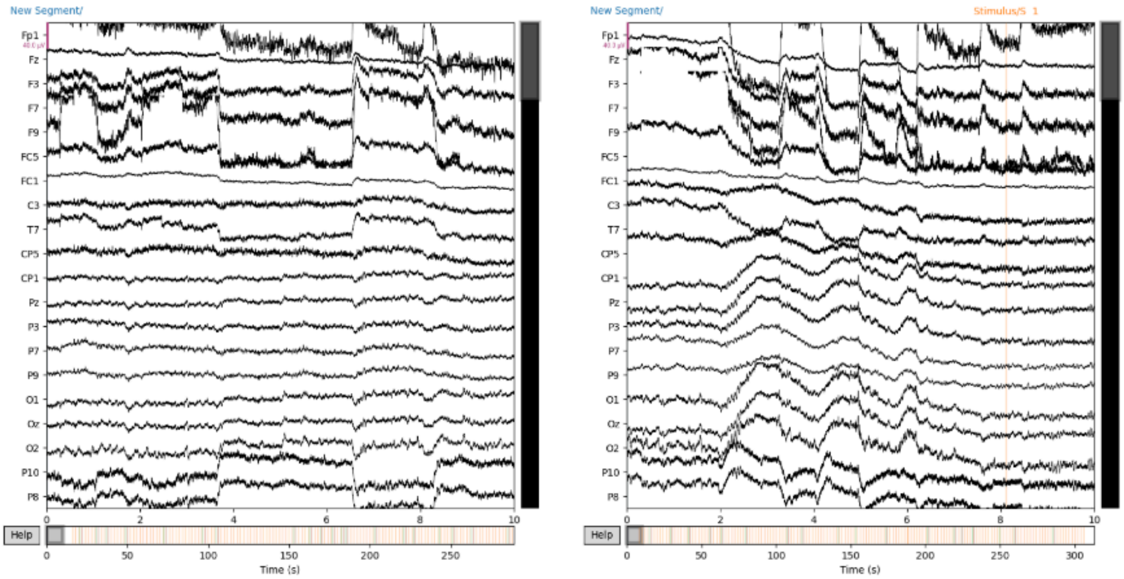

Figure 4. Representative EEG Recordings of Participant 1 and 2 in the Active 2-Back Group

Figure 4 shows sample EEG Recordings from Participants 1 and 2 in the Active Group.

4.2. Preprocessing

4.2.1. Independent Component Analysis (ICA) for Artefact Removal

To remove artefacts such as muscle activity, eye movements, and electrical noise, ICA was used as a required process (Srishyla et al., 2025). This method ensures the separation of the EEG signals into statistically independent components that allow the identification of non-neural sources. Components related to artefacts were eliminated manually and by algorithms to improve the data quality.

a. The Method:

ICA is a blind source separation technique which separates multichannel EEG signals into statistically independent components (Hyvärinen & Oja, 2000). It assumes that observed EEG signals are linear combinations of temporally independent and spatially fixed sources. In this case, if X is the matrix of observed EEG data, we would want to obtain Matrix W such that (1)

$$S = W \cdot X \quad (1)$$

where:

- X is the multichannel EEG signal (channels × time),
- W is the unmixing matrix,
- S contains the independent source components.

ICA takes advantage of the non-Gaussianity and statistical independence of the available sources so as to extract the underlying components. Both brain-related signals and artifacts, e.g., electrooculographic (EOG) and electromyographic (EMG) activity, form these components.

b. Preprocessing Pipeline

The EEG preprocessing in our study was implemented using the MNE-Python library (Shlens, 2014)..

1. Bandpass Filtering: A filter that retains the frequency range of cognitive EEG activity is applied. The filter is set between 1-40 hz to slow down the drifts and high frequency noise.

2. Montage Setting: Electrode positions were standardised by setting a 10–20 montage layout (standard_1020).

3. Notch Filtering: In order to suppress the power line interference, the raw data were passed through a 50Hz notch filter.

4. ICA Decomposition: ICA was performed with 20 components based on a fast iterative algorithm that can separate neural and non-neural components within the raw data.

5. Electrooculography (EOG) to Remove Artefacts: The EOG channels were utilised to detect and reject eye movements.

The remaining (retained) components were used to reconstruct the cleaned EEG signals. (2)

$$X_{cleaned} = A_{retained} \cdot S_{retained} \quad (2)$$

where:

- $X_{cleaned}$ is the artefact-free EEG,
- $A_{retained}$ is the mixing matrix with only the retained components,
- $S_{retained}$ are the corresponding independent sources that were not removed.

6. Epoching: The cleaned EEG data were segmented into epochs aligned with stimulus and response markers (S1, S2 and S3) using a time window ranging from -200 ms to +800 ms.

4.3. Post-Processing and Segmenting Markers

The preprocessed and cleaned data was now grouped into epochs to ensure the successful passing of training data.

S1 (Stimulus Onset): indicates the presentation of a letter during the n-back task.

S2 (Response):indicates the participant's key press in response to a stimulus, reflecting a match with a letter presented two or three positions earlier in the sequence.

