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Effect of Modulated Anodal Stimulation over the Dorsolateral Prefrontal Cortex on Working Memory: A Preliminary Study



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Abstract

Objectives: Working memory has two components: temporary storage and manipulation of the information necessary for cognitive behavior through the central executive system. This study aimed to investigate the effects of modulated anodal transcranial direct current stimulation (mtDCS) of the dorsolateral prefrontal cortex on working memory.

Materials and Methods: Twelve volunteers without neurological or psychiatric disorders and without drug use participated. Working memory performance was assessed with a visual 3-back task using consonant letters during stimulation. A 20-minute training session was conducted to facilitate learning, and participants who achieved an accuracy rate of 50% or higher proceeded to the main experiment. Four stimulation conditions were applied, each lasting 10 minutes: mtDCS-11 Hz and mtDCS-22 Hz (1.70 mA offset, 0.35 mA peak-to-peak), direct current (DC) (2 mA), and sham. Reaction times and total correct responses were recorded.

Results: Statistical analysis of the left dorsolateral prefrontal cortex (DLPFC) stimulation revealed a significant difference in the mean numbers of correct responses among the mtDCS and transcranial direct current stimulation (tDCS) conditions, but not compared with sham. The mean number of correct responses under mtDCS-11Hz and mtDCS-22Hz was significantly lower than under DC stimulation; however the performance decrement under 11 Hz mtDCS was the most pronounced among the active conditions.

Conclusion: mtDCS-11Hz and mtDCS-22Hz negated the subtle facilitation of tDCS might have provided.

Keywords

Working memory • Transcranial direct current stimulation • Electric stimulation therapy



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INTRODUCTION

Neuroimaging studies have demonstrated that during tasks involving active working memory, there is an increase in blood flow and oxygenation within the prefrontal cortex, particularly in the dorsolateral prefrontal cortex (DLPFC) (1).

The effect of transcranial direct current stimulation (tDCS) on cognitive functions has been demonstrated in studies with both healthy and clinical populations. It is thought that tDCS exerts its effects by polarizing groups of neurons via the electric field generated in the brain, thereby altering their activity thresholds at low current levels. Anodal tDCS increases cortical excitability, whereas cathodal tDCS decreases it (2).

Fregni et al. (3) demonstrated that anodal tDCS enhanced working memory performance in healthy participants. However, subsequent studies have yielded mixed findings. For instance, Teo et al. (4) reported that tDCS did not directly affect working memory accuracy but was associated with changes in reaction times. In contrast, a recent meta-analysis revealed a significant overall effect of anodal tDCS on working memory accuracy compared with sham stimulation across 36 studies using correct responses as the outcome measure (5).

The effects of tDCS on cognitive functions depend on changes in neuronal excitability. While tDCS primarily affects neuronal excitability, transcranial alternating current stimulation (tACS) is thought to enhance rhythmic neural activity by promoting synchronization. Unlike tDCS, tACS can modulate ongoing neural oscillations, making it particularly suitable for influencing the oscillatory brain activity during cognitive tasks (6).

Brain oscillations are considered functional markers of cognition. A prevailing perspective suggests that oscillations arise from the coordinated network activity of synchronized neuronal assemblies (7). During both rest and task engagement, the characteristics of brain oscillations vary substantially across brain regions, depending on the individual's mental state and the task demands. According to one view, alpha synchronization increases during visual tasks but decreases during verbal tasks (8).

Studies have also demonstrated the effect of memory load on alpha activity during the delay period. Most findings indicate that alpha activity increases with higher memory load, particularly during the delay phase. This effect is not specific to particular alpha sub-bands but tends to show right-hemisphere lateralization, reflecting the engagement of right parietal networks in visuospatial processing (9). Under certain conditions, alpha activity consistently increases in amplitude, a phenomenon known as event-related synchronization (ERS). ERS occurs when subjects inhibit or regulate response

execution, likely involving cortical regions engaged in top-down inhibitory control processes.

Recent research has reported that increased frontal alpha and widespread beta power following learning are negatively associated with long-term memory retention. These findings showed that heightened post-learning alpha and beta activity may hinder memory consolidation processes (10). This should not be confused with the temporary alpha increase observed during the retention phase, which refers to post-task resting-state alpha/beta activity. Studies reported that, beginning with early studies on mental arithmetic where subjects showed suppression of alpha activity alongside increased beta activity, numerous studies have since considered alpha to be a task-irrelevant oscillation (11). It was found that alpha oscillations increased at rest after neurofeedback (NFB) sessions were associated with improved performance in motor inhibition tasks (12). This finding may seem to contradict post-learning phase results (10); however, the task domains were different. Alpha oscillations appear to differentiate between the verbal and motor domains.

