Research Article

Impact of Anodal Transcranial Direct Current Stimulation on Listening Effort, Mental Fatigue, Tinnitus Annoyance, Working Memory, and Expression of NMDA Receptor Subunits in Adults with Tinnitus

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Short running title: Impact of Anodal Transcranial Direct Current…

Highlights:

- Transcranial direct current stimulation improved working memory in tinnitus patients
- tDCS reduced listening effort, mental fatigue, and tinnitus annoyance in tinnitus
- tDCS led to NR1/NR2 upregulation in tinnitus patients

ABSTRACT

Background and Aim: Tinnitus is associated with increased listening effort and reduced Working Memory (WM) during speech comprehension. A practical approach to enhance cognitive processes is transcranial Direct Current Stimulation (tDCS). This study aimed to evaluate the effect of tDCS on listening effort, mental fatigue, tinnitus annoyance**,** WM, and expression of N-Methyl-D-Aspartate (NMDA) receptor subunits (NR1 and NR2) in the blood of individuals with tinnitus.

Methods: Thirty-two chronic tinnitus participants (aged 30–60 years) were randomly assigned to intervention and control groups. The intervention group received anodal tDCS over the left Dorsolateral Prefrontal Cortex (DLPFC) with electrode on F3 for 20 minutes at 1.5 mA over 10 sessions, while the control group underwent sham tDCS. Pre- and post-intervention assessments included audiometry, tympanometry, tinnitus matching, a dual-task paradigm for measuring listening effort, the visual analogue scale for measuring mental fatigue, the Tinnitus Functional Index (TFI) for measuring tinnitus annoyance, and the N-Back test for WM assessment. Western blot analysis of blood samples measured NR1 and NR2 protein expression.
Results: Compared to the control group, active tDCS significantly reduced listening effort, mental fatigue, tinnitus annoyance ($p<0.001$) and improved WM ($p<0.001$). After intervention, the intervention group showed a 27% increase in NR1 and a 50% increase in NR2 expression.
Conclusion: The tDCS over DLPFC can reduce listening effort, mental fatigue, and tinnitus annoyance, and enhance WM in adults with chronic tinnitus, which is associated with improved NR1/NR2 protein expression in blood. These findings highlight the potential role of tDCS in tinnitus rehabilitation.
Keywords: Tinnitus; transcranial direct current stimulation; working memory; listening effort; N-methyl-D-aspartate receptor 1,2

Introduction

Tinnitus, defined as the perception of sound without an external source, affects approximately 15–20% of the adult population and can negatively influence attention, memory, and overall cognitive function [1]. Neuroimaging and behavioral studies suggest that tinnitus involves abnormal activity in auditory, limbic, attentional, and memory-related networks, as well as the default mode network [2]. These alterations are believed to contribute to tinnitus perception, persistence, and loudness, and they may impair higher-level cognitive processes such as attention control and Working Memory (WM) [3]. A growing body of evidence indicates that patients with tinnitus often experience elevated listening effort, defined as the attentional and cognitive resources required to extract information from weak or noisy auditory signals. Increased listening effort not only reduces the efficiency of speech comprehension but also leads to mental fatigue and diminished quality of life [4]. WM, which is responsible for the temporary storage and manipulation of information, is also compromised in tinnitus patients, with studies showing slower reaction times and reduced performance under demanding conditions. Together, heightened listening effort and impaired WM reflect the significant cognitive burden associated with tinnitus [5].

Transcranial Direct Current Stimulation (tDCS), a non-invasive brain stimulation technique, has recently been investigated as a potential intervention for tinnitus-related cognitive difficulties. Anodal stimulation of the Dorsolateral Prefrontal Cortex (DLPFC) has been shown to enhance speech perception, attention, and WM in both healthy individuals and clinical populations [6]. These effects are thought to involve modulation of brain N-Methyl-D-Aspartate (NMDA) receptor subunits such as N-Methyl-D-Aspartate Receptor 1 (NR1) and N-Methyl-D-Aspartate Receptor 2 (NR2), which play a central role in synaptic plasticity, long-term potentiation, and the sustained neuronal activity underlying WM [7]. Despite promising preliminary findings, research specifically addressing the influence of tDCS over DLPFC on listening effort, WM, and blood NMDA receptor-related biomarkers in patients with tinnitus remains limited. The present study, therefore, aimed to examine whether tDCS (anodal stimulation) over DLPFC can reduce listening effort, mental fatigue, and tinnitus annoyance, improve WM, and increase the level of blood NR1 and NR2 expression in patients with chronic tinnitus.