S3 (Correctness Evaluation): marks trials with correct responses, enabling differentiation between correct and incorrect trials.

According to these marker values, the data were prepared for behavioural analysis and further classification.

4.4. Data Interpretation

4.4.1. Behavioural Data Analysis

Based on the marker values; in order to assess the performance of the working memory, we take into consideration two major parameters, accuracy and reaction time of response (Dodonova & Dodonov, 2012).

*Accuracy (%):*defined as the percentage of correct responses (S3) relative to the total number of responses (S2), as shown in (3).

$$Accuracy : \frac{Number\ of\ Correct\ Responses\ (S3)}{Total\ number\ of\ Responses\ made\ (S2)}\ x\ 100 \quad (3)$$

This measure indicates the extent to which participants are able to perform the task in terms of giving right responses.

Reaction Time (s): defined as the time (in seconds) taken by a participant to respond following stimulus presentation during the cognitive task, as shown in (4).

$$Reaction\ Time : \frac{\Sigma(Reaction\ Times\ by\ Each\ participant)}{Total\ Reaction\ Times} \quad (4)$$

This measure reflects the speed of participants' responses to stimuli before and after the stimulation session.

4.4.2. Statistical Analysis

To assess the impact of iTBS on working memory performance, a statistical analysis was performed on behavioural metrics response accuracy and reaction time, at various time intervals (prior to stimulation, 10 minutes after stimulation, and 30 minutes after stimulation).

Given that the data comprised repeated measurements from the same participants and the sample size was limited, both parametric and non-parametric statistical methods were utilised.

a. Pairwise Comparisons

To evaluate performance variations over time, pairwise comparisons were conducted between:

- Before vs. 10 minutes post-iTBS
- Before vs. 30 minutes post-iTBS

We used a paired t-test to evaluate differences in means, assuming they were close to normal. The test statistic is given by (5):

$$t = \frac{\bar{d}}{\frac{s_d}{\sqrt{n}}} \quad (5)$$

where:

- $\bar{d}$ is the mean of the paired differences
- s_d is the standard deviation of the differences
- n is the number of participants

b. Non-parametric Analysis

The Wilcoxon Signed-Rank Test was used as a robust alternative to the paired t-test due to the limited sample size and the possibility of deviations from normality. This test evaluates if the median difference between two pairs of observations is not equal to zero.

The Wilcoxon test uses the signed rank sum to rank the absolute differences between paired observations and computing the signed rank sum: (6)

$$W = \sum signed\ ranks\ of\ differences \quad (6)$$

To quantify the magnitude of observed changes, Cohen's d was calculated for paired samples: (7)

$$d = \frac{\bar{d}}{s_d} \quad (7)$$

where:

- $\bar{d}$ is the mean difference between paired observations
- s_d is the standard deviation of the differences

Statistical significance was defined at $p < 0.05$. In addition to significance testing, effect size and direction of change were highlighted to analyse performance trends.

4.4.3. Feature Extraction and Time Series Analysis

Once the preprocessing was done, we extracted meaningful information from the brain signals so as to understand iTBS affects the working memory. This facilitated the analysis of how electrical brain activity is distributed across frequency bands over time in both groups. We used the *Morlet wavelet transform* (Cohen, 2019) to convert raw EEG signals into features that show how power changes in different frequency bands (delta, theta, alpha, beta) during each session. These bands are linked to different brain states which can be seen in Table 1.

The Morlet wavelet is defined as: (8)

$$\varphi(t) = \frac{1}{\sqrt[4]{\pi}} e^{i2\pi f_0 t} e^{\frac{-t^2}{2\sigma^2}} \quad (8)$$

- f_0 is the central frequency of the wavelet
- σ is the standard deviation of the Gaussian window

This enables accurate localisation in both the time and frequency domains, which is essential for capturing oscillatory dynamics during n-back tasks.

For every EEG epoch (termed an epoch), we estimated the average power in these bands over several regions of the brain. These powers were used as features that define the patterns of brain activity at that time (Morales & Bowers, 2022; D'Avanzo et al., 2009).

Let $X(t, f)$ be the wavelet transform based time-frequency representation of EEG signal. Then the average band power for each band B is given by (9):

$$P_B = \frac{1}{T}\sum_{t=1}^{T} |X(t,\ f \in B)|^2 \quad (9)$$

where:

- T is the total number of time points
- $f \in B$ denotes frequencies within the desired band

The resulting features are $P_\delta, P_\theta, P_\alpha, P_\beta$.

These features were then used to train an ensemble learning model, the Random Forest Classifier to distinguish between:

- Active iTBS group vs. Sham group, and
- Pre- and post-stimulation time points (before, 10 min, and 30 min).

The Random Forest algorithm was employed due to its ability to handle high-dimensional, non-linear EEG data.

Random Forest Classifier

Random Forest is an ensemble learning technique which is responsible for combining multiple decision trees to enhance the performance of classification. Here we trained separate Models with respect to each category (Breiman, 2001).

