Cognitive enhancement induced by anodal-tDCS drives circuit-specific cortical plasticity

Running title: cortical excitability and brain stimulation in cognition

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Abstract

Increasing evidence shows that anodal-tDCS enhances cognitive performance in healthy and clinical population. Such facilitation is supposed to be linked to plastic changes at relevant cortical sites. However, direct electrophysiological evidence for this causal relationship is still missing. Here we show that cognitive enhancement occurring in healthy human subjects during anodal-tDCS is affected by ongoing brain activity, increasing cortical excitability of task-related brain networks only, as directly measured by Transcranial Magnetic Stimulation combined with electroencephalography (TMS-EEG). Specifically, TMS-EEG recordings were performed before and after anodal-tDCS coupled with a verbal fluency task. Modulation of cortical excitability occurred only at left Brodmann areas 6, 44 and 45, a key network for language production, and was positively correlated to the degree of cognitive enhancement. Our results suggest that anodal-tDCS specifically affects task-related functional networks active while delivering stimulation, and this boost of specific cortical circuits is correlated to the observed cognitive enhancement.

Keywords: anodal tDCS, Cortical excitability, TMS-EEG, Verbal Fluency.

Transcranial direct current stimulation (tDCS) is a brain stimulation technique, which is able to non-invasively increase (anodal-tDCS) or decrease (cathodal-tDCS) the excitability of the human cerebral cortex (Nitsche and Paulus, 2000). In the last decade, several studies successfully applied tDCS to modulate a wide range of motor, perceptive and cognitive processes, as well as to treat neurological and psychiatric diseases (Nitsche and Paulus, 2011; Jacobson et al., 2012). Although human and animal works have provided several hints on the biological mechanisms driving anodal tDCS offline effects (Fritsch et al., 2010; **Bikson et al., 2004;** Liebetanz et al., 2002; Bindman et al., 1964), and despite its increased popularity, the neural underpinnings of tDCS-induced effects on task performance still remain elusive. Motor evoked potential studies showed that the neurophysiological effects induced on-line by anodal tDCS rely on the sub-threshold depolarization of the primary motor cortex neuronal membrane, mediated by NA+ voltage dependent ion channels activation (Liebetanz et al., 2002; Nitsche et al., 2003). Similarly, **previous** in vitro studies reported that this modulation of neurons excitability increased spontaneous cortical activity (Bindman et al., 1964). Off-line effects, instead, have been shown to be mediated by glutamate NMDA receptors activation, which results in a greater CA++ postsynaptic concentration, which triggers cortical plasticity (Nitsche and Paulus, 2000; 2011). However, in humans, outside the motor domain, no evidence is reported which could directly link tDCS plastic modulation of cortical excitability to its effects on cognition. Moreover, tDCS low spatial resolution seems to be in contrast with focal effects on cognitive performance (Nitsche and Paulus, 2011; Jacobson et al., 2012; Monti et al., 2012) and electrophysiological measures (Wirth et al., 2011; Keeser et al., 2011) described in many studies. Current modeling research, indeed, found that electrical currents delivered through tDCS spread well far away from the stimulation site and that micro-anatomical differences may vary its path (Bikson et al., 2012; Datta et al., 2010; Opitz et al., 2015). A**n activity-selectivity hypothesis for tDCS enhancement of human behavior has been repeatedly proposed but never directly tested (for a perspective review see Bikson and Rahman, 2013).** The present study examines **the specificity of** anodal tDCS

effects on brain connectivity and cortical excitability during a task execution, by means of TMS-EEG **recordings**.