Methods

Participants

This is a cross-sectional study. A cohort of 32 adult males and 17 females (age=48.88±9.17 years) with chronic tinnitus was recruited Participants experienced either unilateral or bilateral tinnitus for at least six months, and their condition was validated by an audiologist. The inclusion criteria were no epilepsy, neurological or neuropsychiatric disorders and no metal implants, cardiac pacemakers, or cranial infections. All participants were native Persian speakers. They were randomly assigned to the control (n=14) or intervention (n=18) groups using a random number table. Figure 1 shows the study process.

Assessments

After recording demographic characteristics, participants underwent otoscopic and acoustic reflex examinations, pure-tone audiometry (250 Hz–8 kHz), and Speech Recognition Threshold (SRT) and Speech Recognition Score (SRS) tests. Tinnitus assessments were conducted using a CA86 audiometer (Pejvak Ava Company, Iran) with TDH 39 headphones in an acoustic chamber. Stimuli were presented in 1/3-octave steps with pure tones or narrowband noise. Loudness matching and Minimal Masking Level (MML) were measured following standard procedures [8]. Finally, participants completed the Tinnitus Functional Index (TFI) and Visual Analog Scale (VAS), and their blood samples were collected for Western blot analysis (Figure 1).

The TFI is a questionnaire designed to evaluate tinnitus and measure treatment outcomes. It has 25 items rated on an 11-point Likert scale from 0 to 10. Items 1 and 3 are exceptions, and their answers range from 0 to 100%. For calculation, answers are converted into percentages on a scale of 0–10. The total TFI score is determined by multiplying the average score of all items by 10. At least 19 items must be answered to calculate a total TFI score. The total TFI score ranges from 0 to 100%, classifying the groups into five levels of tinnitus intensity: not a problem (0–17%), small problem (18–31%), moderate problem (32–53%), big problem (54–72%), and very big problem (73–100%). In addition, the items can be grouped into eight subscales: intrusiveness (items 1–3), sense of control (items 4–6), cognition (items 7–9), sleep (items 10–12), auditory (items 13–15), relaxation (items 16–18), quality of life (items 19–22), and emotional distress (items 23–25). The method for calculating the subscale score is similar to the total score calculation method, which involves taking the average of the answered questions within a subscale and multiplying it by 10 [9, 10]. The VAS is a self-report tool. A simple ruler-like scale with the numbers 0 to 10 written on it was used in this study [11]. We measured mental fatigue of participants using this tool, as proposed by Alhabanli et al. [12]. The participants were asked to determine their level of mental fatigue using this tool. The zero value indicates the lowest level of fatigue, and the number 10 indicates the highest level of mental fatigue.

The dual-task paradigm was used to measure speech recognition and WM. It involved the simultaneous execution of two cognitively demanding auditory tasks. The primary task was a speech recognition test with lists of 5×10 two-syllable words recorded in a female voice at 60 dB HL, presented with multitalker noise at 75 dB HL. Noise was delivered 5 seconds before and after word presentation via two loudspeakers at 45° angles, 1 meter from the participant, signal-to-noise ratio=–15 dB HL. The secondary task was an auditory 1-back test for WM, in which participants pressed a button whenever a word was repeated in the primary task list. Participants were trained on ten lexical items to ensure auditory acuity and task familiarity. Baseline measurements of the primary and secondary tasks were obtained separately [13], followed by the simultaneous dual-task test using the AP12 speaker (Pajvak Ava Co., Iran) in the open sound field.

For western blotting testing before and after the brain stimulation, 7 mL of venous blood was collected from each participant. Samples were collected in serum separation tubes containing anticoagulant and gently mixed to prevent clotting. Blood was then diluted 1:1 with phosphate-buffered saline**,** layered over Ficoll-Paque, and centrifuged at 1000 rpm for 20–25 minutes to isolate the mononuclear layer. The upper Ficoll-Paque layer was carefully extracted using a plastic pipette, washed three times with serum, centrifuged, and stored at –70°C until analysis [14]. Western blotting was performed to quantify protein levels. Total protein content of 60 µg per sample was determined by the Bradford method using bovine serum albumin as a reference standard. Samples were separated by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Membranes were blocked with 2% Tris-buffered saline with Tween 20-nonfat dry milk, incubated overnight with specific primary antibodies, and detected using enhanced chemiluminescence. Densitometric analysis was performed using Image software [15].