Each tree is trained on a bootstrap sample of the dataset and uses a random subset of features at each split. The final prediction is made by majority voting: (10)

$$\hat{y} = mode(\hat{y}_1, \hat{y}_2, \ldots, \hat{y}_N) \quad (10)$$

Where:

- $\hat{y}_i$ is the prediction of the i^{th} decision tree
- N is the total number of trees in the forest

a. Decision tree Splitting Criterion

At each node, the decision tree selects a feature x_j and threshold t that best splits the data by maximising information gain or minimising Gini impurity (11):

$$G = 1 - \Sigma(p_k^2) \quad (11)$$

where p_k is the probability of class k in a node.

The split is chosen to minimise the weighted sum of impurities in the child nodes. Table 1 summarises EEG frequency bands and their associated cognitive states.

Table 1. EEG Amplitudes and associated memory states

	EEG Amplitudes	Frequency Range	Associated Memory State	Typical Topography
1	Delta (δ)	0.5 to 4 Hz	Deep sleep, slow-wave activity. and large-scale cortical coordination	Frontal (adults)
2	Theta (θ)	4 to 8 Hz	Working memory processing, cognitive control, and memory encoding	Frontocentral / Midline
3	Alpha (α)	8 to 13 Hz	Alert Mode, Relaxed wakefulness and inhibition of task-irrelevant regions	Posterior (occipital)
4	Beta (β)	14 to 26 Hz	Active Thinking, attention, and executive control	Frontal and parietal
5	Gamma (γ)	> 30 Hz	Higher-order processing, feature integration, and perception	Distributed

This classification of different frequency bands is with respect to the time component associated with different memory states (Bandal et al., 2026). Figure 5 shows the experimental workflow of the system.

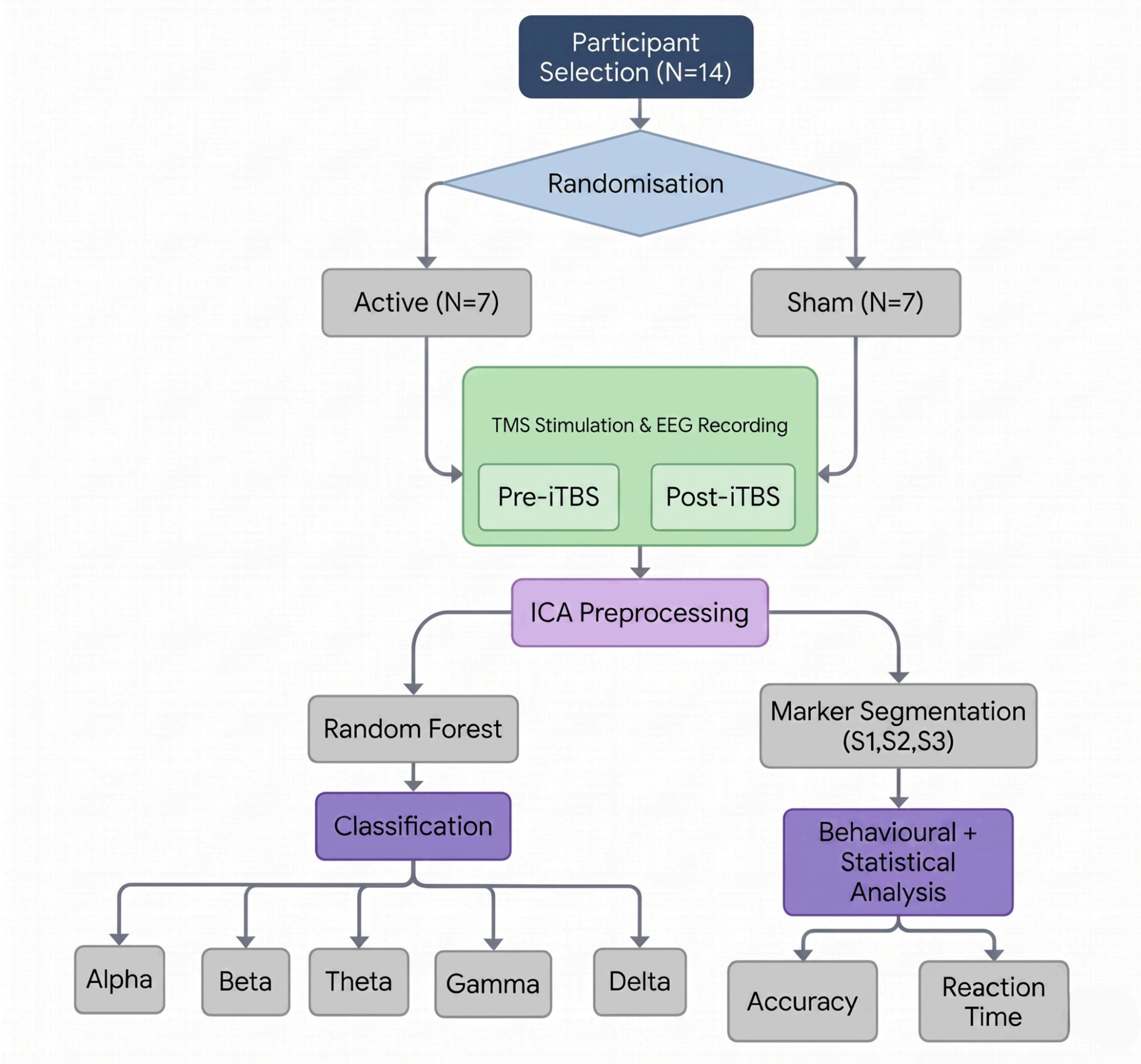

Figure 5. Experimental workflow

5. Results and Discussion

5.1. Behavioural Analysis

In summary, all four groups who underwent the TMS session and completed the n-back tests were analysed using statistical and comparative methods. The results of the study can be given in four divisions.

