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Brain networks modulation in young and old subjects during transcranial direct current stimulation applied on prefrontal and parietal cortex

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Evidence indicates that transcranial direct current stimulation (tDCS) has the potential to transiently modulate cognitive function, including age-related changes in brain performance. Only a small number of studies have explored the interaction between stimulation sites on the scalp, task performance, and brain network connectivity within the frame of physiological aging. We aimed to evaluate the spread of brain activation in both young and older adults in response to anodal tDCS applied to two different scalp stimulation sites: prefrontal (PFC) and posterior parietal (PPC) cortex. EEG data were recorded during tDCS stimulation and evaluated using the Small World (SW) index as a graph theory metric. Before and after tDCS, participants performed a behavioural task; a performance accuracy index was computed and correlated with the SW index. Results showed that the SW index increased during tDCS of the PPC compared to the PFC at higher EEG frequencies only in young participants. tDCS at the PPC site did not exert significant effects on performance, while tDCS at the PFC site appeared to influence task reaction times in the same direction in both young and older participants. In conclusion, studies using tDCS to modulate functional connectivity and influence behavior can help identify suitable protocols for the aging brain.

Keywords: EEG; tDCS; task; network.

1. Introduction

In the last 30 years, non-invasive brain stimulation (NIBS) methods have become crucial tools for elucidating how motor and cognitive behavior causally depends on specific aspects of neural network activity in the human brain. These methods allow for direct modulation of neural processes in the healthy and pathological brain, enabling researchers to directly study the consequences of experimentally altered neural activity [1].

One of the most common NIBS methods used in both basic and clinical contexts is transcranial electrical stimulation (tES), which involves the application of weak, painless electrical currents to the scalp (current intensities of ~1–3 mA). The most popular variant of tES is transcranial direct current stimulation (tDCS), which utilizes a constant current between electrodes that partially passes through the cortical tissue and affects relatively large cortical areas [2].

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Depending on the stimulation setting, it is assumed that the current depolarizes (anodal montage) or hyperpolarizes (cathodal montage) the resting membrane potentials and thereby alters the cortical excitability of those neurons/networks impacted by the stimulus. Some evidence has demonstrated that anodal tDCS increases neuronal excitability. Thus, theoretically, it could modify (enhance/decrease) behavioral performance if applied to task-facilitating neural networks. For this reason, anodal stimulation has been widely employed [3].

Recently, tDCS has been proposed as an efficacious, safe, and innovative treatment that can be added to conventional therapies for several neurological disorders such as stroke, chronic pain, and certain psychiatric disorders [4, 5].

Interestingly, a growing body of evidence indicates that tDCS has the potential to transiently modulate –either enhancing or impairing– cognitive function in humans, including older adults, depending on the specifics of experimental settings [6, 7].

Physiological brain aging is known to adversely affect cognitive skills in various domains (sensorimotor, coordination, cognition, balance…). tDCS has been employed to modulate such age-related changes in brain performance, however, only a small number of studies have systematically explored the interaction between stimulation sites on the scalp, task performance, and brain network connectivity within the frame of physiological aging [8-11]. It is worth considering that in any network activity-dependent approach, tES-induced effects will be extremely sensitive to the specific state of the network(s); the brain is never in a truly steady state condition. This means that the effects of stimulation will depend on the level of ongoing brain activity and the time-varying excitability of the stimulated brain network(s). For this reason, detailing the level of pre-stimulus network activity is essential to predicting the final outcome of tES [12].

Previous research has demonstrated that stimulation of the Posterior Parietal Cortex (PPC) alters attentional processes [13-15], while stimulation of the Prefrontal Cortex (PFC) affects executive control processes [16-18], suggesting that tDCS to these areas may act on neural mechanisms underlying attentional and working memory processes [8, 11, 19-21].

An important recent breakthrough is the option to collect functional neuroimaging information while applying tDCS [22]. This permits the researcher to target specific cortical areas and allows for the online evaluation of tDCS-induced effects on neural excitability and connectivity [23-25].

Recent functional neuroimaging research has begun to focus on interactions between neural networks, in addition to isolated brain regions. In fact, the analysis of functional connectivity encompasses both task-dependent and -independent synchronous activity in the brain, and thus reflects the organization of the brain into distinct performance-relevant networks.

