International Journal of Physical Medicine & Rehabilitation

Barassi et al., Int J Phys Med Rehabil 2016, 4:5
DOI: 10.4172/2329-9096.1000364

Research Article OMICS International

Bilateral Transcranial Direct-current Stimulation (tDCS) of Dorsolateral Prefrontal Cortex during Specific Working Memory Tasks

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Received date: August 03, 2016; **Accepted date:** August 04, 2016; **Published date:** August 08, 2016

Abstract

Many authors analyzed the effect of direct current stimulation (tDCS), but the interest in tDCS effect on the human central nervous system for clinical application developed only in the recent years. Aim of current study is to evaluate the impact of tDCS on WM performance and better understand neuromodulatory effects of tDCS, in order to hypothesize applications for the treatment of memory deficits in stroke, Parkinson's and Alzheimer's diseases.

This study was conducted at the Centre of Physical and Rehabilitation Medicine, "Gabriele d'Annunzio" University in Chieti, in accordance with the Helsinki Declaration of 2013. 20 healthy subjects between 20 and 30 years old, 7 females and 13 males were enrolled. The population was divided into two groups; Group A, that underwent transcranial stimulation and Group B, that underwent sham stimulation. During the stimulation patients carry out specific test to asses working memory (Dual n-Back game). We performed 3 sessions in one week, every other day. An improvement in performance was recorded in both groups, statistically significant in the experimental group in 1 Back test. tDCS prefrontal stimulation can modulte working memory performance: further studies are required that would expand the search field and application of tDCS mainly in pathological conditions such as stroke and Parkinson's disease.

Keywords: Working memory; Transcranial direct-current stimulation; Rehabilitation working memory impairment

Introduction

The interest *in vivo* of effects of transcranial direct current stimulation (tDCS) is widely spreading nowadays. Many authors have been study the effect of tDCS [1-4] but the interest in tDCS effects on the human central nervous system for clinical application has developed only in the recent years. tDCS is a non-invasive form of brain stimulation that involves the application of mild electrical currents to the scalp via large electrodes. tDCS alters cortex excitability through modulating, rather than directly stimulating, neuronal activity [5]. A small direct electrical current, typically 1-2 mÅ is induced across the brain from an anode to a cathode electrode [6] However, the effects of tDCS are not limited to modulations in cortical excitability during stimulation, and may outlast the stimulation period by several minutes or even hours [7-10]. These after effects of tDCS are associated with a number of different physiological modifications, including local changes in ionic concentrations (hydrogen, calcium) and levels of cyclic adenosine monophosphate (cAMP), alterations in protein synthesis, and modulation of N-methyl-D-aspartate (NMDA) receptor efficacy [11-17]. Thus increasing or decreasing the firing rate of the neurons depending upon their alignment with the current flow.

Recent studies use non-invasive brain stimulation (NIBS) techniques, such as repetitive transcranial magnetic stimulation (rTMS) [18-20] and tDCS [21,22], to increase dorsolateral prefrontal cortex (DLPFC) activity and, consequently, working memory (WM) performance. Working memory is the capacity to keep in mind some information for a short time to allow monitoring and manipulating information in short-term memory, providing the interface between perception, long-term memory and action that enables goal-directed behavior [23,24].

Some studies, thanks to the development of neuroimaging techniques, indicate that WM is localized across multiple regions in the brain particularly in the dorsolateral region of the prefrontal cortex [25,26].

Barbey et al. [27] showed that anatomical site of general intelligence and working memory is circumscribed, concentrated within the core of white matter fiber tracts that connect DLPFC with the inferior parietal cortex and that terminate in the superior parietal lobe. The observed reliance upon white matter fiber tracts suggests that working memory and other high-level cognitive processes are supported by the interregional communication among many brain areas, emphasizing the central role of the DLPFC and parietal cortex [28]. It has been demonstrated that 10 minutes [29] of 1 mÅ anodal tDCS, applied to the left DLPFC, increase verbal performance of a WM task completed during the last 5 minutes of stimulation, compared with sham stimulation. Previous studies of normal healthy people who had no neurological abnormality were also carried out [15,30]. Forthermore tDCS-induced changes in excitability or tDCS application preceding learning processes have been shown to cause inverse or preventative effects on proceeding manipulations of neuronal excitability and synaptic plasticity [31].

Others reports of tDCS effects on WM suggest that enhancement is possible through anodal stimulation of the left the dorsolateral prefrontal cortex (DLPFC), a finding that makes sense given the tendency for anodal stimulation to enhance neuronal excitability [32,33] and the role of left DLPFC in WM [34].