a. Active 2-back test

The active 2-back iTBS group showed a variable pattern of results. Some participants showed improvements in accuracy and RT, whereas others showed no change or even a decline in performance. This inconsistency shows us that iTBS delivered concurrently with a 2-back task may have a less robust and less consistent effect on working memory performance, which may be due to the relatively lower cognitive load of the 2-back task. Table 2 shows the aggregate accuracy and reaction time of active 2-back test:

Table 2. Aggregate Accuracy and Reaction Time of Active 2-Back Test

	Time Point	**Accuracy (%)**	**Reaction Time(s)**
1	Before iTBS	91.47	8.76
2	After 10 minutes iTBS	83.50	6.10
3	After 30 minutes iTBS	88.27	5.69

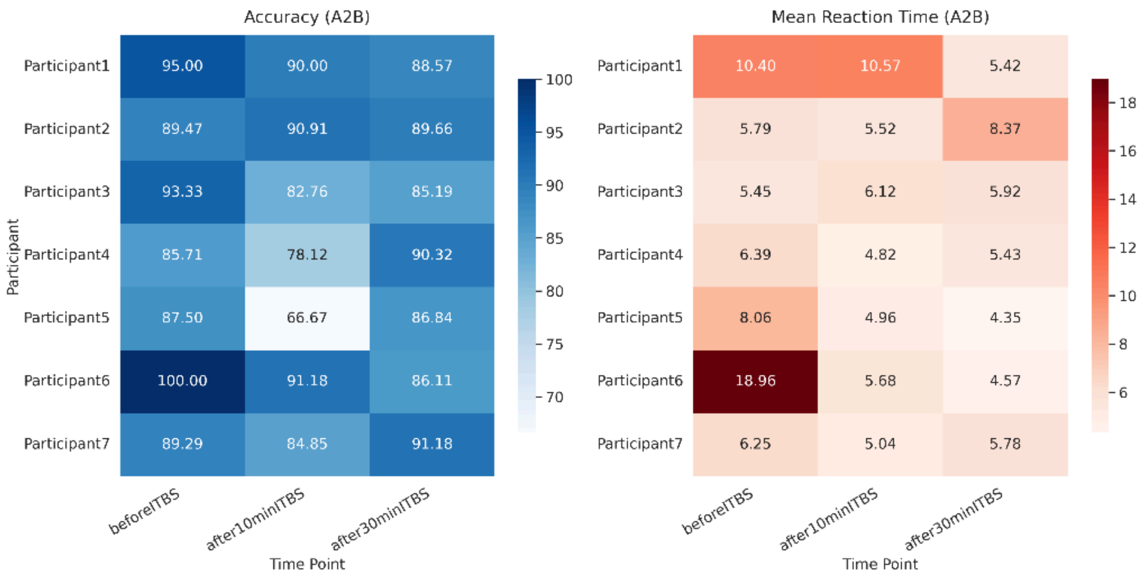

Figure 6. Heatmap representing Accuracy and Reaction Time of Active 2 Back Group

b. Active 3-back test

In contrast to the previous condition, the active 3-back iTBS group showed a clearer trend of improvement in both accuracy and RT across all three time points, before and after iTBS. Participants in this group exhibited less variability in performance as compared to that of the 2-back group. This suggests a more consistent and robust effect in the test. If the demands are higher, a higher cognitive load is observed, thus indicating the effectiveness of iTBS in enhancing working memory performance as seen in Figure 7.

Table 3. Aggregate Accuracy and Reaction Time of Active 3-Back Test

	Time Point	**Accuracy (%)**	**Reaction Time(s)**
1	Before iTBS	82.50	8.53
2	After 10 minutes iTBS	88.44	7.60
3	After 30 minutes iTBS	91.56	7.49