As recently demonstrated, indeed, TMS-EEG recordings are able to highlight, by analyzing TMS-evoked potentials (TEPs), plastic changes in cortical excitability and connectivity during and after anodal tDCS, applied at resting state over the motor and parietal cortices (Pellicciari et al., 2013; Romero Lauro et al., 2014; 2016). However, it is unknown whether and how specific task-related spontaneous cortical activity interacts with the electrical stimulation and how this is linked with the behavioral modulations found in the literature. Animal models showed that anodal tDCS **is able to modify synaptic efficiency, by inducing repetitive firing in target neurons (Bikson et al., 2004) causing an increase in extracellular ionic activity and possibly protein expression. Accordingly, further in vitro studies showed that** offline effects are the result of an interaction between the spontaneous ongoing cortical activity and electrical stimulation, with the latter modulating plasticity and excitability only in those neurons, which are more active during the stimulation protocol (Fritsch et al., 2010). If this is true also for humans, we should expect an increased response in terms of cortical excitability and connectivity only after testing task-related areas. Otherwise, if anodal tDCS alone is enough to modulate brain functioning, electrical stimulation should a-specifically increase cortical excitability of both task related and unrelated areas. **In order to test this hypothesis, w**e chose a verbal fluency task as our experimental tDCS – behavioral protocol since previous evidence (Cattaneo et al., 2011; Meinzer et al., 2012) suggested that anodal tDCS over the left inferior frontal gyrus (LIFG) led to a better performance in verbal fluency compared to a placebo condition. TMS-EEG recordings were performed measuring cortical response to magnetic perturbation of areas **included (left BA 6), or not (left BA 7), in the functional network underlying verbal fluency; in this way we aimed to assess whether the electrical stimulation protocol can induce site specific plastic changes, or whether the neurophysiological modulation affected broader cortical regions not related to task execution.**

Materials and methods

Participants

Eighteen neurologically unimpaired individuals (**8 Males, mean age 27.7 years, SD 5.3, range 21-38; mean years of formal education 16.2, SD 2.1, range 13-18 years**) took part in the experiment. All participants were native Italian graduate students; they were naïve as to the experimental procedure, and the purpose of the study. All subjects were right-handed (**mean EHI=0.95; SD= 0.06; range= 0.79 - 1**) and with normal or corrected-to-normal vision. Participants had no history of chronic or acute neurologic, psychiatric, or medical disease; no family history of epilepsy; no current pregnancy; no cardiac pacemaker; no previous surgery involving implants to the head (cochlear implants, aneurysm clips, brain electrodes); and did not take acute or chronic medication. Written informed consent was obtained from all participants. Each subject underwent three different experimental **sessions designed as following: 1) anodal tDCS (a-tDCS) over the LIFG and TMS over the left BA6; 2) sham tDCS and TMS over the left BA6; 3) anodal tDCS over the LIFG and TMS over the left PPC (for a schematic representation of experimental sessions, see Fig. 1a). While session 1 can be considered as the main experimental condition, session 2 and 3 served as controls. In particular, condition 2 controlled for specific effects of the tDCS protocol, by comparing TMS-EEG recordings performed pre and post anodal vs sham stimulation, with TMS applied over BA6. Condition 3, instead, tested the same tDCS protocol used in condition 1, but controlled for possible effects of coil proximity on neurophysiological measurements. To safely exclude this possibility, anodal tDCS was delivered over the LIFG as in session 1, but TMS targeted the left PPC, corresponding to left BA7, i.e. an area not involved in the task (Weiss et al., 2003; Birn et al., 2010).** Stimulation order was counterbalanced across subjects and each session was separated by a 1 week washout period. The local ethical committee of the University of Milano-Bicocca approved the experiment and subjects were treated in accordance with the Declaration of Helsinki.

tDCS

tDCS protocol was delivered by introducing the electrodes under the EEG cap. A battery driven costant current stimulator (Eldith, Neuroconn, Ilmenau Germany) delivered the stimulation. The anode (16 cm^2) was placed over the LIFG, while the cathode (25 cm^2) was placed over the right supraorbital region. The LIFG was localized on the individual structural MRI of the subject through the integrated neuro-navigation system of the TMS-EEG instrument (Eximia Nexstim, Helsinki, Finland). Stimulation intensity was set at .75 mA resulting in a current density of .47 A/m^2 and charge density of 562 C/m2 for the anode and a density of .3 A/m^2 and charge density of 360 C/m^2 for the cathode; the duration of stimulation was 20 min with a fade in/fade out period of 30s. For Sham stimulation, the electrodes were placed in the same positions as real tDCS, but the duration was set at 30s. **Electrodes were applied by using a conductive paste (Ten20, Weaver and co.), which lowered electrodes impedance and helped in keeping the electrodes adherent to the scalp. After the tDCS protocol, stimulation electrodes were removed, and for the few EEG electrodes, which were displaced with this procedure (2 for the right supraorbital region and 3 for the left frontal region), impedance was controlled and adjusted to obtain optimal values (<5kΩ). This procedure took ~3 minutes.**

