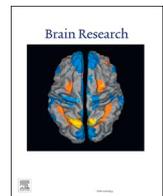


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## tDCS over posterior parietal cortex increases cortical excitability but decreases learning: An ERPs and TMS-EEG study

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### ABSTRACT

The application of anodal transcranial direct current stimulation (AtDCS) is generally associated with increased neuronal excitability and enhanced cognitive functioning. Nevertheless, previous work showed that applying this straight reasoning does not always lead to the desired results at behavioural level. Here, we investigated electrophysiological markers of AtDCS-mediated effects on visuo-spatial contextual learning (VSCL). In order to assess cortical excitability changes after 3 mA AtDCS applied over posterior parietal cortex, event-related potentials (ERPs) were collected during task performance. Additionally, AtDCS-induced effects on cortical excitability were explored by measuring TMS-evoked potentials (TEPs) collected before AtDCS, after AtDCS and after AtDCS and VSCL interaction. Behavioural results revealed that the application of AtDCS induced a reduction of VSCL. At the electrophysiological level, ERPs showed enhanced cortical response (P2 component) in the group receiving Real-AtDCS as compared to Sham-AtDCS. Cortical responsiveness at rest as measured by TEP, did not indicate any significant difference between Real- and Sham-tDCS groups, albeit a trend was present. Overall, our results suggest that AtDCS increases cortical response to incoming visuo-spatial stimuli, but with no concurrent increase in learning. Detrimental effects on behaviour could result from the interaction between AtDCS- and task-mediated cortical activation. This interaction might enhance cortical excitability and hinder normal task-related neuroplastic phenomena subtending learning.

### 1. Introduction

Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation (NIBS) technique consisting of a simple device that delivers low intensity electric current for brain stimulation. This technique effectively modulates a wide range of perceptual, motor and cognitive functions, and it is widely used for rehabilitation treatment in several clinical settings (Berryhill & Martin, 2018; Moffa et al., 2018; Stagg & Nitsche, 2011; Utz et al., 2010). It has been shown that at the neuronal level, the excitability of neurons is decreased and increased by cathodal and anodal-tDCS (AtDCS), respectively (Bindman et al., 1964). Moreover, after-effects occur because the neural activity rate remains at increased/decreased levels even after stimulation (Gartside, 1968; Purpura & McMurtry, 1965). It has been suggested that tDCS-induced changes in cortical excitability and activity are based on long-term

potentiation and depression-like phenomena (Stagg & Nitsche, 2011). Therefore, the alteration (i.e., enhancement or reduction) of the state of cortical excitability induced with neuromodulation, likely affects also basic mechanisms of neural circuits associated with learning (Man-svelter et al., 2019).

Although tDCS is considered relatively easy to use, the complex interactions occurring between its application and the brain's activity are very often underestimated (see Fertonani and Miniussi, 2017). In this regard, several works have highlighted that it is important to carefully consider tDCS-brain interactions, as the effects of tDCS can be even reversed by the ongoing level of activity within the stimulated area and/or network (e.g., Benwell et al., 2015; Bortoletto et al., 2015a; Cantarero et al., 2013; Siebner et al., 2004). For instance, studies on the motor domain show that tDCS applied in close temporal sequence with another NIBS protocol or a task involving motor learning produced behavioural

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and neurophysiological outcomes that do not match the expected anodal-excitation/cathodal-inhibition effects (for a review see Müller-Dahlhaus & Ziemann, 2015). However, there is only a limited number of studies that have systematically examined the effects of tDCS on learning or cognition and cortical excitability outside the motor areas (Gibson et al., 2020; Pirulli et al., 2013; Raveendran et al., 2020). Investigating these topics in cortical regions involving a more distributed network of connections is crucial for a deeper understanding of tDCS-brain interactions when complex circuitries are involved. We recently showed that applying 3 mA offline AtDCS over posterior parietal cortex (PPC) produces a significant reduction of visuo-spatial contextual learning (VSCL) formation (Grasso et al., 2020). The paradigm used has been classically administered to investigate implicit statistical learning within the visuo-spatial domain and consists of a visual search task in which half of the search arrays are repeated across blocks (Chun & Jiang, 1998). The repetition of the arrays produces consistent ameliorative behavioural responses mirroring visuo-spatial implicit learning processes. The reduced VSCL reported in our previous work after the application of AtDCS was interpreted in the framework of an excessive increase in neuronal excitability produced by the interaction between the stimulation and the task which could have hindered normal task-related neuroplastic phenomena within the involved brain network.