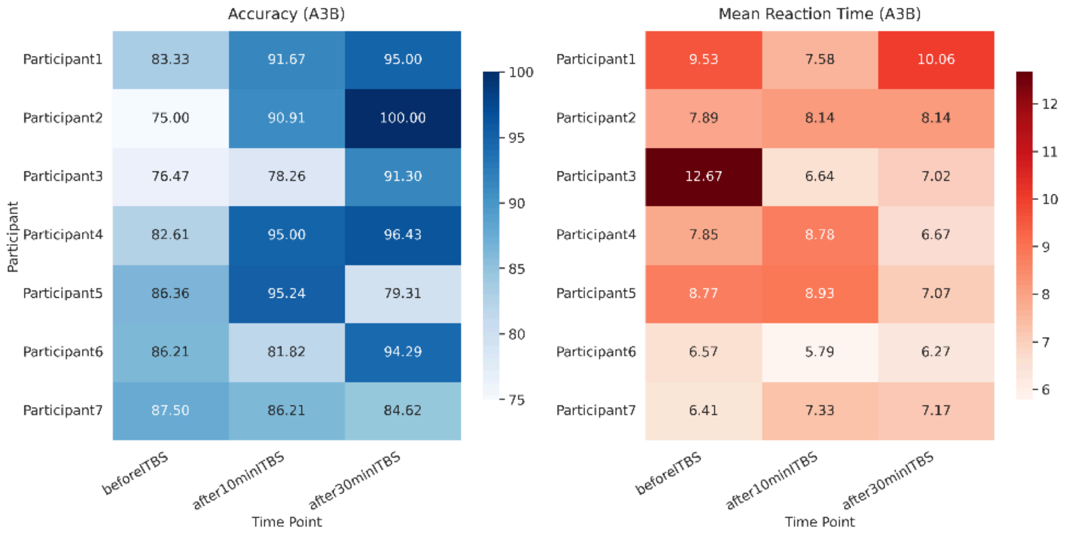

Figure 7. Heatmap representing Accuracy and Reaction Time of Active 3 Back Group

c. Sham 2-back test

In this test, both parameters exhibit minimal variability, reflecting an absence of consistent enhancement in performance. The observed improvement could be a result of a placebo effect or a passage of time.

Table 4. Aggregate Accuracy and Reaction Time of Sham 2 Back Test

	Time Point	**Accuracy (%)**	**Reaction Time(s)**
1	Before iTBS	87.59	5.93
2	After 10 minutes iTBS	88.13	5.82
3	After 30 minutes iTBS	85.50	6.80

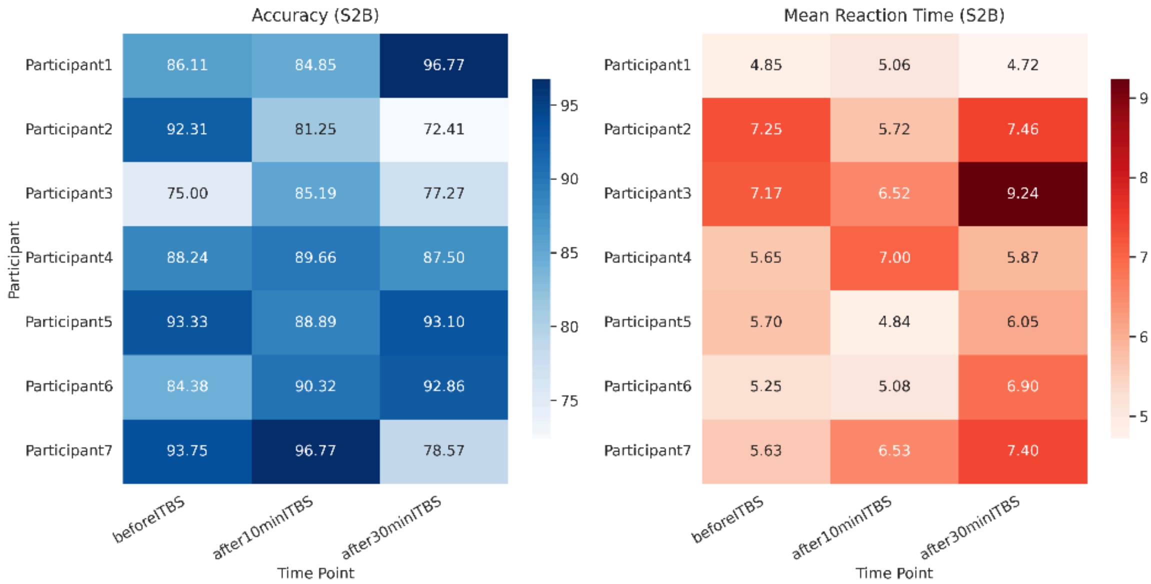

Figure 8. Heatmap Representing Accuracy and Reaction Time of Sham 2-Back Group

d. Sham 3-back Test

Similar to Sham 2-back, no such clear trend is observed, thus indicating that the sham procedure has no clear evidence of effect on accuracy in the 3-back task as observed in Table 5.

Table 5. Aggregate Accuracy and Reaction Time of Sham 3 Back Test

	Time Point	Accuracy (%)	Reaction Time(s)
1	Before iTBS	86.36	10.18
2	After 10 minutes iTBS	86.86	11.48
3	After 30 minutes iTBS	85.83	8.74