---------- Insert Figure 1 about here ----------

TMS – EEG

In each session two different TMS recordings were performed, before and after the tDCS-task experimental protocol. TMS was delivered by means of an Eximia™ TMS stimulator (Nexstim™, Helsinki, Finland) using a focal figure of eight 70-mm coil. The frontal TMS hotspot was located over the left premotor cortex (LPMC, BA 6, MNI coordinates: x -16, y 4, z 68, see Fig. 1b). Other

imaging studies reported similar coordinates for BA6 (Meinzer et al., 2013; Kircher et al., 2011; Costafreda et al., 2006). This area was chosen as TMS hotspot according to previous studies in which a greater activation of BA6 was reported for verbal fluency with respect to word repetition (Meinzer et al., 2012). The TMS hotspot (for sham and real frontal sessions) was selected in a pilot session as the site in BA6 where stimulation induced TEPs without muscular artefacts. The parietal TMS target was set over the left superior parietal lobule (BA 7, MNI coordinates: x -34, y -74, z 50, see Fig. 1b), an area not involved in the functional network specific for verbal fluency **(Weiss et al., 2003; Birn et al., 2010). High-resolution (1x1x1 mm) structural magnetic resonance images (MRI) were acquired for each participant using a 3 T Intera Philips body scanner (Philips Medical Systems, Best, NL). The TMS target was identified on individual MRIs using an integrated Navigated Brain Stimulation (NBS) system (Nexstim™, Helsinki, Finland) which employs infrared-based frameless stereotaxy, in order to map the position of the coil and of the participant's head, within the reference space of the individual's MRI space.** T**he NBS system allowed to continuously monitor the position and orientation of the coil, thus assuring precision and reproducibility of the stimulation across recordings. Moreover, the NBS system estimated on-line the intensity (V/m) of the intracranial electric field induced by TMS at the stimulation hotspot, accounting for the head and brain shape of each participant, and taking into consideration the distance from scalp and coil position.** In each session TMS intensity was delivered at an intensity eliciting an estimated electrical field at the hotspot of 95 V/m. This resulted in a mean intensity of **62**% of the maximum stimulator output (SD **5.7**; range **50-70**%). Critically, Wilcoxon non-parametric tests showed no difference in TMS intensity between pre and post recordings within the three experimental sessions and between sessions (all ps>.11). TMS **single pulses were delivered** at an inter-stimulus interval randomly jittering between 2100 and 2300. 180 trials were acquired for each recording.

EEG Recording during TMS

EEG signal was continuously recorded using a TMS compatible 60-channels amplifier (Nexstim Ltd., Helsinki, Finland), which prevents saturation by means of a proprietary sample-and-hold circuit which holds the amplifier output constant from 100 μs pre to 2 ms post-TMS pulse (Virtanen et al., 1999). Two electrodes placed over the forehead were used as ground. Eye movements were recorded by means of two additional electrodes placed near the eyes in order to monitor ocular artifacts. As in previous studies, during EEG recordings, participants wore earplugs and heard a continuous masking noise to cover TMS coil discharge (Massimini et al., 2005; Casarotto et al., 2010; Romero Lauro et al., 2014), avoiding thus the emergence of auditory evoked potentials. Electrodes impedance was kept below 5 kΩ, and EEG signals were recorded with a sampling rate of 1450 Hz.

Data pre-processing was carried out using Matlab R2012a (Mathworks, Natick, MA, USA). Data were down-sampled to 725 Hz, continuous signal was split in epochs starting 800 ms pre- and ending 800 ms post-TMS pulse. Trials with excessive artifacts were removed by visual inspection (Casali et al., 2010) and a band-pass filter between 2 and 80 Hz was applied as well as a notch filter at 50Hz. TEPs were computed by averaging selected artifact-free single epochs. Bad channels were interpolated using spherical interpolation function of EEGLAB (Delorme & Makeig, 2004). TEPs were then referenced and baseline corrected between -300 and -50ms before the TMS pulse.