In the present work, we have employed the same stimulation protocol and the same visuo-spatial learning task used in our previous study and added the collection of event-related potentials (ERPs) and transcranial magnetic stimulation during electroencephalography (TMS-EEG) data. We used a between subject design to compare the behavioural and the electrophysiological effects produced by Real- and Sham-AtDCS. On the one hand, ERPs recorded during task execution allowed us to directly assess the effect produced by AtDCS while processing repeated vs. non-repeated stimuli. On the other hand, TMS-EEG co-registration provides real-time information on cortical reactivity and connectivity through the analysis of TMS-evoked potentials (TEPs). Therefore, TEPs allowed us to directly investigate the link between functional activity and behaviour by examining how cortical excitability at rest is shaped by AtDCS alone and the combination of AtDCS and VSCL. In short, TEPs reflect how tDCS induced effects vary as a function of neuronal state (i.e., excitability and pattern of connectivity) in which the stimulated area is embedded (Bortoletto et al., 2015b; Ilmoniemi et al., 1997; Miniussi & Thut, 2010). Recent studies have confirmed the effectiveness of TMS-EEG measurements, administered alone or in combination with a task, to investigate tDCS-induced excitability changes (Pellicciari et al., 2013; Pisoni et al., 2018; Romero Lauro et al., 2014; Varoli et al., 2018). Thus, we measured TEP in the posterior parietal cortex as well as its propagation to functionally connected regions at baseline, after AtDCS and after AtDCS/VISCL interaction.

The overall goal of this work was to identify neurophysiological markers associated with neuroplasticity induced by means of tDCS during a learning process. In particular, we aimed to determine the correlation between neurophysiological parameters affected by tDCS and learning performance changes evaluated by several EEG indexes. Based on previous findings, we predict a reduction of VSCL associated with an increase in cortical activity indexed by ERP and TEP measures. Such a result would confirm the need to reevaluate the current dependent brain stimulation effects (i.e., anodal stimulation induces behavioural facilitation vs. cathodal stimulation induces inhibition) in favour of a network activity-dependent approach (Fertonani and Miniussi, 2017).