If the aim is to target specific functional networks rather than specific brain areas, it is necessary to understand how (or even whether) brain networks respond during and after application of tDCS. In fact, the notion that tDCS stimulation only results in increases or decreases in activation of within the stimulated area has been challenged by studies showing that its effects go beyond the stimulated sites [26-28]. This finding makes sense if brain areas that are anatomically remote, are nevertheless functionally connected to the stimulated areas. Moreover, some recent studies have showed that tDCS affects brain connectivity patterns both during task and rest [26, 29-33]. This suggests that tDCS has an impact not only on target areas, but also on the related brain networks, as can be evaluated using graph theory parameters such as small world index [34].

Brain connectivity is affected by the process of physiological aging as reflected in increased local information processing, decreased long-range interactions with other neural populations [35-38], less complex dynamics, and more regular fluctuations in brain and behavior [39, 40]. In fact, changes to the balance of the excitation-inhibition mechanisms that regulate brain network performance through synaptic transmission [41-43] could be the cause age-related motor and cognitive functional decline [44]. Moreover, integration/segregation balance and small-worldness have been extensively investigated in relation to physiological and pathological aging [45-47]. A disruption of integration properties may be linked to long range and associative fiber damage, while a concomitant alteration of integration and segregation may be related to both short and long connection damages including intracortical involvement.

In the current study, the analysis of brain networks modulation has allowed a direct observation of the mechanisms by which the tDCS modulates areas that are functionally activated during the stimulation and the effects of a given brain network in shaping cortical connectivity as computed by graph theory measures, such as small world index [48].

Electroencephalographic recordings (EEG) provide highly time-sensitive and non-invasive measures of the activity of neuronal assemblies. Analysis of EEG oscillations using advanced computerized methods and algorithms that compute coherence, synchronization, connectivity, etc., has helped pinpoint how age-related changes develop in parallel with the loss of task-related skills. The next step is to identify the brain mechanism that underly these marked changes in EEG activity, and

how these mechanisms are differentially impacted by tDCS with aging.

Stimulation of a cortical region induces spreading activation. This activation can be traced through the propagation of EEG signals across the scalp over time or via cortical source reconstruction of this signal, which reflects the levels of synchronous EEG activity in different brain regions. Distinct frequency bands, each closely associated with a specific function, can be identified in oscillatory EEG activity. Therefore, any tES approach can be tuned to interact with a specific EEG frequency band produced by a brain region that is thought to be involved in a given function. It is also possible to use a combined approach, correlating EEG measurements of tDCS-induced modifications and participants' performance on experimental tasks. tDCS and EEG coregistration can track stimulation-induced modulations to cortical activity that correspond to different stages of information processing with high temporal resolution. Moreover, the tDCS-EEG method allows the researcher to assess how stimulation affects neural processing in distal brain regions. In fact, the modifications caused by electrical stimulation spread to connected areas, and simultaneous EEG recordings permit tracking these activations across the entire brain. Recent evidence have demonstrated that PFC stimulation affects the brain organization of specific functional networks at specific frequency bands differently depending on the age of participants [49] and that task-related performance in the elderly improved after anodal tDCS was applied over the left DLPFC [50].

Keeping in mind these concepts, here we aimed to evaluate the brain response to anodal tDCS applied to two different scalp stimulation sites: PFC and PPC in both young and older participants. Moreover, how stimulation at these two sites differentially affect the performance of these two age groups on simple behavioral tasks were analyzed. The novel approach of this study was to analyze the EEG data recorded during the tDCS applied on two different sites and to compute innovative index of graph theory, i.e. the SW index, to obtain measures of brain networks' modulation in relation to aging. We have used the anodal stimulation, as it has already shown able to facilitates behavioral performance respect to the cathodal one [51, 52].

2. Methods

2.1. *Participants*

Eighteen healthy young (average age 24.7 ± 3.2 s.d. years, average education 13.94 ± 2.34 years, 9 females) and fifteen older adult participants (average age 70.1± 5.1 years, average education 9.8 ± 3.78 years, 9 females) gave informed consent and took part in this study, which was approved by the local Ethical Committee.