Aim of current study is to evaluate the impact of tDCS on WM performance and better understand neuromodulatory effects of tDCS, train the use of tDCS device, in order to hypothesize applications for the treatment of memory deficits in stroke, Parkinson's and Alzheimer's diseases.

Materials and Methods

This single-blind control procedure was conducted at the Centre of Physical and Rehabilitation Medicine, "Gabriele d'Annunzio" University in Chieti in accordance with the Helsinki Declaration of 2013. 20 healthy subjects between 20 and 30 years old, 7 females and 13 males were enrolled. The population was divided into two groups; Group A (10 subjects) underwent trans-cranial stimulation and Group B (10 subjects) underwent sham stimulation. These subjects had never performed tDCS before, they did not know their group membership and all participants signed an informed consent.

Exclusion criteria were: a history of epilepsy, metal prosthesis, pace maker implant, cardiac or neurologic disease and any medication therapy or pregnant. Each subject performed 3 sessions (T1-T2-T3) in one week every other day. In Group A, tDCS was transferred using saline-soaked electrode in buckskin (35 cm^2) and it was delivered using a battery-driven, constant-current stimulator HDCstim system (Newronika EN-ITA 00 2010).

During left DLPFC stimulation, the active electrode was over F3 and the reference above the right side- F3-F4 according to the 10–20 international system for electroencephalogram electrode placement [35-44]; the intensity was set at 1.5 mÅ (starter peak time of 5 sec) for a total time of 13 minutes. Intensity of electric current was kept under safety thresholds. Sham stimulation (Group B) was conducted with 120 s of tDCS applied at the onset, the same electrode placement was used (F3-F4 according to the 10–20 international system for electroencephalogram electrode placement), after 120 sec the device was programmed to stop.

During session subjects carry out after a Dual N-Back game test which is commonly used to study working memory.

The Dual N-Back game involves remembering a sequence of spoken letters and a sequence of positions of a square at the same time, and identifying when a letter or position matches the one that appeared earlier [35-39].

For the current study dual N-Back test was administered on a personal computer and structured in to two levels of difficulty: 1-Back first level and 2-Back second level. In 1-Back test 24 visual and auditory stimuli were provided, each one lasted 1, 5 sec with a gap between one stimulus and the next one of 2, 3 sec, for a total time of 91 sec. 1 Dual-Back: an auditory stimulus (a,b,c etc.) and simultaneously one visual-spatial stimulus consists of a blue square that changes position within a 9-panes grid is presented to the subject. The subject is instructed to press a button when there is a match is in position, the square reappears in a position where it was previously, that sound, when repeating a letter already heard earlier. 2-Back test had three kinds of stimuli: a spatial visual, simultaneously visual and an auditory stimulation. Visual spatial stimulus consisted in an image that, with each apparition, changed its position in a space grid, the visual stimulation consisted in the same image that changed shape; the auditory stimulation consisted of an alphabetic letter. Dual 2-Back: it has the same characteristics of the Dual n 1-Back, but with additional difficulty. The subject does not see the square that changes position but a figure; it may introduce a car, a boat, a plane, a star etc., which changes position and shape. So subjects have to remember: the earlier listened letters, position, and figure previously appeared. Stimuli provided were 24; each one lasted for 1.5 sec with latency between one and the other of 2.3 sec, for a total time of 91 sec. (Figure 1)

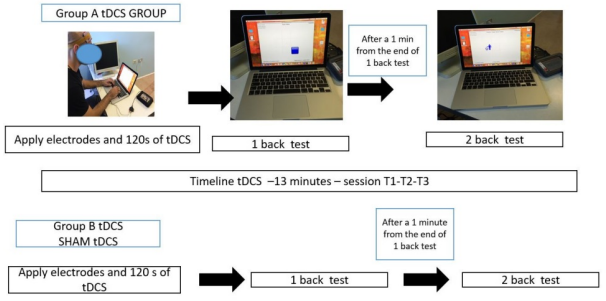

Figure 1: Timeline and sequence of the session both for group A that group B.

During the first session all participants were instructed on the use of n-Back test with 48 total trial stimuli between level 1 Back and level 2 Back tests, these results were not considered for data analysis. After 8 minutes the subjects underwent dual n-Back test and this time data was considered. During the second and the third session subjects did not undergo training but they directly did 1 and 2 Back test. At each session were recorded the obtained scores (number of correct answers) is 1 to 2 for the Back which Back testing.