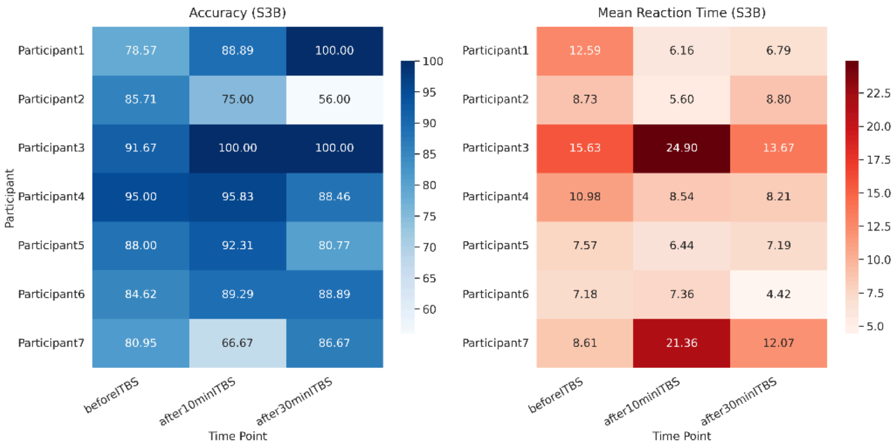

Figure 9. Heatmap representing Accuracy and Reaction Time of Sham 3 Back Group

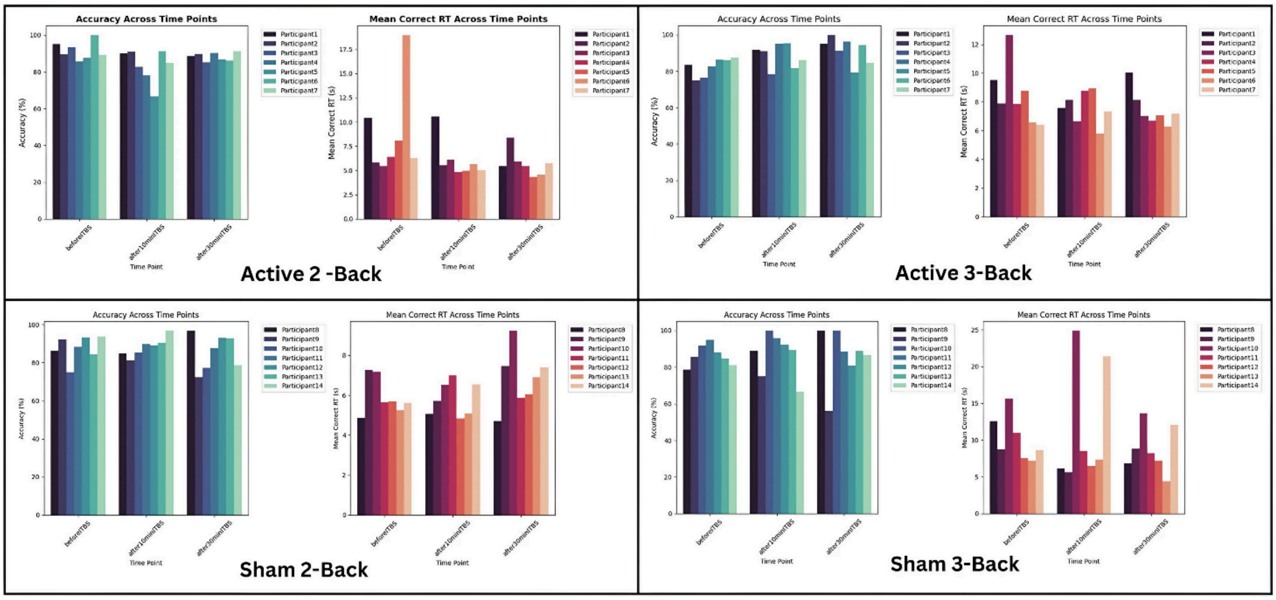

Figure 10. Group-wise comparison of mean accuracy and reaction time for 2-back and 3-back tasks across baseline, 10-minute, and 30-minute intervals

The bar plots in Figure 10 display the changes in mean accuracy (left column in each block) and mean correct reaction time (RT) (right column in each block) across three time points (before iTBS, Post-10 minutes iTBS and Post-30 minutes iTBS) for both active and sham iTBS groups.

5.2. Statistical Analysis

5.2.1. Active iTBS Group

In the A2B condition, there was a significant difference in reaction time between baseline and 10 minutes after stimulation (Wilcoxon: $p = 0.031$), with a large effect size ($d \approx 1.05$). No significant differences were observed at 30 minutes post-stimulation.

In the A3B condition, accuracy showed trends approaching significance at both 10 minutes ($p \approx 0.109$) and 30 minutes ($p \approx 0.078$), with large effect sizes ($d \approx 0.80$).

5.2.2. Sham Group

In the S2B condition, a significant difference in reaction time was noted at 30 minutes post-stimulation (Wilcoxon: $p = 0.031$), accompanied by a substantial effect size ($d \approx 0.96$). There were no significant differences in accuracy.

In the S3B condition, there were no statistically significant differences in either reaction time or accuracy across time points, with minimal effect sizes.

5.3. Time Series Analysis (Intergroup Differences)

5.3.1. Random Forest Classification

For classifying EEG data, a Random Forest classifier was used. To make sure the model could be run again, hyperparameters *n_estimators* were set to 50 and *random_state* was set to 42. Before training the model, all features were standardised with a Standard Scaler. To make the classifier work as quickly as possible, it was built using parallel processing (*n_jobs* = -1).