All participants were right-handed as assessed by the Edinburgh handedness inventory test (Oldfield, 1971) and none of them reported any previous history of neurological or psychiatric disorders or any metal implants. Older participants underwent a neuropsychological evaluation to make sure that cognitive functioning was within the normal parameters for their age and years of schooling. The study was performed in compliance with the ethical guidelines outlined in the 1964 Declaration of Helsinki and received prior approval by The Saint John of God Clinical Research Centre Ethical Committee and the international safety guidelines for NIBS [53, 54]. The experimental procedures were explained to all participants who volunteered to take part in the study. Informed and written consent was obtained from all participants.

2.2. *Experimental design*

The participants underwent two anodal tDCS sessions on separate days at one-week intervals to avoid any tDCS carry-over effects.

At each session, stimulation was delivered during continuous recording of electroencephalographic (EEG) signals.

During the experiment, participants were seated in a comfortable armchair in a sound-proofed room within a Faraday-cage. They were instructed to keep their eyes open, avoid blinking, and to keep their eyes on a stationary point at the center of a computer screen.

The tDCS protocols were performed in accordance with safety guidelines 51. Anodal tDCS was delivered by a battery-driven electrical stimulator (Brain Stim) with the conductive rubber electrode (area of 16 cm2) placed over two different scalp stimulation sites: the left parietal cortex (corresponding to the the P3 position on the EEG cap) and the left prefrontal cortex (corresponding to the F3 position on the EEG cap). The intensity of the current was fixed at 1.5 mA for 13 minutes, with a ramping period of 8 seconds both at the beginning and at the end of the stimulation. The return electrode (50 cm2) was fixed extra-cephalically on the right arm (Figure 1).

2.3. *Behavioral tasks*

Before and after each EEG-tDCS session, the participants executed two different behavioral tasks without EEG recordings: a working memory task in the PFC stimulation session, and a word recognition task in the PPC session. The tasks were selected because they activate brain areas that were also subject to stimulation [50, 55].

In order to avoid that the order of stimulation sites and the tasks could influenced the results, the sequences of sites stimulation and tasks were randomized.

2.4. *Working memory task*

Participants performed a brief practice sequence consisting of 80 trials before the main PFC experiment began. They were allowed to practice the task until they had achieved approximately 60% accuracy or better. Data from participants who did not achieve 60% accuracy after three blocks of practice was discarded. Before and after each stimulation condition, participants performed an n-back (3-back for young adults; 2-back for older adults) working memory (WM) letter task with concurrent EEG recording. We have chosen different versions of the n-back (i.e., 3-back for young and 2-back for older adults) in order to match the level of difficulty for both groups of participants. This is important to exclude subjective level of difficulty as a source of variability contributing to the differences observed between young and older groups in network activation [56, 57]. Stimulus presentation was controlled by Presentation software. Participants viewed a sequence of white letters in 48-point Arial font presented at the center of the screen on a black background. The distance between the participant and the projection screen was approximately 90 cm. A series of 12 consecutive letters were presented (these were the only letters used: A, B, C, D, E, F, G, H, I, J, K, L). The letters during the task appeared in a random order. Each block started with the task instructions. Then participants viewed a continuous stream of 160 random letters; each letter was presented for 500 ms, followed by a delay period of 2000-2500 ms. Participants were asked to respond to each letter as quickly and accurately as possible, indicating with a response button, whether the currently presented letter matched the letter presented n-trials before ("n" was defined in the instructions). There were two blocks of n-back tasks with pseudo-random presentation of 160 trials in each (a total of 320 trials); 25% of trials were correct n-back targets (Figure 1). Importantly, participants reported no using any particular strategy beyond having the key number of letters in mind.

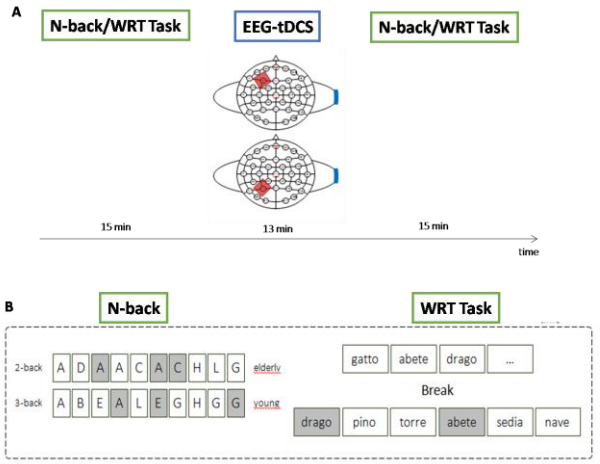

Fig. 1. Panel A, Experimental protocol: subjects underwent task session before and after the EEG-tDCS session of 13 minutes duration; Panel B: schematic view of 2 back and 3 back working memory task (left) and of word recognition task (WRT) (right).