A magnetoencephalography (MEG) study revealed the event-related desynchronization (ERD) of alpha oscillations during encoding and an increase ERS during memory retention (13). Taken together, these findings demonstrate that alpha and beta oscillations play distinct and context-dependent roles across cognitive domains. Alpha ERS facilitates inhibitory control while potentially hindering memory consolidation when excessively synchronized post-learning.

In this sense, modulated tDCS (mtDCS) provides a useful tool for investigating the mechanisms underlying tDCS-related improvements in working memory. Anodal occipital tDCS increased the parieto-occipital alpha amplitude during the resting state in a previous study (14). Anodal tDCS is thought to facilitate memory performance by increasing cortical excitability and inducing alpha-band (8–13 Hz) activity (15). Extending this evidence, in-phase versus anti-phase alpha-tACS over the parietal cortex revealed that desynchronizing alpha oscillations impair working memory retention, providing causal support for the functional role of parietal alpha activity in memory maintenance (16).

The application of tDCS over the left DLPFC affected event-related potentials (ERPs) at the occipito-parietal region during a working memory task. An increase and enhancement of the oscillatory power in the beta and alpha bands were observed following anodal oscillated tDCS (17).

This study aimed to examine the effects of frequency-modulated transcranial stimulation on working memory performance. We used the n-back paradigm to measure



working memory and applied mtDCS, tDCS, and sham stimulation over the DLPFC region.

MATERIALS AND METHODS

This study was approved on ethical grounds by the Clinical Research Ethics Committee of Istanbul University (approval number: 1422).

Participants

Twelve healthy volunteers (7 women, 5 men; 23–30 years) with no psychiatric or neurological medication use were participated. All were graduate-level students (master's or PhD). The participants provided informed consent. The sham procedure was described, and a single-blind design was used, with only the researcher aware of the condition order.

Working Memory Assessment

The n-back task is widely used to assess working memory. In this study, a visual 3-back version with consonant letters was created using Scilab. Consonants appeared pseudo-randomly for 600 ms, followed by a 2000 ms blank interval. Letters (2.4 cm, black on white) were viewed from 50 cm. Participants pressed *Enter* when a letter matched the one three trials earlier; no response was required otherwise. Training continued until participants reached $\geq 50\%$ accuracy within 20 min; others were excluded. The training phase included five sets of 50 letters (17 targets each; 250 total, 85 targets). Targets were pseudo-randomly placed (interval = 1–4 trials, median ≈ 2.5 –3) with a balanced letter frequency. In the experimental session, the participants completed four 3-back blocks (91 letters, 30 targets) using distinct letters (A, B, C, D, E, G, H, J, K, M). Different letters were used to sustain alertness and prevent stimulus-specific priming referring to previous research (18).

tDCS Device and Modulated Current

The stimulator (TESTi, Teknofil Ltd., Istanbul, Turkiye) featured a two-conductor connection (+/-), manual polarity switching, and an 8-bit PWM A/D converter with a 250 Hz sampling rate. It included digital potentiometric inputs for amplitude, frequency, and offset adjustments, providing a 0–3.5 mA output current and 7 V compliance voltage. Both direct (DC) and modulated (mtDCS) currents were applied. The modulated current consisted of a sinusoidally varying direct current at specific frequencies. Using a headband, the anode was positioned over the left dorsolateral prefrontal cortex corresponding to F3 according to the international 10–20 electroencephalogram (EEG) system, and the cathode over the right mastoid. This is an approach validated by neuronavigation for accurate DLPFC targeting (19). Saline-

soaked sponge electrodes (5×7 cm; 35 cm^2) were used with impedance maintained below 10 k Ω . The stimulation parameters were set as follows: DC = 2 mA, and for mtDCS-11 Hz and mtDCS-22 Hz, offset = 1.70 mA and sine amplitude = 0.35 mA (Figure 1).

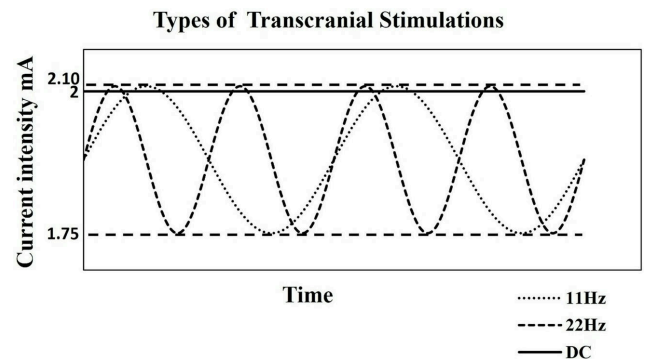


Figure 1. Types of transcranial stimulations

Experimental Protocol

After a brief practice run, the participants performed the 3-back task during stimulation. Each session lasted 10 min, with the task presented in the final 4 min. Four stimulation conditions were applied: tDCS, mtDCS-22 Hz, mtDCS-11 Hz, and sham.