The intervention and control groups were blinded to the ratio and the method of group allocation. This ensured that both groups remained unaware of their respective groups. The control group had tDCS electrodes placed on their head, but the electric current stopped after just 30 seconds, without the participants' knowledge. In contrast, the intervention group received active tDCS for 20 minutes per session with a constant current intensity of 1.5 mA and a current ramp-up phase of 20 seconds at 10 consecutive sessions (five sessions per week) using the ActivaDose II device (Activa Tek Co., USA). The placement of the electrodes within a pair of sponge pads (area 35 cm^2) effectively facilitated the conduction of current while simultaneously reducing the potential damage incurred by the current passing through normal saline, a solution comprised of 10 grams of salt in 1000 cc of water. Direct current was then transferred through these pads to the head. For all subjects receiving tDCS, the negative electrode (cathode) was positioned over the right DLPFC (F4) and the positive electrode (anode) over the left DLPFC (F3), as determined by the International 10-20 Electroencephalogram System [16].

Data analysis

Statistical analyses were conducted in SPSS v.17, considering a significance level set at 0.05, using the following tests: Pearson's correlation test, repeated measures analysis of variance (ANOVA), paired t-test, and independent t-test. Prior to analysis, assumptions of normality and sphericity were checked and confirmed.

Results

The psychoacoustic characteristics of tinnitus were as follows: tinnitus loudness=32±15.8 dB HL, tinnitus pitch=3925±3027 Hz, and MML=28±18 dB SPL. Table 1 presents the mean scores of the mental fatigue caused by tinnitus (VAS score), tinnitus annoyance (TFI score), listening effort, and WM (1-back test) for patients with tinnitus in the two groups in the pre-test and post-test phases.

The results of repeated measures ANOVA revealed a significant within-subject effect on mental fatigue ($F_{(1,30)}$=92.86, p=0.001, η^2=0.75), indicating that active tDCS caused a reduction in VAS scores over time. A significant between-subject effect was also observed ($F_{(1,30)}$=10.80, p=0.003, η^2=0.26), reflecting overall differences between intervention and control groups. Importantly, the interaction effect of time and group was significant ($F_{(1,30)}$=81.91, p=0.001, η^2=0.73), demonstrating that reductions in mental fatigue were greater in the intervention group than in controls.

The results of repeated measures ANOVA revealed a significant within-subject effect on tinnitus annoyance ($F_{(1,30)}$=193.47, p=0.001, η^2=0.86), indicating that active tDCS caused a reduction in TFI score over time. A significant between-subject effect was also observed ($F_{(1,30)}$=38.08, p=0.001, η^2=0.55), reflecting overall differences between the two groups. Importantly, the interaction effect of time and group was significant ($F_{(1,30)}$=189.86, p=0.001, η^2=0.86), demonstrating that reductions in tinnitus annoyance were greater in the intervention group than in controls.

The results of repeated measures ANOVA revealed a significant within-subject effect on listening effort ($F_{(1,30)}$ 171.73, p=0.001, η^2=0.85), indicating that active tDCS caused a reduction in listening effort over time. A significant between-subject effect was also observed ($F_{(1,30)}$=137.16, p=0.001, η^2=0.82), reflecting overall differences between the two groups. Importantly, the interaction effect of time and group was significant ($F_{(1,30)}$=140.35, p=0.001, η^2=0.82), demonstrating that reductions in listening effort were greater in the intervention group than in controls. The t-test confirmed this effect (t=15/88, p<0.001).

The results of repeated measures ANOVA revealed a significant within-subject effect on WM($F_{(1,30)}$=294.86, p=0.001, η^2=0.90), indicating that active tDCS caused improvements in WM accuracy scores over time. A significant between-subject effect was also observed ($F_{(1,30)}$=57.10, p=0.001, η^2=0.65), reflecting overall differences between the two groups. Importantly, the interaction effect of time and group was significant ($F_{(1,30)}$=227.42, p=0.001, η^2=0.88), demonstrating that WM improvements were greater in the intervention group than in controls the t-test also showed a significant difference (t=13/46, p<0.001).

The effect of tDCS on the levels of NR1 and NR2 in the blood was also assessed. In the intervention group, NR1 blood protein expression increased from 1.1 to 1.4 (27%), and NR2 expression increased from 1.0 to 1.5 (50%) following anodal tDCS. In the control group, no comparable changes were observed (Figure 2). These results describe the observed changes in protein expression after active tDCS.

Discussion

The present study investigated the effects of anodal tDCS over DLPFC on listening effort, WM, mental fatigue, tinnitus annoyance, and expression levels of NR1 and NR2. The findings indicated that active tDCS significantly reduced listening effort and cognitive load during challenging listening conditions, reduced tinnitus annoyance, and improved WM performance. These improvements were associated with increased NR1 and NR2 blood protein levels.