2.5. *Word recognition task*

Before and after parietal tDCS, participants performed a computer-based word recognition task (WRT). First, the participant had to read aloud 20 words presented on a black background in the center of the screen. Each word was presented for 2 sec, followed by a 1500 ms inter-stimulus interval with a central fixation cross. Five minutes later in the recognition phase, these 20 words were randomly mixed with another 30 words the participant had not seen in the first presentation. The participant had to respond to each of these 50 words, indicating YES if the word had been presented previously (true positive response) and NO if it had not (true negative response). Each word list was presented only once in each trial. Thus, the WRT was performed before and after parietal tDCS for the baseline evaluation and for the post-stimulation evaluation. However, the word lists used for the baseline and post-stimulation evaluations differed. We generated a total of six alternative word lists from a standardized database collected from Italian participants, randomizing the use of these wordlists across groups to avoid learning.

The words were selected so as to have the same familiarity, frequency, and number of letters. Correct responses were measured as the number of true positive responses (the number of times participant responded YES to previously presented words) plus true negative responses (the number of times the participant responded NO to words not previously seen) for a maximum of 50 correct answers (Figure 1).

Performances on both tasks were evaluated by considering both reaction times (RT) and accuracy. Accuracy was calculated by considering correct and missed responses to the target stimulus as well as erroneous responses to the non-target stimulus (false alarms). This was done using the d prime index (d'), calculated as follows:
d' = Z(hit rate) - Z(false alarm rate), with hit and false alarm rates transformed into z scores using the standard normalized probability distribution. A higher d' thus indicates better performance. The d' value could be increased by increasing the number of hits for the target stimulus (i.e., accuracy) and/or correct rejections of the non-target stimulus as well as by minimizing missed responses to the target stimulus or erroneous responses to the non-target stimulus (i.e., false alarms) [50, 55].

2.6. *EEG recordings and preprocessing*

Electroencephalographic (EEG) data were recorded during the tDCS protocol, from a standard montage of 31 electrodes (Easycap, GmbH, Brain Products) positioned according to the augmented 10–20 International System (Fp1, Fp2, AF7, AF8, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, CP5, CP1, CP2, CP6, P7, P3, Pz, P4, P8, PO7, PO8, O1, O2), with a sampling rate of 5000 Hz. The ground electrode was placed in mid-frontal (Fpz) position. The right mastoid electrode (M2) served as an online reference for all electrodes, whereas the left mastoid electrode (M1) was used offline to re-reference the scalp recordings to the average of the left and the right mastoid. Eye movements were monitored from two electrooculogram channels. Skin/electrode impedances were below 5 KΩ.
Data were analyzed with Matlab software using scripts from EEGLAB toolbox (Swartz Center for Computational Neurosciences, La Jolla, CA) [58, 59].
In order to identify and extract visible artifacts (i.e., eyes movements, cardiac activity, scalp muscles contractions, line noise), EEG data were down-sampled at 512 Hz and filtered with a Finite Impulse Response (FIR) filter from 0.1 to 47 Hz. Following the procedure already used in EEG data recorded during tDCS, data were segmented in 2-second epochs and analyzed using an Independent Component Analysis (ICA) procedure with a blind source decomposition algorithm that enables the separation of statistically independent sources from multichannel data [60]. Finally, the epochs were further revised by an expert in EEG and removed if contaminated by artifacts.