## 2. Results

### 2.1. Behavioural

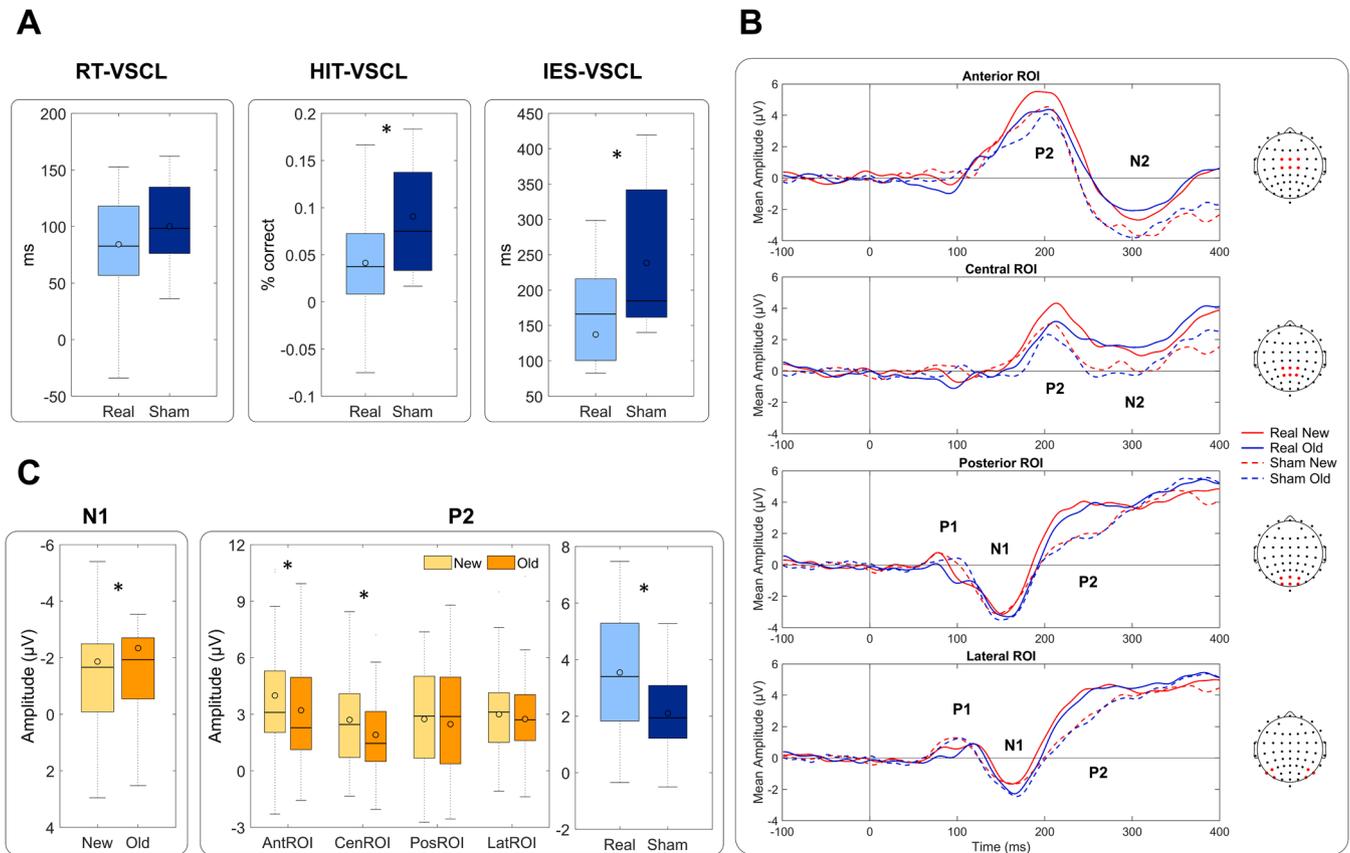
The overall performance (*new* and *old* trials averaged) in terms of reaction times (RT) and hits (HIT) was 891.6 ms (standard deviation - SD: 103.9 ms) and 0.87 (SD: 0.12) for the Real-tDCS group and 877.8 ms

(SD: 93.6 ms) and 0.84 (SD: 0.13) for the Sham-tDCS group. VSCL was determined as the difference between responses to *new* and *old* trials in terms of reaction times (i.e., new-old; RT-VSCL), percentage of correct responses (i.e., old-new; HIT-VSCL) and inverse efficiency score (an index merging RT and HIT scores, i.e., RT/HIT; IES). The IES allowed us to weight in a single index the impact of speed and accuracy provided that the two measures point to the same direction (Vandierendonck, 2017). Three separate 2x10 mixed design ANOVAs with the between factor Stimulation (Real-tDCS and Sham-tDCS) and the within factor Blocks (ten levels) were performed on RT-VSCL, HIT-VSCL and IES-VSCL scores, after confirmation that RT-VSCL data were normally distributed. The analysis of RT-VSCL revealed neither a main effect of Stimulation ( $F(1, 30) = 1.078; p = 0.307; \eta_p^2 = 0.03$ ) or Blocks ( $F(9, 270) = 1.687; p = 0.092; \eta_p^2 = 0.03$ ) nor a Stimulation  $\times$  Blocks interaction ( $F(9, 270) = 0.880; p = 0.544; \eta_p^2 = 0.03$ ). The analysis of HIT-VSCL revealed a significant main effect of Stimulation ( $F(1, 30) = 5.551; p = 0.025; \eta_p^2 = 0.16$ ) that was explained by a reduced HIT-VSCL in the Real-tDCS group as compared to the Sham-tDCS (Real: 0.041; Sham: 0.091; Fig. 1A). Neither the main effect of Blocks ( $F(9, 270) = 1.342; p = 0.215; \eta_p^2 = 0.04$ ) nor the Stimulation  $\times$  Blocks interaction ( $F(9, 270) = 0.686; p = 0.721; \eta_p^2 = 0.02$ ) were significant. Finally, the analysis of IES-VSCL also revealed a significant main effect of Stimulation ( $F(1, 30) = 6.983; p = 0.013; \eta_p^2 = 0.19$ ) with reduced IES-VSCL in the Real-tDCS group as compared to the Sham-tDCS (Real: 148.8 ms; Sham: 249.7 ms; Fig. 1A), but no significant main effect of Blocks ( $F(9, 270) = 0.691; p = 0.597; \eta_p^2 = 0.02$ ) or Stimulation  $\times$  Blocks interaction ( $F(9, 270) = 1.107; p = 0.356; \eta_p^2 = 0.04$ ; Fig. 1A). As a brief note, we would like to point out that the lack of a significant main effect of Block in the employed indices could look odd at a first glance as one might expect that the repetition of the task produced a gradual increase in VSCL. Although an increase was indeed evident in RT-VSCL data (73 ms at Block 2, 116 ms at Block 11) this only yielded a trend towards significance in the statistical analysis ( $p = 0.09$ ). One possibility is that the exclusion of the first block robustly reduced the effect as part of RT-VSCL increase was evident between the first and the second block (47 ms at Block 1 and 73 ms at Block 2). Regarding HIT-VSCL, it is possible that the relatively high scores in both new (0.82) and old (0.88) trials already evident at Block 2 precluded a further enhancement to occur.