In the sham condition, the current was applied manually for 30 s to reproduce the initial tingling sensation and then switched off. Because the device lacked an automated sham mode, the current was manually stopped after 30 s without ramp-up or ramp-down phases. Sham stimulation employed the same electrode placement and intensity parameters as the active tDCS condition.

To prevent carryover effects, the stimulation order was randomized and counterbalanced, and the sessions were separated by 30 min. This interval was considered sufficient because short stimulations (9–13 min) can induce aftereffects lasting up to 30 min (20). The experimental design is presented in Figure 2.

Statistical Analyses

Analyses were conducted using SPSS 16 (SPSS Inc., Chicago, IL, USA). Given the sample size (<30), non-parametric tests were used. The Friedman test examined within-subject differences across the four stimulation conditions (mtDCS-11 Hz, mtDCS-22 Hz, DC, and sham). Post hoc pairwise comparisons were performed using Wilcoxon signed-rank tests. To control multiple comparisons (three pairs), the Bonferroni correction was applied, setting the adjusted significance level at $p < 0.017$.

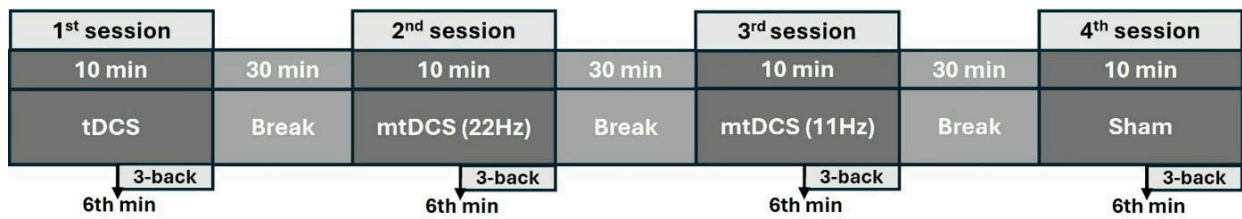


Figure 2. Experimental design (mtDCS: Modulated transcranial direct current stimulation; tDCS: transcranial direct current stimulation; Sham: Placebo stimulation; min: Minutes).

RESULTS

Demographic Variables

Twelve volunteers (7 women, 58%; 5 men, 42%) aged 23–30 years ($M = 25.66$, $SD = 2.1$) participated. Eleven participants (91.7%) were master's students and one (8.3%) was a doctoral student. Ten participants (83.3%) were right-handed and two (16.7%) were left-handed.

Correct Responses

The number of correct responses to 30 target stimuli was analyzed. The mean correct responses were 22.33 ± 4.33 in the sham, 24.00 ± 3.93 in DC, 20.92 ± 4.37 in mtDCS-11 Hz, and 22.33 ± 4.00 in mtDCS-22 Hz conditions. A Friedman test revealed a significant difference among the four conditions, $\chi^2(3)=9.00$, $p=0.029$, with a moderate effect size (Kendall's $W=0.25$). Pairwise comparisons were limited to the active stimulation conditions (DC, mtDCS-11 Hz, mtDCS-22 Hz), as comparisons with the sham condition were non-significant.

Post hoc Wilcoxon signed-rank tests with Bonferroni correction ($\alpha=0.017$, $0.05/3$) showed that DC stimulation produced significantly higher correct responses than both mtDCS-11 Hz ($Z=-2.56$, $p=0.011$) and mtDCS-22 Hz ($Z=-2.52$, $p=0.012$). No significant difference was found between mtDCS-11 Hz and mtDCS-22 Hz ($Z=-1.50$, $p=0.134$). The effect sizes were large for DC vs. mtDCS-11 Hz ($d=-1.04$, 95% CI $[-1.73, -0.31]$) and DC vs. mtDCS-22 Hz ($d=-0.97$, 95% CI $[-1.65, -0.26]$), but non-significant for mtDCS-11 Hz vs. mtDCS-22 Hz ($d=-0.32$, 95% CI $[-0.78, 0.15]$). The comparison of correct responses across conditions is illustrated in Figure 3.