For each recording, as a measure of cortical excitability, Global Mean Field Power (GMFP) was computed on the averaged TEP signal recorded from all 60 EEG channels (as in Romero Lauro et al., 2014). GMFP is considered a reliable measure of cortical excitability and connectivity of the targeted area and of its functional network (Massimini et al., 2005; Rosanova et al., 2008; Casarotto et al., 2010; Mattavelli et al., 2013; Romero Lauro et al., 2014). Similarly, Local mean field power (LMFP) was computed to specifically assess cortical excitability of a restricted scalp area (Pellicciari et al., 2013; Romero Lauro et al., 2014). In particular, LMFP was computed for six different electrode clusters, defined on the basis of their anatomical

position. The first one included the two electrodes directly interested by the a-tDCS, over the LIFG (C1, electrodes F5-F7). C2 included the electrodes above the frontal TMS hotspot (BA6), therefore under the TMS coil (electrodes F1-FC1). C3 included the electrodes over the parietal TMS hotspot (CP1-P1). C4, C5 and C6 represented the contralateral sites of C1 C2 and C3. (See Fig. S.2). GMFP and LMFP were computed for the whole considered TEP duration (0-150ms) and for three time windows, identified in order to separately analyse early and late TEPs components: 0-30ms; 30-65ms and 65-150ms.

To better refine the spatial resolution of the highlighted findings and to account for possible effects of volume conduction in the EEG signal, source modelling was performed following the procedures in Casali et al. (2010) and Romero-Lauro et al. (2015). First, meshes of cortex, skull and scalp compartments (containing 3004, 2000 and 2000 vertices, respectively) were obtained starting from individual MRIs to represent conductive head volume, and were modelled following the 3-spheres BERG method (Berg and Scherg, 1994), which is implemented in the Brainstorm software package (http://neuroimage.usc.edu/brainstorm). This method includes three concentric spheres with different homogeneous conductivities, each representative of the best-fitting sphere of inner skull, outer skull and scalp compartments. Then the model was constrained to the cortex, reconstructed as a 3D grid of 3004 fixed normally oriented dipoles with respect to the cortical surface. Finally, EEG sensors positions recorded during the TMS-EEG sessions were co-registered with the meshes, using rotations and translations of digitized landmarks identified on the individual MRI (nasion, left and right tragus). Then, the inverse transformation was applied to the MNI canonical mesh of the cortex for approximating to real anatomy. Then, for each participant, the inverse solution was computed on each artefact-free TMS/EEG trial using the weighted minimum norm estimate with Gaussian geodesic smoothness prior (Casali et al., 2010). After source reconstruction, a statistical threshold was computed in order to assess when and where the post-TMS cortical response differed from pre-

TMS activity (i.e. to identify TMS-evoked response). To do so, a non-parametric permutation-based procedure was applied (Pantazis et al., 2003). A binary spatial-temporal distribution of statistically significant sources was obtained and thus only information from significant cortical sources was used for further analyses. As indices of cortical activity, we cumulated the absolute Significant Current Density (global SCD, measured in µA/mm2, Casali et al., 2010) over all 3004 cortical vertexes and over the three time windows of interest (0-30ms; 30-65ms and 65-150ms) for each recording session (6: pre and post each experimental session). Finally, in order to mirror the LMFP analysis of the sensor data, for each experimental condition, we computed a local SCD in the vertexes within six different Brodmann's areas (BAs), identified by means of an automatic tool of anatomical classification (WFUPickAtlas tool; http://www.ansir.wfubmc.edu; Maldjian et al., 2003 and Maldjian et al., 2004). These BAs approximately corresponded to the 6 LMFP clusters identified in sensor analysis (left/right BA 44/45, 6, and 7, as in Casali et al., 2010; Romero Lauro et al., 2015).