Statistical analysis

Data are analysed by Wilcoxon-Mann-Whitney test with NCSS9® software and one way Anova for Windows. The minimum level of significance was set to $p<0.05$.

Results

In group A for the 1 Back: the average number of correct answers (indicated as a score) has significant increased from T1 (51.8 ± 21.61) to T2 (63.4 ± 24.38) p=0.04883 (p<0.05.). The increase is confirmed to T3 compared to T1 (71.5 ± 21.9) p=0.045 ($p \leq 0.05$). Instead, B group (sham group) shows decrease in the average score from T1 (55 ± 21.9) to T2 (33.2 ± 22.2) p=0.068 and at T3 (67 ± 27.12) the increase is not significant (Figure 2).

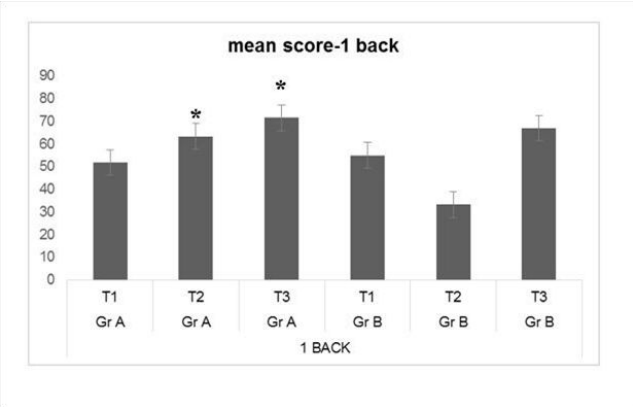

Figure 2: Avarenge score trend in 1 Back at T1-T2-T3 in Group A and Group B.

There was a significant effect of tDCS evaluated with one way ANOVA as shown in Table 1.

ANOVA					
Anova one way	d.f.	SS	MS	F	P value
Between groups	5	3.187,3333	637.4667	2.5093	0.0409
Intra group	54	13.718	254.037		
Total	59	16.905,3333			

Table 1: One way Anova for 1 Back. df degrees of freedom; SS deviance; MS variance; F test F.

Average of 2 Back scores: the group A showed a non-significant increase in T2 to T3 (T1 50.2 ± 13.4, T2 55.7 ± 13.8, T3 p=0.89791). In group B: there was a stabilization of the averages of scores to T2 (T1 46.1 ± 21.39, T2 46.7 ± 15.4) and an increase at T3 (63.4 ± 15.6) that is not significant p=0.013 (Figure 3).

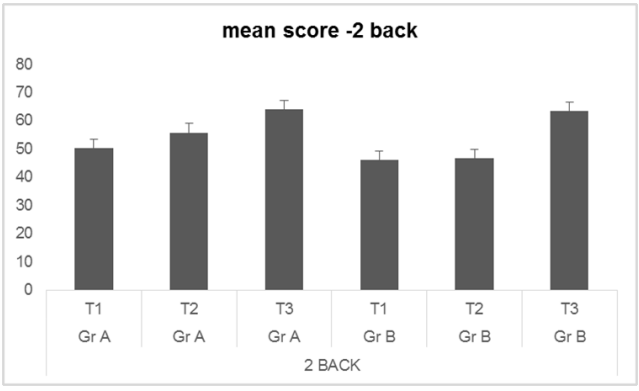

Figure 3: Avarenge score trend in 2 Back at T1-T2-T3 in Group A and Group B.

These data are confirmed by the analysis of variance with one way ANOVA (Table 2).

ANOVA					
Anova one way	SS	df	MS	F	p value
Between groups	937.4	2	468.7	1.105	0.3457
Intra group	11.452,9	27	424.1815		
Totale	12.390,3	29			

Table 2: One way Anova for 2 Back. df degrees of freedom; SS deviance; MS variance; F test F.

The improvements in Group A are shown to be steady and homogeneous, meanwhile the improvement in group B Seem to have a spike in T3 probably two to the training effect.

Discussion

The selective influence of tDCS on different cognitive processes, after the bilateral application has already been amply demonstrated in the primary motor cortex [40], in the prefrontal cortex [41,42], in the anterior temporal lobe, in the fronto-temporal region [43] and posterior parietal cortex [30].