The Random Forest Classifier was rained using extracted time–frequency features and by excluding the outlier participants as identified in behavioural analysis.

A Stratified 5-Fold Cross-Validation was used to check how well the model worked, making sure that the class distribution stayed the same across all folds. The model was trained on 80% of the data in each fold and tested on the other 20%. We calculated performance metrics like accuracy, precision, recall, and F1-score for each fold and then averaged them to get solid estimates.

The model was successful to classify and differentiate the two inter groups 2-back and 3-back with respect to active and sham participants.

a. Active Group

The Active Group 2v3 Classifier achieved a mean accuracy of 87.33% (SD = ±0.63%) across the five folds and the individual fold accuracies ranged from 86.68% to 88.25% as seen in Table 6.

The performance metrics indicate a high degree of separability between the 2-back and 3 -back conditions.

Table 6. Cross Validation Analysis for Active 2v3 Classifier

Stratified K-Fold	2Back (Class 0) 3Back (Class 1)	Precision (%)	Recall (%)	F1- Score (%)	Active 2V3 Accuracy (%)
1	0	86.0	91.0	88.0	86.97
	1	88.0	82.0	85.0	
2	0	87.0	89.0	88.0	86.82
	1	87.0	84.0	85.0	
3	0	89.0	89.0	89.0	87.91
	1	87.0	86.0	87.0	
4	0	88.0	91.0	89.0	88.25
	1	89.0	85.0	87.0	
5	0	87.0	89.0	88.0	86.68
	1	86.0	84.0	85.0	

b. Sham Group

In contrast, the Sham Group 2v3 Classifier achieved a mean accuracy of 81.43% (SD = ±0.62%) across the respective five folds and the individual fold accuracies ranged from 80.36% to 82.18% as seen in Table 7, with overall slightly lower performance compared to the active group.

Table 7. Cross Validation Analysis for Sham 2v3 Classifier

Stratified K-Fold	2Back (Class 0) 3Back (Class 1)	Precision (%)	Recall (%)	F1- Score (%)	Sham 2V3 Accuracy (%)
1	0	81.0	87.0	84.0	82.18
	1	84.0	76.0	80.0	
2	0	79.0	88.0	83.0	81.25
	1	84.0	73.0	78.0	
3	0	81.0	87.0	84.0	81.86
	1	83.0	76.0	80.0	
4	0	80.0	87.0	83.0	81.48
	1	83.0	75.0	79.0	
5	0	79.0	87.0	83.0	80.36
	1	83.0	73.0	78.0	

EEG time-series features were successfully used to classify 2-back versus 3-back task epochs, resulting in higher accuracy in the active group (87.3%) compared with the sham group (81.4%). The higher 2v3 separability in the active group shows that iTBS suggests the distinctiveness of EEG signals under different working memory loads, which is because of the modulation of neural efficiency or synchrony in task-related circuits within the frontoparietal networks caused by iTBS. This difference points to functional changes associated with iTBS that were not observed in the sham group.

Although the dataset is limited in terms of participants, the use of Stratified K-Fold cross-validation and consistent performance across folds (low standard deviation) shows us that the model generalises well within the dataset and is not significantly affected by overfitting.