2.7. *Network analysis*

We used graph theory, which has emerged as an important tool for modeling and quantifying the global properties of brain networks [35, 61-65] to investigate the organizing principles of the recorded brain networks.
In graph theory, networks are mathematically represented as a set of nodes, which can be represented by either brain regions or EEG electrodes. These nodes are connected by edges, which can be either directed or undirected and weighted or unweighted [66].
In the present study, we built undirected weighted networks with the Brain Connectivity Toolbox (BCT, brain-connectivity-toolbox.net). The nodes of the network were the Brodmann Areas (BAs) in the space of EEG cortical sources and the edges were weighted by the Lagged Linear connectivity value between each pair of nodes.
Starting from the EEG scalp electrodes, the cortical sources of the EEG signal were obtained by exact low resolution electromagnetic tomography software (eLORETA) [67]. eLORETA was also used to compute EEG functional connectivity analysis on the cortical 3-D distribution of current density. The connectivity values were obtained using the Lagged Linear Coherence algorithm as a measure of functional physiological connectivity not affected by volume conduction. eLORETA is based on low-resolution brain electromagnetic tomography and was chosen because it is a good method for source reconstruction in the presence of both biological and measurement noise.
In particular, brain connectivity was computed in 84 regions, positioning the center in the available 42 Brodmann Areas (BAs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47) in the left and right hemispheres.
The signal at each of the cortical BAs consisted of the average electric neuronal activities of all voxels belonging to that BA, as computed with eLORETA. the intracortical Lagged Linear Coherence between the eLORETA current density time series for the 84 ROIs, extracted via the "all nearest voxels" method [68], was computed between all possible BA pairs for each of the seven independent EEG frequency bands. The main EEG frequency bands of interest are [69-71] delta (2–4 Hz), theta (4–8 Hz), alpha 1 (8–10.5 Hz), alpha 2 (10.5–13 Hz), beta 1 (13–20 Hz), beta 2 (20–30 Hz) and gamma (30-45 Hz) and they were computed from EEG data recordings. The lagged linear coherence between time series x and y in the frequency band ω was computed using [68] formula,

$$LagR^2_{xyw} = \frac{[ImCov(x,y)]^2}{Var(x) * Var(y) - [ReCov(x,y)]^2}$$

which is based on the cross-spectrum given by the covariance (Cov) and variance (Var) of the signals. It

was developed as a measure of true physiological connectivity not affected by volume conduction and low spatial resolution [68].
The *Lagged Linear connectivity* values computed between all pairs of BAs for each EEG frequency band were the weights assigned to the edges between each pair of nodes in the brain networks.
We computed some of the most common measures used to characterize these networks in neuroscience [72, 73]: the *Clustering coefficient* (C), the C*haracteristic Path Length (L) and the Small World (SW)* index. The *Clustering coefficient* characterizes the tendency of the nearest neighbors of the i-th node to be inter-connected; the C*haracteristic Path Length* is the average minimum number of edges that need to be traversed between two nodes. The ratio between the normalized *C* and the normalized *L* gives the *SW* index [35]. The *SW* index is defined as a measure of the balance between local connectedness and global integration in a network and can be used to characterize brain network organization [72]. A it has been demonstrated that the SW is an index of representation of the key organizational principles of the brain processes of integration and segregation [74, 75] in the current study we aimed to explore the SW index modulation. In order to reach this aim, for each participant, normalization was performed by dividing *C* and *L* respectively by the average values of *C* and *L* computed for all the EEG frequency bands. This kind of normalization means the data is no longer dependent on the number of participants nor on the number of nodes, diminishing any bias due to differences in network structure [76-78].

2.8. *Statistical evaluation*

Statistical evaluations were conducted with analysis of variance (ANOVA) using Statistica Software (StatSoft Inc., statsoft.com).
The first ANOVA was designed to address the statistical evaluation of *SW* index differences between the factors Group (young, older), Site (PPC, PFC), and Frequency Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, and gamma).
In order to evaluate whether the gender of the subjects could have affected the results, two corresponding repeated-measures ANOVAs for gender values were carried out separately for young and older subjects and the factors, Site (PPC, PFC), and Frequency Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, and gamma).

The second ANOVA was applied for the statistical evaluation of *RT and d'* index differences with the factors Group (young, older), Site (PPC, PFC) and Time (before-tDCS, after-tDCS).

The Kolmogorov-Smirnov test was used to test data normality. The hypothesis of Gaussianity could not be rejected so Duncan post-hoc analysis was performed with the significance level fixed at 0.5.