Ohn et al. [10] reported that a 20-or 30-min anodal stimulation boosted short-term memory performance significantly more than 10 min of stimulation did, demonstrating that the effects of tDCS intensify with a longer stimulation period. Lally et al. [44] confirmed these results in a larger cohort, but only when subjects were tested during the stimulation session (online), without a persisting effect 48 h later. Marshall et al. [45] reported increased reaction time in the same task during both anodal and cathodal bilateral intermittent stimulation over the DLPFC. Moreover, Andrews et al. [46] investigated the impact of 1 session of anodal tDCS delivered during a WM task (n-Back task) on performances on a subsequent WM task (digit span forward). Upon completion of the n-Back task, they observed a significant improvement in performance on the digit span forward task. In Javadi and Walsh's [47] and Javadi et al. [48] studies, anodal tDCS that was applied during initial encoding enhanced performance on a subsequent word memory task. This raises the possibility that anodal tDCS used during initial encoding can be combined with anodal tDCS administered during subsequent consolidation in order to bring larger memory improvements to people. Alonzo et al. [49] explored the effects of anodal stimulation (0.0571 mA/cm^2 current density; M1/contralateral orbit montage; 20 min duration) on MEP amplitude over the course of 5 days (Monday–Friday). Although these researchers reported variable baseline levels across the week, the ratio of pre- to post-stimulation group average MEP amplitudes did not significantly change from day-to-day. Using a similar protocol, Gálvez et al. [50] reported similar findings: namely, whereas baseline levels changed throughout the week, the group averaged after-effects of daily stimulation did not significantly vary across 5 days. Jean et al. [51] investigated the effect (tDCS) applied over the prefrontal cortex on the improvement of verbal, visuo-spatial working memory and naming in healthy adults. The subjects were divided into four groups randomly and they underwent sham or anodal tDCS over the left or right prefrontal cortex, for 20 minutes at a direct current of 1 mA. The word/interference significantly improved in the left prefrontal tDCS group compared with that of the sham group while the visuospatial attention

significantly increased in the right prefrontal tDCS group compared with that of the sham group. tDCS can induce verbal working memory improvement and naming facilitation by stimulating the left prefrontal cortex. Donckery at al. [52] in a study on healthy subjects showed that TDSC is able to increase planning performance and cognitive skill learning. This study concluded that excitability decreasing cathodal tDCS mediates its beneficial effect through early noise reduction of neuronal activity. Nelson et al. [53] have shown the efficacy of tDCS in a study in which participants performed a task of air traffic control simulation requiring detect possible collision paths.

Tremblay et al. [54] show that the position of electrodes determine different effects. Previous studies have shown that stimulation with bilateral assembly determines an increase of mental flexibility: [55], language comprehension, generation of untruthful answer [56], attention and language performance [57], automaticity for learned materials [58].

One possible way to reduce uncertainty about effect of TDCS is to monitor the brain impact of tDCS separately and independently of behavioural and cognitive effects. Techniques such as EEG, TMS-EEG, magnetic resonance spectroscopy, functional magnetic resonance imaging and modelling of induced currents have all been shown to be effective in characterizing the physiologic effects of tDCS.

We only performed stimulation and test for only 1 week, if we performed more follow up tests with different periods, we could have detected persistent durations of tDCS effects and the changing patterns of each test more accurately. If you replicated with a larger sample, more numerous sessions and with a follow up, the data may eventually be confirmed, with potential long-term effects that also extend in the hours or days following the experimental session. Moreover we must remind that the subjects in exam are not from a clinical population, thus making the individual differences more relevant, and the number of patients is not huge so we cannot make a statement that can be valid in general. However, more research needs to be conducted to examine if the beneficial effects of tDCS during encoding and reactivation are additive, or if there is a limit on the extent to which tDCS can enhance memory performance.

Concluding our data could be explained according to Teo et al. [59] and Hoy et al. [60] could be that the greater cortical excitability needed to perform the n-back task with its consistent WM load resulted in a cumulative effect above which the cortex is not over excited in the last part of tDCS stimulation session.

Furthermore the idea of the study is also born to learn more about the use of the machine and then start trials in those diseases characterized by an impairment of working memory. WM impairment is a major feature of a number of neurologic and psychiatric disorders, including schizophrenia, Alzheimer's disease, Parkinson's disease, and major depression [61-64]. Some studies have indicated that antipsychotics, particularly the newer atypical antipsychotics, may improve cognitive functioning in schizophrenia [65,66]. Despite this, no effective treatment has yet been established so new and thorough research will be needed in this field.

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