Verbal Fluency

In each session, participants performed the fluency task with 2 semantic and 2 phonemic cues. In particular, they were asked to produce in one minute as many words as they could beginning with a given letter or belonging to a specific semantic category. Subjects were also asked not to produce the same word twice and to stick as much as possible to the noun grammatical category. Letters were presented in fixed pairs ('P' and 'G', 'D' and 'L', 'F' and 'C') balanced according to the relative frequency of names beginning with each pair of letters, as derived from the Corpus and Frequency Lexicon of Written Italian (COLFIS, see http://www.istc.cnr.it/material/database/colfis/index_eng.shtml). Category pairs were 'Clothing' and 'Vegetables', 'Animals' and 'Tools', and 'Vehicles' and 'Fruits'. As for letters, they were matched according to a pilot study performed on 10 healthy subjects in order to have (i) a similar number of words produced per each category pair and (ii) a living and a non-living category in each session.

Letters and categories pairs order was counterbalanced across sessions and stimulation condition, in order to have subjects performing the fluency task with different letters and categories in each experimental session.

Analyses

Analyses were run with the statistical programming environment R (R core team, 2014). Linear mixed effects models were adopted as the main statistical procedure (Baayen et al., 2008). As our data involved a continuous dependent variables, namely number of produced words, TEP values and SCD values, a series of linear mixed effects regression using LMER procedure in "lme4" R package (version 1.1-5, Bates et al., 2014) were performed. Fixed effects inclusion in the final model has been tested with a series of likelihood ratio tests, including each effect which significantly increased the model's goodness of fit (Gelman and Hill, 2006). Concerning the behavioural performance, the considered fixed effects were stimulation session (**factorial, 3 levels: Real tDCS-frontal TMS; Real tDCS-parietal TMS and sham stimulation**) and fluency type (factorial, 2 levels: semantic and phonemic fluency) and their interaction. Concerning the random effect structure, a by-subjects random intercept was included. GMFP, LMFP and SCD values were submitted to a similar procedure. **Concerning GMFP, models were estimated by including stimulation session (factorial, 3 levels: anodal tDCS - TMS BA6, anodal tDCS - TMS BA7 and sham stimulation) and recording time (factorial, 2 levels: pre and post tDCS) as fixed effects on each time window. Concerning the random effect structure, a by-subjects intercept was included**. The same procedure was adopted for global SCD. Concerning LMFP and local SCD, the same procedure was adopted, and data were separately analysed for clusters and time windows. Once the final model was defined, an ANOVA was run on it, which will be reported with significance levels based on Satterthwaite's degrees of freedom approximation in "lmerTest" R package (version 2.0-6, Kuznetsova et al., 2015). Lastly, to directly contrast single levels of the significant interactions and main effects, post-hoc procedures were carried out on the best fitting final model with the "phia" R package (version 0.2-0, De Rosario-Martinez, 2015), applying **Bonferroni - Holm** correction for multiple comparisons. To assess if the

increase in indices of cortical excitability was associated with the behavioural performance in the verbal fluency task, one-tailed correlations were run between increase **in neurophysiological responses and behavioural performance. For the neurophysiological increment index, we subtracted the increment in local SCD** between pre and post **sham** tDCS recordings (SCD post tDCS- SCD pre tDCS) **to the increment in local SCD between pre and post real tDCS recordings, separately for both BA6 and BA7 sessions**. **We then computed the index of behavioural enhancement by subtracting the verbal fluency score in the sham session to the verbal fluency score in the real sessions, separately for BA6 and BA/ sessions**. **Correlations between the behavioural and neurophysiologic enhancement (in each considered BA)** were run and 90% confidence intervals were obtained for significant correlations by a 1000 permutation bootstrap procedure in R with the Boot function.