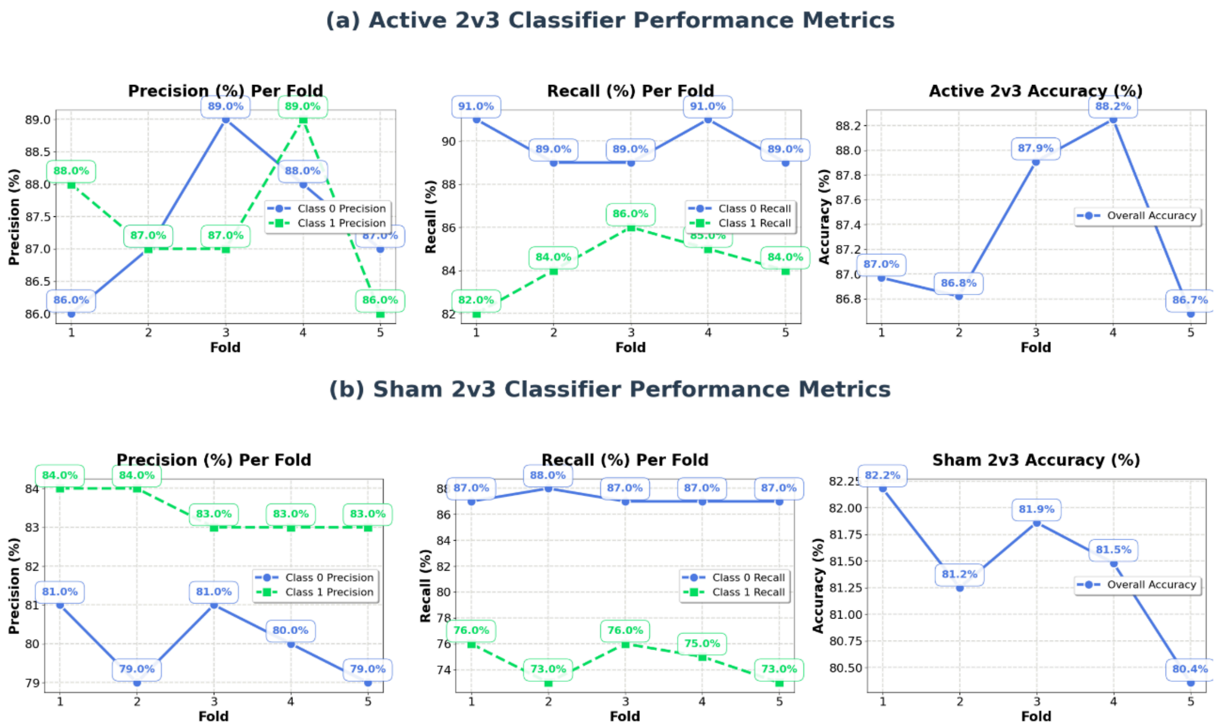

Figure 11. Performance measures of the Classifier across Different Folds

Figure 11 shows the scatter plot of the Active 2v3 and Sham 2v3 classifiers across all the 5 stratified folds.

5.3.2. Band-wise Feature Importance Analysis

The analysis of feature importance by band showed that beta-band features had the largest effect on classification performance in both the Active (35.4%) and Sham (32.1%) groups. The Active group showed a small rise in beta-band contribution compared to the Sham group. The alpha and theta bands showed almost similar contributions in all conditions. The importance of the theta band stayed stable between the Active (22%) and Sham (21%) groups. Additionally, the delta-band contribution was slightly higher in the Sham group (22.3%) compared to the Active group (20.3%).

The marginally elevated contribution of beta-band features in the Active group indicates heightened activation of frontal executive processes subsequent to iTBS stimulation of the dorsolateral prefrontal cortex. Beta oscillations are frequently linked to active cognitive processing and top-down control. This rise in beta-band contribution may be linked to the Active group's better performance in n-back tests, which included faster reaction times and slightly better accuracy after iTBS stimulation.

Theta-band contributions, generally associated with working memory load, exhibited minimal variation between Active and Sham conditions. The relatively higher delta-band contribution in the Sham group indicates greater low-frequency activity in the non-stimulated condition. This stability may suggest that iTBS does not significantly modify fundamental oscillatory mechanisms associated with working memory, but rather exerts subtle effects on higher-order control processes as evidenced by beta activity (Tan et al., 2024).

Table 8. Band-wise Feature Importance (%) for Active and Sham Groups

	Frequency Band	Active Group	Sham Group
1	Beta	35.4%	32.1%
2	Alpha	24.03%	24.21%
3	Theta	22%	21%
4	Delta	20.3%	22.3%

5.4. Limitations

This study employed a controlled cohort and structured experimental design, with two groups (Active and Sham), cognitive loads (2-back and 3-back), and repeated measurements at several time points (baseline, pre-, post-10 minutes, post-30 minutes). While this design facilitates within-subject analysis and captures variability across tasks and time, it also introduces certain constraints, such as limited external validity due to the controlled setting.

The small sample size (N = 14; 7 per group) reduces statistical power and limits the generalisability of the findings. The use of a single session iTBS protocol in the Active group also precludes assessment of long-term or cumulative neuromodulatory effects.

The inclusion of only female participants was intended to reduce gender-related neurophysiological variability. However, this restricts the applicability of results to broader, mixed-gender populations and may overlook important gender differences.

Machine learning analysis was performed on a limited dataset, which likely affects model generalisability. While Stratified K-Fold cross-validation was used to support robustness within the dataset, the risk of overfitting and limited predictive power remains a concern.

Future research should address these limitations by recruiting larger and gender-diverse cohorts, employing multi-session stimulation protocols, and expanding datasets to improve statistical power, generalisability, and validation of observed effects.

6. Conclusion

The research investigated the effect of intermittent theta-burst stimulation (iTBS) on the working memory of healthy young adults focusing on accuracy and response time as key parameters of study during the n-back tests. The results suggest that iTBS had a positive impact on working memory performance under higher cognitive load conditions, as observed by the increase in test accuracy and reduced response times in the active 3-back group. In contrast, the 2-back condition had inconsistent outcomes among the participants. The 2-back tasks are less cognitively demanding than that of the 3-back tasks, resulting in greater variability and reduced sensitivity to stimulation effects. The inconsistent results in the sham group suggest that observed changes may be attributable to placebo effects or the passage of time rather than stimulation.

The statistical analysis supports the trend of observed behavioural patterns, especially in the 3-back condition, which proves a facilitatory impact of iTBS on the working memory performance. Our time series analysis of behavioural and neural measures at all the three time points (baseline, post-10 min, post-30 min) helps us to capture temporal dynamics of iTBS-induced effects exhibiting both short term improvements as well as long term changes in neural and behavioural responses.

Overall, the findings support the use of the application of combined neurostimulation and EEG-based machine learning techniques to study cognitive modulation. With further expansion on the limited dataset, several challenges identified, such as individual differences to responses, outliers within limited data, and the time-specific change within effects, could be addressed with comparative analysis and more comprehensive exploration of the intergroup differences.

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