In order to examine whether the reported differences could be due to a priori between groups differences in visual search performances, three separate mixed design ANOVAs with 2 (between factor Stimulation)  $\times$  5 (within factor Blocks) were performed on RTs, HITs and IES collected during the execution of Training Blocks. Results revealed no main effect of Stimulation (RTs:  $p = 0.237$ ; Hits:  $p = 0.758$ ; IES:  $p = 0.408$ ) and no Stimulation  $\times$  Blocks interaction (RTs:  $p = 0.558$ ; Hits:  $p = 0.234$ ; IES:  $p = 0.700$ ) in any of the tested measures. Similarly, three mixed design ANOVAs with 2 (between factor Stimulation)  $\times$  10 (within factor Blocks) were performed on RTs, HITs and IES in response to *new* trials only collected during the Experimental Blocks. Again, results revealed no main effect of Stimulation (RTs:  $p = 0.766$ ; HITs:  $p = 0.094$ ; IES:  $p = 0.505$ ) and no Stimulation  $\times$  Blocks interaction (RTs:  $p = 0.109$ ; HITs:  $p = 0.602$ ; IES:  $p = 0.116$ ) in any of the tested measures, suggesting that Real and Sham-tDCS groups were not different in terms of visual search performances.

### 2.2. Sensations questionnaire

The Kruskal-Wallis test was used to compare sensations induced by Real- and Sham-tDCS. The analysis showed that the two stimulation protocols elicited statistically comparable sensations as indicated by the lack of any significant difference (all  $ps > 0.128$ ; see Table 1 for further details). Participants were mostly unaware whether they received Real or Sham-tDCS when asked to guess about it and were mainly convinced they received a real stimulation. More specifically, in the group receiving Real-tDCS, 75% of participants believed they received a real stimulation, 6% a sham stimulation and 19% did not know. Similarly, in



**Fig. 1.** A. Boxplots depicting VSCL measured in terms of RTs, HIT and IES (RT/HIT). Horizontal lines represent median values while circles represent mean values. B. ERPs resulting from Anterior, Central, Posterior and Lateral ROIs depicted on the right. Continuous lines depict ERPs from the group receiving Real-tDCS, dashed lines ERPs from the group receiving Sham-tDCS, red lines ERPs in response to New Trials and blue lines ERPs in response to Old Trials. C. Boxplot depicting amplitude values of N1 and P2 components of the ERPs in the specified conditions. Horizontal lines represent median values while circles represent mean values.

**Table 1**

Mean value and standard errors of each reported sensation separated for the stimulation protocols. The sensation intensity is presented on a 5-point scale as follows: 0 = None, 1 = Mild, 2 = Moderate, 3 = Considerable, and 4 = Strong.

|           | Irritation  | Pain        | Burning     | Heat     | Itch        | Iron taste  | Fatigue     |
|-----------|-------------|-------------|-------------|----------|-------------|-------------|-------------|
| Real tDCS | 1.25 ± 0.32 | 0.31 ± 0.20 | 1.19 ± 0.29 | 1 ± 0.26 | 1.5 ± 0.26  | 0.5 ± 0.24  | 0.06 ± 0.06 |
| Sham tDCS | 0.63 ± 0.24 | 0.25 ± 0.11 | 1.06 ± 0.25 | 1 ± 0.24 | 1.25 ± 0.23 | 0.56 ± 0.30 | 0.5 ± 0.24  |

the group receiving Sham-tDCS, 81% believed they received a real stimulation and 19% did not know.