Commentato [cp1]: Prima hai sempre usato lo spelling USA in cui non ci vuole la u. Decidi quale spelling usare

Results

Verbal fluency

At a behavioral level, scores were higher for semantic fluency (17.9 words, sd=3.3) as compared to phonemic one (15.8 words, sd=3.5; F(1,85)=10.5; p=.002). Interestingly, as expected, stimulation significantly enhanced verbal fluency. The main effect of stimulation, indeed, was significant [F(2,85)=7.4; p=.001]. In particular, placebo stimulation sessions resulted in lower fluency scores (15.2 words, sd=2.7) compared to both sessions in which anodal tDCS over the LIFG was delivered (TMS-BA6: 17.9 words, sd=4.2; p<.001; TMS-BA7: 17.5 words, sd=3.3; p=.003). As previously reported (Cattaneo et al., 2011), the stimulation by type of fluency interaction was not significant [F(2,85)=0.18; p=.84].

GMFP and LMFP

Concerning global cortical excitability, measured as GMFP, the stimulation **by recording time interaction resulted significant [F(2,85)=3.59; p=.03]. Post-hoc analyses showed that cortical excitability** significantly increased in post anodal tDCS compared to pre-tDCS recordings **when TMS was applied over BA6** (p=.**013**), while no change was detected in sham sessions (p=.**99**) **and in anodal tDCS sessions, when TMS was applied over BA7 (p=.93)**. These results strongly corroborate the hypothesis that anodal tDCS acts by increasing cortical excitability of the cerebral cortex even outside the primary motor cortex.

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In order to better assess how cortical excitability was modulated by the application of tDCS, we analyzed the modulation of TEPs within three time windows based on the grand average of the GMFP: 0-30 ms (early-latency), 30-65 ms (middle-latency) and 65-150 ms (late-latency). **The stimulation by recording time interaction was significant in the early-latency TEP component [F(2,51)=3.78; p=.03], which reflects cortical excitability of the targeted area (Ilmoniemi and Kicic, 2010; Pellicciari et al., 2013), where an** increase in global cortical excitability was detectable in post-tDCS as compared to pre-tDCS recordings only in real tDCS**-BA6** sessions (p<.001, see Fig. 3a). S**imilarly, TEP increased in the middle-latency component [F(2,68)= 3.8; p=.026], only after anodal tDCS TMS over BA6 sessions (p=.01) while no difference was highlighted in the other sessions (sham: p=.95; BA7:p=.84).**

In order to roughly localize the cortical excitability increase, we computed, for each time window, the LMFP for different electrodes clusters, namely a cluster near the anode (C1), a cluster near the TMS coil (C2), and a cluster over an area which was not involved in the task but near coil location in the control session (i.e. the left posterior parietal cortex, PPC; C3). Homologous clusters on the contralateral hemisphere were also investigated (C4, C5 and C6; see Supplementary material Fig S1).

For the early latency component, LMFP analyses showed a significant increase in LMFP in C1, i.e. near the anode location only after real tDCS sessions **with TMS applied over BA6 (p<.001**), confirming tDCS specific effect on the stimulated area, while no effect was highlighted in sham (p=1**) sessions or when TMS was applied over BA7 (p=.1). Similarly, C2 showed the same increase in LMFP in the early latency component (Anodal tDCS-BA6: p=.007; Anodal tDSC-BA7: p=1; Sham: p=1).** Concerning the middle-latency component, which reflects functional network cortical excitability properties (Casarotto et al., 2010; Ilmoniemi and Kicic, 2010; Veniero et al., 2012), a greater post-tDCS TEP was found for C2, near the TMS coil (i.e. left BA 6) p<.001) **only in anodal tDCS sessions with TMS applied over BA6**. In sham sessions, when TMS was applied over BA7 no increase was reported between pre- and post-tDCS recordings (both ps=1). For the late-latency components, no increase was highlighted in any considered cluster (Fig. 3b).

---------- Insert Figure 3 about here ----------

SCD and Local SCD

Confirming the spatial specificity of the effects of stimulation (see Fig 4c-f), left BA 6 and BA 44/45 were the only cortical sites in which an increase in cortical excitability was detectable. In particular, for left BA6, the stimulation by recording session interaction was significant [F(2,51)= 3.9; p=.027], since post-tDCS recordings in anodal stimulation sessions with TMS over BA6 resulted in an increase in SCD when compared to pre tDCS recordings (p=.014), while no difference was present for sham (p=.96) and anodal sessions with TMS over BA7 (p=.97). The same result was found for left BA44/45 [F(2,51)= 3.1; p=.05]; Post hoc analysis showed a significant difference between pre and post real tDCS sessions with TMS applied over BA6

(p=.01), while no difference was present between pre and post sham (p=.97) and BA7 (p=.98) sessions (see Fig.4).