3. Results

The results of the ANOVA showed statistically significant interactions ($F(6, 192)=2.163$, $p=0.048$) of the SW index and the factors Group (young, older), Site (PPC, PFC), and Frequency Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, and gamma) (Figure 2).
In the young, Duncan planned post hoc testing showed higher values of SW in the beta1 ($p=0.030$), beta2 ($p=0.010$) and gamma ($p=0.0001$) bands for the PPC than the PFC stimulation site. No differences were found in the older adults.
No significant gender effect was found for neither young $F(6, 96) = 1.3842$, $p=0.228$ nor older subjects $F(6,78)=1.3846$, $p=0.231$.

The second ANOVA showed no significant interactions of RTs ($F(1, 30)=2.653$, $p=0.1138$) or d' scores ($F(1, 30)=0.3557$, $p=0.5553$) and the factors Group (young, older), Site (PPC, PFC) and Time (before-tDCS, after-tDCS). The repeated measures ANOVA (Group x Site x Time) for the d' index revealed a Group effect $F(1, 30)=4.8521$, $p=0.0354$, as the d' index was higher in older than in young participants. The repeated measures ANOVA (Group x Site x Time) for the RT index revealed a Group effect $F(1, 30)=27.292$, $p=0.00001$, as the RT index was higher in older than in young participants. The analysis also revealed a Site effect $F(1,30)=17.683$, $p=0.0002$, as the PPC stimulation was higher than PFC. In addition, a Group x Site effect $F(1, 30)=9.8725$, $p=0.0037$ was found, as in the PPC stimulation the older were higher than young ($p<0.00006$) and in the Older, the PPC stimulation were higher than the PPF one ($p<0.00015$).

The results of ANOVAs on behavioral data performance under PFC stimulation showed significant interactions of d' and the factors Group ($F(1, 31)=5.3339$, $p=0.0277$) and Time (Current effect: $F(1, 31)=8.6772$, $p=0.0061$). Moreover, significant results were found of RTs and the factor Time (Current effect: $F(1, 31)=4.0158$, $p=0.0539$). In particular, after the tDCS PFC stimulation the d' indices were higher in the older than young participants, and were higher after the stimulation in both groups, while RTs were lower after the stimulation in both groups.

The results of the ANOVAs on the behavioral data after PPC stimulation showed significant interactions of RTs and the factor Group (Current effect: F(1, 31)=24.206, p=0.00003), with lower values for RTs in the young compared to the older participants, regardless of the time of stimulation. No significant results were found for the d' index. Behavioural scores RT and d' are reported in Table 1.

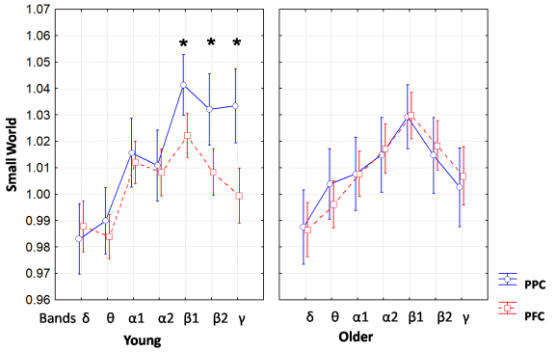

Fig. 2. A significant interaction was found (F(6, 192)=2.163, p=0.048) between the small world (SW) index and the factors Site (PPC, PFC), Group (young, older), and Frequency Band (delta, theta, alpha 1, alpha 2, beta 1, beta 2, and gamma). In the young, Duncan planned post-hoc testing showed higher values for SW in beta1 (p=0.030), beta2 (p=0.010) and gamma (p=0.0001) bands in the PPC (continuous blue line) than the PFC (dashed red line) stimulation site. No differences were found in the older adults.

Table 1 Mean and standard deviation (St Dev) of Reaction Rime (RT) and d' values in pre and post Posterior Parietal Cortex (PPC) and Prefrontal Cortex (PFC) stimulation in young and older subjects.

		PPC				PFC			
		RT		d'		RT		d'	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
Young	Mean	792.12	817.91	2.23	2.09	754.70	728.75	1.39	1.59
	St Dev	98.94	142.35	98.94	142.35	236.68	222.62	1.50	1.65
Older	Mean	1290.87	1237.26	2.29	2.47	850.52	830.11	2.32	2.67
	St Dev	421.11	348.16	0.80	0.77	141.59	161.52	0.76	0.75

4. Discussion

The present study investigated the effects of both PPC and PFC anodal tDCS on brain network architecture in young and older adults. To reach this aim, we recorded EEG activity during the stimulation protocols and evaluated the SW index for EEG data to estimate modulations of brain networks.