Anodal tDCS likely enhances cognitive performance by modulating glutamatergic signaling and synaptic plasticity, facilitating NMDA receptor activation, and supporting short-term memory and executive functions within the DLPFC [17, 18]. Modulation of temporal cortex activity may further contribute to improved speech perception and reduced listening effort, given its role in auditory processing and linguistic information encoding [19, 20]. These effects align with long-term potentiation-like synaptic potentiation, strengthening neural networks essential for memory, attention, and cognitive control [21]. Despite the overall positive effects observed on WM, some studies have reported null or negative impacts of tDCS on WM and cognitive performance, this discrepancy may be due to differences in stimulation parameters (current intensity, duration, electrode placement), task complexity, or individual factors including age, cognitive baseline, and overall health status [22, 23].

Our findings reinforce the concept that listening effort is a multidimensional construct, dependent on the interaction of cognitive components and brain networks. In tinnitus patients, elevated listening effort demands additional cognitive resources, potentially causing mental fatigue and impairing daily cognitive function [4, 24]. By reducing listening effort and enhancing cognitive performance, prefrontal tDCS demonstrates potential as a non-invasive intervention for cognitive difficulties associated with tinnitus [25, 26].
Further research is warranted to clarify underlying neural and molecular mechanisms, optimize stimulation protocols, and examine long-term effects in diverse populations.
The current research had many limitations or disadvantages, such as non-cooperation of some patients in the blood sampling and tDCS stages. Also, due to the COVID-19 pandemic, it was not possible to follow up with all patients. Also, it should be noted that serum levels of NR1 and NR2 may not directly reflect their expression in the DLPFC; therefore, interpretations regarding cortical mechanisms should be made with caution.

Conclusion

Anodal transcranial Direct Current Stimulation (tDCS) over dorsolateral prefrontal cortex reduces listening effort, mental fatigue, and tinnitus annoyance and improves VM performance in challenging listening conditions. These behavioral benefits are accompanied by increased N-Methyl-D-Aspartate Receptor 1 (NR1) and N-Methyl-D-Aspartate Receptor 1 (NR2) blood protein levels, suggesting potential involvement of glutamatergic mechanisms. These results highlight the potential of prefrontal tDCS as a non-invasive intervention. Further research is recommended to refine stimulation protocols and investigate their long-term cognitive benefits in broader patient populations.

Ethical Considerations

Compliance with ethical guidelines

All procedures were approved by the Ethics Committee under the ethical approval code IR.UT.IRICSS.REC.1399.007. Before the intervention, written informed consent was obtained from all participants.

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Authors' contributions

AI: Study design, acquisition of data, interpretation of the results, statistical analysis, and drafting the manuscript; MN: Study design, interpretation of the results, statistical analysis; HJ: Study design, interpretation of the results; MRZ: Study design, interpretation of the results; SK: Study design, interpretation of the results.

Conflict of interest

The authors declare that they have no conflict of interest. No financial or non-financial incentives were offered.

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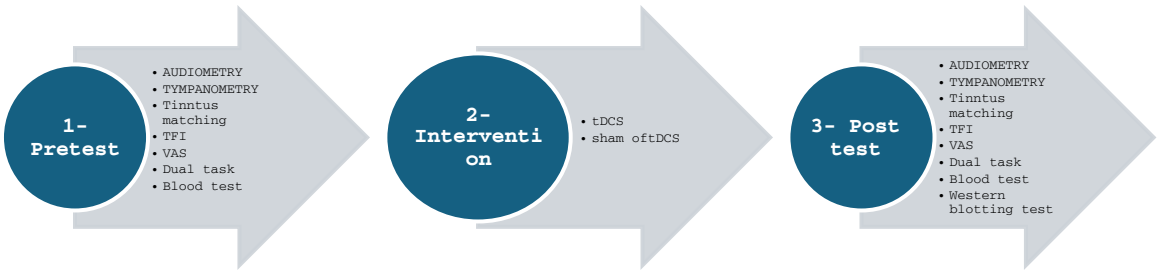
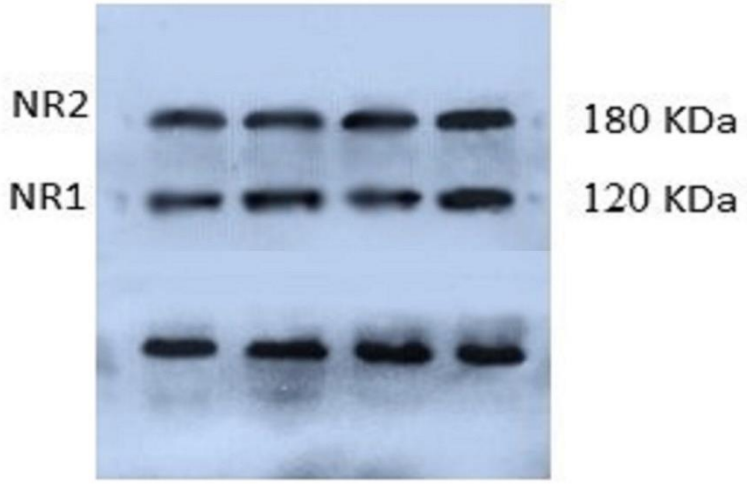