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Finally, to further investigate the link between cognitive and neurophysiological tDCS-driven enhancement, we computed the correlation between the enhancement in verbal fluency performance and cortical excitability increase between pre and post-tDCS protocols. Our results indicate **only** a positive **correlation between the increase in SCD in left BA 44/45 after anodal tDCS and TMS applied over left BA6 and verbal fluency performance in that session (r=.53; p=.012, Bootstrap 90% CI=.38 .79; see Fig. 5).** To our knowledge, this is the first time that a direct measure of brain excitability is linked to a modulation of a cognitive performance, and the first, in vivo, evidence that neurophysiological and cognitive effects of tDCS are correlated.

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Discussion

The present results define how, at a functional level, tDCS affects cortical circuits when the stimulation is applied during task performance. Both global and local **neurophysiological measurements** showed a significant increase after anodal tDCS in the early- and middle-latency TEP components which are considered, respectively, the most reliable markers of cortical excitability of the targeted area (Ilmoniemi and Kicic, 2010; Pellicciari et al., 2013) and of regions which are strictly connected to it. This means that the LIFG **and BA6** showed a direct increase of cortical excitability, while BA6 showed **also** an increased response as a part of a functional network activated by the task

(Casarotto et al., 2010; Ilmoniemi and Kicic, 2010; Veniero et al., 2012). Similarly, source analysis confirmed the **specificity** of the effects of stimulation (see Fig 4c-f), since left BA 6 and BA 44/45 were the only cortical sites in which an increase in cortical excitability was detectable between pre and post-anodal tDCS recordings when TMS was applied over BA6. By performing source analysis we also controlled for possible volume conduction effects, which could have spread over different scalp sites the underlying cortical increase in excitability (Romero Lauro et al., 2016). **As Fig. 4b shows, the topography of the tDCS-induced cortical enhancement when TMS was applied over BA6 is restricted to functionally related sites, and the peak of activation is not directly under the tDCS patch but, as suggested by current modelling studie**s **(Bikson et al., 2012; Datta et al., 2010; Opitz et al., 2015), rather between the anode and the cathode.** No increase of cortical excitability, instead, was detected when TMS was delivered over BA7, a region not involved in the task, thus ruling out the possibility that tDCS local effects were due to magnetic stimulation proximity. Similarly, no change was detected when sham tDCS was delivered, confirming that the increase in cortical excitability recorded in real tDCS sessions was due to an interaction between neurophysiological modulation and cortical activity elicited by cognitive processing. Overall, the present results showed that, while performing a language production task, anodal tDCS induces cortical plastic changes only in those areas, which are relevant for task execution. The implication of the present findings are striking, since they suggest that even if electrical currents delivered by tDCS spread far away from the stimulation site, as suggested by modeling studies (Bikson et al., 2012; Datta et al., 2010; Opitz et al., 2015), their functional effects are restricted to those areas which are more active during the stimulation protocol. This evidence seems at odds with a previous TMS-EEG study showing that at rest, after right parietal tDCS, cortical excitability increased in bilateral frontal and parietal sites (Romero Lauro et al., 2014). However, this fronto-parietal cortical pattern overlaps the default mode network, which is assumed to be active when no specific task is performed.