To test if the order effect was significant, repeated measures ANOVA was used. This analysis revealed no significant main effect of order on correct responses ($F(3, 9)=2.76$, $p=0.104$). However, pairwise comparisons (LSD) indicated that the performance at the 4th order ($M=23.83 \pm 1.16$) was significantly higher than that at the 1st ($M=21.08 \pm 1.11$, $p=0.015$) and 3rd ($M=21.75 \pm 1.28$, $p=0.025$) orders.

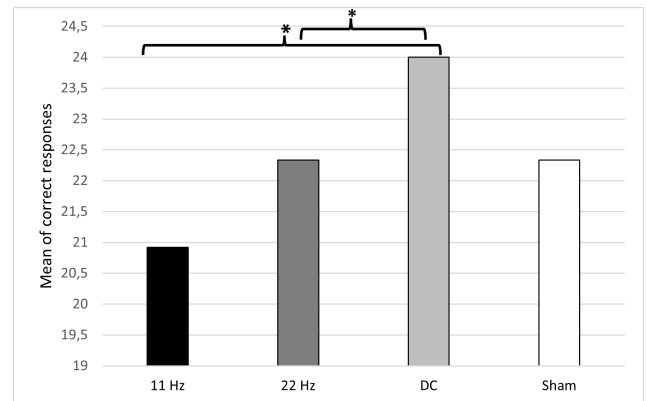


Figure 3. Mean of correct responses across conditions (11 Hz: mtDCS; 22 Hz: mtDCS; DC: Direct current; Sham: Placebo stimulation; *: $p<0.05$)

Errors

Incorrect key presses were counted as errors. A Friedman test compared the mean error counts across the four conditions (mtDCS-11 Hz, mtDCS-22 Hz, Sham, DC). The difference was not statistically significant ($\chi^2(3)=4.486$, $p=0.214$). The number of errors for sham, DC, 22 Hz, and 11 Hz were 3.58 ± 3.05 , 4.66 ± 4.33 , 3.83 ± 2.03 , and 5.08 ± 3.82 , respectively.

Response Time

A Friedman test examined differences in the mean reaction times for correct responses across conditions (mtDCS-11 Hz, mtDCS-22 Hz, Sham, DC). No significant differences were found among conditions. mtDCS-11 Hz 776 ms (± 220), mtDCS-22 Hz 756 ms (± 224), DC 759 ms (± 201) and sham 755 ms (± 215) (Friedman test: $\chi^2=0.328$, $p=0.955$) (Figure 4).

DISCUSSION

This study provides preliminary evidence on the effects of mtDCS on working memory. Although none of the active conditions differed significantly from sham, the overall pattern suggested differences among the active stimulations. The mean accuracy was slightly lower under 11 Hz mtDCS compared with 22 Hz mtDCS and DC stimulation. These results indicate that frequency modulation, regardless of the rate, may reduce the subtle facilitatory effects typically

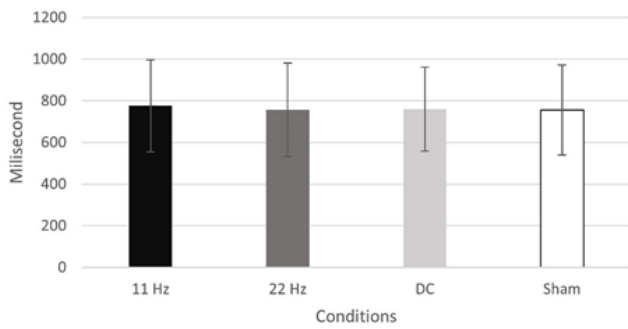


Figure 4. Response times through conditions (Mean \pm SD). DC: direct current; Sham: placebo stimulation.

observed with standard anodal tDCS, rather than impairing performance. While the error rates and reaction times did not differ significantly, a mild performance decline was observed under the 11 Hz mtDCS condition. Recent studies indicate that posterior alpha ERS is widely interpreted as an active inhibitory mechanism that sustains attention by suppressing task-irrelevant inputs (9). Under the highest memory load, attentional capacities are likely exceeded, requiring inhibitory control processes to complete the task. These increased cognitive demands are reflected by enhanced 10–12 Hz alpha desynchronization in the anterior (frontal) regions (21). As the task load increases in the n-back paradigm, power-spectral analyses show decreased alpha power over the frontal channels (22, 23), whereas the posterior parietal cortex exhibits alpha synchronization, a pattern associated with the suppression of task-irrelevant information during selective attention (24). Widespread beta power following learning has also been reported to be negatively associated with long-term memory retention (10).