Significant differences during PPC and PFC tDCS were found only in young participants, with an increase in the SW index during tDCS of the PPC compared to the PFC, but only at higher EEG frequencies (i.e., beta and gamma bands).

The SW parameter has been selected as it is a graph theory index which captures brain network architecture well. In fact, the small world model provides a summary of both specialized and integrated information processing in the brain, representing the balance between local and global processes[35]. Moreover, integration/segregation balance and small-world have been extensively investigated in relation to physiological and pathological aging [45].

Furthermore, electrophysiological methods –such as the EEG method– measure neuronal activity more directly than other methods (e.g., fMRI), providing high temporal resolution at many frequencies. This provides a more sensitive measure of SW, making it possible to link modulations in SW to cognitive performance and normal aging [45].

In the current study, SW at higher EEG frequency bands involved in sensory motor processes increased during PPC relative to PFC stimulation in the young subjects. These higher frequency bands are involved in the sensory motor processes: going beyond mere motor control, modulation of beta-band functional connectivity reflects the endogenous rhythm of the motor system, and is necessary for its proper functioning. Beta activity should also be considered in the wider context of information gating, which favors maintenance of the status quo of the selected neuronal system. Gamma activity is supposed to facilitate the synchronization and information transfer necessary for cognitive processes, memory, and sensory-motor integration [79-81].

Accordingly, our results support the hypothesis that anodal tDCS to PPC is better able to induce functional changes in the brain, modulating ongoing oscillatory activity with respect to PFC stimulation. Furthermore, increases in the SW index in higher EEG bands is associated with enhanced brain performance, as we have previously demonstrated [82].

The fact that neither PPC nor PFC stimulation elicited brain network modulation in the older adults may be due to alterations in the efficacy of cognitive performance and plasticity mechanisms during healthy aging.

Previous evidence has already demonstrated a relationship between age-related cerebral atrophy and modifications of functional activity and connectivity [83-85]. Other studies have reported that grey matter atrophy in aging may be at least partly responsible for changes in BOLD fMRI activity and may also affect the distribution of current density [85-87] in EEG.

Moreover, some researchers have suggested that brain modifications underlying the decline of cognitive functions (associated with physiological aging) are not only caused by neuronal loss but also by alterations in synaptic connectivity [88-90].

Atrophy and decreased grey matter volume with increased age results in a greater scalp-brain distance that may allow less current to enter the brain [86, 91, 92]. This would imply that age-related structural and

functional deterioration could affect the patterns of current flow in older adults, [91, 93, 94] and may reduce the effects of tDCS. In our study, it is possible that tDCS stimulation at two sites was able to modulate cortical activity in the brain networks of the young, while any effects in older participants did not reach significance in the EEG network connectivity analysis.

Changes in the brain excitation-inhibition balance with advancing age –involving alterations in synaptic transmission [41-43, 95, 96] – have been proposed as one of the underlying mechanisms for age-related motor and cognitive functional decline [44]. Anodal tDCS has differential effects on resting-state inhibition with a relative decrease in younger and a relative increase in older participants [97]. Moreover, stimulation-induced change in event-related modulation of inhibition has only been observed in older participants who typically demonstrate decreased capacity to release inhibition during movement preparation. Dexterous manual performance has, in general, been positively influenced by anodal tDCS.

In accordance with these previous findings, the stimulation effects varied with the nature of the task performed (task-specificity) and the beneficial effect was also target-group specific, occurring more in older than younger participants.

A further study using EEG to compare the neurobiological effects of left dorsolateral prefrontal cortex tDCS in younger and older adults, found that while younger adults showed modulations in both cortical reactivity and task-related activity after tDCS, older ones showed modulations only in task-related activity [6]. Moreover, younger adults appeared to exhibit a larger degree of post-tDCS change in terms of performance speed than older ones.