One plausible reason determining the site specificity of functional effects can be found in tDCS on-line and off-line mechanisms of action: neurophysiological modulation induced by the stimulation are strictly connected to spontaneous firing and **synaptic efficacy** (Fritsch et al., 2010; **Bikson et al., 2004;** Nitsche and Paulus, 2000; Bindman et al., 1964). If the area is not activated by task execution, concurrently with tDCS applications, no plastic change could be detectable. Animal model support this view by showing that M1 mouse slices needed simultaneous DC and synaptic activation in order to induce LTP like-changes (Fritsch et al., 2010). According to this view, the areas involved in the proposed task execution (left BA6, BA44 and 45), which more likely exhibited an increase of synaptic activity during the stimulation protocol, showed an increment in cortical excitability, while areas outside the functional network of verbal fluency (left BA7) did not show any neurophysiological modulation. These findings, **by supporting the activity selectivity hypothesis (Bikson and Rahman, 2013),** confirm in humans what was found in animal models, representing a solid theoretical framework for designing future experiments involving anodal tDCS and for interpreting past and future results obtained with this non-invasive brain stimulation technique. **It has to be noted, however, that more than electrodes location, what may be crucial for the observed neurophysiological modulation could be current flow direction, which may alter the neural input/output (I/O) function (Lafon et al., 2016)**. **Technically speaking, thus, defining the present protocol as "anodal" may be misleading, since any tDCS protocol with cephalic reference includes an anode and a cathode. However, while computational models provided evidence for an increased I/O function for the areas under the anode, they do not show significant effects on areas under the cathode, at least for the classical motor cortex montage (Lafon et al., 2016)."**

Another relevant result is that performance at the verbal fluency task and the cortical excitability increase occurring in left BAs 44 and 45 significantly correlated. To our knowledge, this is the first time that a direct measure of brain excitability is linked to the modulation of a cognitive performance, and the first, in vivo, evidence that neurophysiological and cognitive effects of tDCS are correlated.

414 Our data suggest thus a strict link between the tDCS-induced enhancement in performance on the
415 verbal fluency task and plastic changes occurring at specific cortical sites.

416 Taken together, by shedding light on the site-specificity of tDCS neurophysiological effects on
417 cortical plasticity and their relationship with cognitive functions enhancement, the present results
418 offer a theoretical framework in which non-invasive brain stimulation literature could interpret its
419 findings and may help in designing more effective tDCS protocols aimed at treating neurological and
420 psychiatric conditions and study diseases hallmarked by abnormal cognitive functioning and
421 neurophysiological responses.

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Captions to figures

Fig. 1: **a** Schematic representation of the experimental sessions. **b** Neuronavigation in one subject of the left frontal (BA6) and parietal (BA7) TMS hotspots.

Fig. 2: Behavioral results of the verbal fluency tasks. Performance improved after anodal tDCS.

Fig. 3: Resuts from the GMFP and LMFP analyses. Dot-shaded areas indicate significant differences. **a)** GMFP in pre and post tDCS recordings in Sham (upper row) and Real anodal (lower row) tDCS sessions. Global cortical excitability increased after real stimulation in the early TEP component. **b)** LMFP of Cluster 1 (dotted box) and Cluster 2 (solid box), highlighted in the central head model. In C1 LMFP increased after real anodal stimulation in the early component. In C2 LMFP increased after real stimulation in a middle-latency component. Grey-shaded areas indicate significant differences.

Fig. 4: Results from the global and local source modelling. Grey-shaded areas indicate significant differences. **a)** Plots of the SCD over time in pre (blue line) and post (red line) tDCS recordings. Significant difference in pre-post tDCS cortical activity is evident only for anodal tDCS sessions with TMS applied over left BA6 (first plot), while no difference is highlighted for sham tDCS sessions (second plot) or when TMS was applied over the left BA7. **b)** source localization of the global cortical activity. The increment in SCD is evident in left premotor areas after anodal tDCS with TMS over left BA6. **c-f)** SCD in left BA 6 (**c**, orange box), left BA 44 45 (**e**, green box), left BA7 (**d**, yellow box) and left BA 21/22 (**f**, cyan box) in pre (blue line) and post (red line) sham and anodal tDCS

sessions, while probing cortical excitability from left BA 6. Differences between pre and post tDCS sessions are highlighted only for anodal tDCS sessions in left BA 6 and 44/45.

Fig. 5: Scatterplot illustrating the significant correlation between the increase in verbal fluency performance, compared to sham sessions, and the increase in SCD in left BAs 44 45 during the anodal tDCS session with cortical excitability probed from left BA6.