Figure 1. The evaluation processes of studied groups

Table 1. Descriptive indicators of research variables in two groups of patients, separated by pre-test and post-test

Variable	Sessions	Mean±SD	
		Experimental group (n=18)	Control group (n=14)
The degree of mental fatigue caused by tinnitus	**Pre test**	7.66±1.13	7.07±1.32
	Post test	3.11±1.96*	6.92±1.54+
The annoying amount of tinnitus	**Pre test**	0.70±0.13	0.74±0.13
	Post test	0.24±0.12*	0.74±0.13+
Listening effort	**Pre test**	34.72±12.31	37.07±8.80
	Post test	–35.57±13.01	33.57±10.69+
Working memory	**Pre test**	6.61±1.33	6.42±1.39
	Post test	13.22±0.80*	6.85±1.79+

* Shows a significant difference between pre-test and post-test in the intervention group ($p<0.001$)
+ Shows a significant difference between the post-test of the intervention group and the post-test of the control group ($p<0.001$). These revisions are highlighted in the manuscript


NR2
NR1
180 KDa
120 KDa

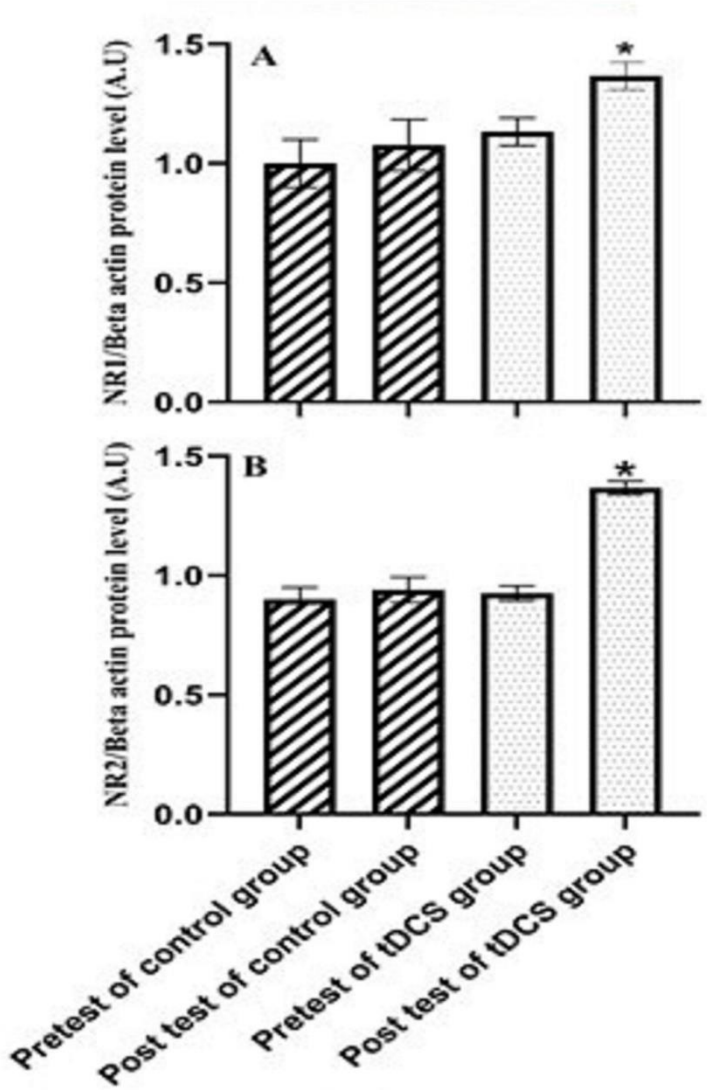

A
B
NR1/Beta actin protein level (A.U)
NR2/Beta actin protein level (A.U)
1.5
1.0
0.5
0.0
*
*
Pretest of control group
Post test of control group
Pretest of tDCS group
Post test of tDCS group

Figure 2. The process of blood protein level change of NR1 (part A) and NR2 (part B) in two groups of transcranial direct current stimulation and control group before and after intervention with transcranial direct current stimulation.