In our study, both 11 Hz and 22 Hz frequency-modulated tDCS applied over the left DLPFC were associated with reduced 3-back performance compared to standard anodal tDCS, a task requiring high attentional control. We propose that frequency modulation—particularly at 11 Hz—may have altered cortical dynamics, limiting neural flexibility in task-relevant regions. Although the EEG was not recorded, this interpretation aligns with prior electrophysiological studies linking rhythmic stimulation and oscillatory synchronization to inhibitory control processes (10, 21). Therefore, our explanation regarding the oscillatory mechanisms remain theoretical and should be interpreted cautiously.

Interestingly, the 22 Hz mtDCS condition produced a performance pattern similar to sham stimulation, suggesting a weaker or less consistent facilitation relative to DC. Given the within-subject design involving four stimulation orders, potential sequence or fatigue effects cannot be entirely ruled

out, and these may have obscured a clearer 22 Hz mtDCS-specific effect.

No significant differences in the reaction times were observed between the conditions. Consistent with our results, Fregni et al. also found no difference between sham and anodal tDCS over the DLPFC (3). Although that study did not explain, recent meta-analyses have reported that tDCS can enhance both accuracy and reaction time in working-memory tasks (5). Conversely, slower reaction times have been linked to tACS in similar paradigms (25), likely reflecting distinct mechanisms: tDCS alters cortical excitability via membrane potential shifts, whereas tACS modulates neuronal timing through oscillatory entrainment (2, 6).

In this study, the sham condition did not differ significantly from the active stimulations (tDCS and mtDCS), limiting interpretability. This may partly reflect the small sample size, which reduced the statistical power and generalizability. With such a limited sample, some non-significant findings, such as the absence of sham is active differences or reaction-time effects may represent false negatives rather than true null results. Accordingly, these findings should be considered preliminary and warrant replication with larger, adequately powered samples.

A key limitation is that all stimulation sessions were conducted on the same day, which may have introduced practice, fatigue, or learning effects. Although the session order was randomized and a practice run was provided, order-effect analyses revealed higher performance in the fourth session compared with the first and third, suggesting a learning effect. This improvement likely reflects task familiarity despite the control measures. Fregni et al. minimized such effects through pre-experiment practice and found no order differences between the two conditions (3). Pre-experiment practice was also applied in our study; however, the inclusion of four stimulation conditions may have prevented full control of learning. Future studies should conduct sessions on separate days with adequate washout periods to avoid cumulative and residual effects.

Another limitation is the within-subject design, in which all participants received each stimulation condition. Although this approach controls inter-individual variability and is common in tDCS studies (26), it cannot fully exclude carryover or expectancy effects. To minimize these, the stimulation order was counterbalanced, and 30-min washout intervals were applied, consistent with previous single-session protocols (27). However, the 30-minute interval may be insufficient to minimize carryover effects. For example, high-definition tDCS after-effects over the motor cortex persist for 30 min and longer lasting after-effects for more than 2 h

(28). Future studies could adopt a between-subject design to eliminate sequence effects entirely.

Although our discussion primarily focused on alpha mechanisms, recent evidence highlights the central role of theta oscillations in working memory. The frontal midline theta activity increases with higher task demands and is closely linked to performance accuracy (29). Therefore, future studies should examine theta oscillations in addition to the alpha and beta bands.

While this study differentiated the effects of 11 Hz and 22 Hz mtDCS relative to standard tDCS, these differences were not consistently observed compared with sham stimulation. The small sample size limits the generalizability of the results; therefore, this study should be considered a pilot study providing preliminary insights and a basis for future, larger-scale research with adequate statistical power.

In conclusion, this study provides preliminary insights into the effects of frequency modulation on the working-memory performance. Future research should include larger samples, separate-day sessions, and between-group comparisons to minimize training effects and better isolate modulation-specific outcomes. Combining tDCS with neuroimaging methods such as fMRI or EEG may further enhance the reliability and interpretability of the findings.

Public significance statement

Our findings indicate that the impact of brain stimulation on memory performance depends on the effect of frequency. In particular, 11 Hz mtDCS over the left dorsolateral prefrontal cortex appeared to hinder performance compared with standard anodal tDCS, indicating that not all brain stimulation exerts facilitative effects.



Ethics Committee Approval	This study was approved by the Clinical Research Ethics Committee of Istanbul University (approval number: 1422).
Peer Review	Externally peer-reviewed.
Authors Contributions	Conception/Design of Study- Z.K., E.T.E., A.K., S.K.; Data Acquisition Z.K., E.T.E., A.K., S.K.; Data Analysis/ Interpretation: Z.K., E.T.E., A.K., S.K.; Drafting Manuscript- Z.K., E.T.E., A.K., S.K.; Critical Revision of Manuscript- Z.K., E.T.E., A.K., S.K.; Final Approval and Accountability- Z.K., E.T.E., A.K., S.K.
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