Another study investigating young and elderly participants, who performed a working memory task before and after receiving anodal, cathodal, and sham tDCS over the left dorsolateral prefrontal cortex, revealed a strong tendency for tDCS to modulate working memory performance. In detail, young, but not elderly participants benefited from additional practice in the absence of real tDCS. The cathodal tDCS had no effect on any group of participants. Importantly, anodal tDCS improved task accuracy in the elderly. These findings suggest that, in elderly participants, improved working memory after anodal tDCS applied over the left DLPFC may be related to the promotion of frontal compensatory mechanisms, which are in turn related to attentional and memory processes [50, 98].

With respect to the behavioral aspects of tDCS, the current study demonstrated that in PFC stimulation the d' index was higher after stimulation in both groups and it increased in older more than in young participants, but while the RTs were faster after stimulation in both groups. PPC stimulation resulted in faster RTs for young compared to older participants, regardless of the time of stimulation. Thus, PPC stimulation did not exert significant effects on performance, while PFC appeared to influence task RTs' in the same direction in both young and older participants. The fact that the index of accuracy (d') was higher for the older than the younger group may be a methodological issue: in the PFC experiment the task paradigm was the same for both age groups but the version performed by younger participants was more difficult. Specifically, before and after each stimulation, the young participants performed a 3-back, while the older adults performed a 2-back working memory letter task. It is possible that the different levels in task complexity could have affected the direction of the results indicating that older participants were more accurate than younger participants, as previously reported by Cespón and colleagues[50]. Further explication may be related to different behavioral performance between young and old participants. For example, Gajewski and collaborators [99] examined performance during lifespan in the n-back task and in other cognitive tests and discovered different cognitive strategies between groups: younger individuals involve mainly executive functions, whereas older subjects' performance is associated primarily with attentional functions, suggesting that broad processing resources are involved in compensating for executive deficits in older age.

Our results on stimulation timing are partially in line with previous findings reported by Fertonani and colleagues regarding the timing of tDCS application during a naming task performed by young and older adults. These researchers compared anodal stimulation with two timings (i.e., online vs. offline) in the same subjects who performed the same tasks in young and older subjects Results showed that online application of tDCS induced facilitation in both groups, but offline application did not induce facilitation in the older [89]. In young adults, this online vs. offline difference in facilitation was inconsistent. The authors concluded that the capacity of aged neural circuits to increase efficiency is maximized if tDCS was applied during the execution of the task, confirming the importance of timing choice when applying tDCS in older participants. Our behavioural results were probably similarly affected by the fact that the task was performed offline and not during stimulation.

As limitations of the current study, it can be mentioned that the scalp EEG and the EEG sources' reconstruction is a partially reflection of the cortical local field

potential (LFP). In fact, the spiking activity is not the source of the LFPs but the dendritic activity and not only dendrites from neurons contribute to the spatio-temporal integration of potential, the glia participle also as source. Moreover, what is being seen in LFPs, and partially reflected in scalp EEG, is the synchronization of these events, while unsynchronized dendritic activity across neuronal population cannot be seen in EEG. Moreover, as the atrophy and decreased grey matter volume could have influenced the spreading of current in the brain, this is also true for the EEG recordings. Future studies could take into account these important issues, for example including the subjects' structural brain imaging, as the magnetic resonance imaging (MRI), which is able to measure the brain atrophy. Also, as the brain connectivity is affected by the process of physiological aging as reflected in increased local information processing, decreased long-range interactions with other neural populations, future studies could be addressed to explore measures of local and global connectivity separately, as for example the Characteristic Path Length and the Clustering coefficient.

Further, it was not possible to compute a within-subjects statistical analysis because not all the subjects underwent both the stimulations. Finally, the low number of participants is justified by the complexity of the experiment. Further studies could be conducted in order to increase the number of subjects and the statistical power.

5. Conclusions

The aim of the current study has been to demonstrate how the brain networks are modulated during the application of tDCS on different sites (PPC and PFC) through the EEG recorded during the stimulation. The results have shown that only in the youngers the PPC and PFC have different effects on SW modulation, while in the older subjects the SW have not showed any differences between PPC and PFC stimulation. Accordingly, the SW modulations due to the two stimulation sites have been influenced by the different reaction of the brain to the stimulation that is affected by the ageing processes.
Concluding, studies using tDCS to simultaneously modulate functional connectivity and influence behavior can help identify suitable protocols for the aging brain, and may also be crucial in future clinical and rehabilitation protocols.

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