### 2.3. Event related potentials

We ran four ANOVAs with between-subject factor Stimulation (Real-tDCS and Sham-tDCS) and within-subject factors Stimulus Type (New Trials, Old Trials) and region of interest (ROI) (2 to 4 levels: Anterior ROI, Central ROI, Posterior ROI, Lateral ROI) on mean amplitude values of the identified ERP components. The P1 and N1 components were evident over Posterior and Lateral ROIs therefore only these two ROIs were considered for the statistical analysis. The P2 component was visible in all the four ROIs that were therefore used for the statistical analysis. Finally, the N2 component was evident with an antero-central distribution and therefore only Anterior and Central ROIs were considered for the analysis (see Fig. 1B and Supplementary Materials for an in-depth description of identified ERP components).

The 2x2x2 ANOVA conducted on the P1 component only revealed a significant main effect of ROI ( $F(1, 29) = 25.924; p < 0.001; \eta_p^2 = 0.47$ ) that was explained by higher amplitude values on Lateral ROI as compared to Posterior ROI (Lateral ROI:  $0.69 \pm 0.32 \mu\text{V}$ ; Posterior ROI:

$-0.20 \pm 0.31 \mu\text{V}$ ). There were no other significant main effects or interactions (all  $ps > 0.223$ ).

The 2x2x2 ANOVA conducted on the N1 component also revealed a significant main effect of ROI ( $F(1, 29) = 10.049; p = 0.003; \eta_p^2 = 0.26$ ), that indicated more negative values over the Posterior as compared to the Lateral ROI (Posterior ROI:  $-2.71 \pm 0.78 \mu\text{V}$ ; Lateral ROI:  $-1.50 \pm 0.46 \mu\text{V}$ ). In addition, there was a significant main effect of Stimulus Type ( $F(1, 29) = 14.061; p < 0.001; \eta_p^2 = 0.32$ ) due to higher negative values in response to old as compared to new trials (New Trials:  $-1.86 \pm 0.62 \mu\text{V}$ ; Old Trials:  $-2.34 \pm 0.63 \mu\text{V}$ ; Fig. 1B and 1C). There were no other significant main effects or interactions (all  $ps > 0.360$ ).

The 2x2x4 ANOVA conducted on the P2 component revealed a significant main effect of Stimulation ( $F(1, 29) = 4.328; p = 0.046; \eta_p^2 = 0.13$ ) explained by higher values in the group receiving Real as compared to Sham-tDCS (Real-tDCS:  $3.54 \pm 0.78 \mu\text{V}$ ; Sham-tDCS:  $2.10 \pm 0.58 \mu\text{V}$ ; Fig. 1C). There were also a significant main effect of Stimulus Type ( $F(1, 29) = 9.793; p = 0.004; \eta_p^2 = 0.25$ ) and a significant Stimulus Type × ROI interaction ( $F(3, 87) = 6.829; p = 0.007; \eta_p^2 = 0.19$ ), which showed lower values in response to old as compared to new trials (New Trials:  $3.11 \pm 0.51 \mu\text{V}$ ; Old Trials:  $2.58 \pm 0.49 \mu\text{V}$ ). This effect was particularly evident over Anterior and Central ROIs (Anterior ROI – New

Trials:  $3.99 \pm 0.63 \mu\text{V}$ ; Old Trials:  $3.21 \pm 0.60 \mu\text{V}$ ; Central ROI – New Trials:  $2.70 \pm 0.42 \mu\text{V}$ ; Old Trials:  $1.91 \pm 0.39 \mu\text{V}$ ; Posterior ROI – New Trials:  $2.74 \pm 0.53 \mu\text{V}$ ; Old Trials:  $2.47 \pm 0.52 \mu\text{V}$ ; Lateral ROI – New Trials:  $3.00 \pm 0.43 \mu\text{V}$ ; Old Trials:  $2.74 \pm 0.43 \mu\text{V}$ ; Fig. 1B and 3C). There were no other significant main effects or interactions (all  $ps > 0.118$ ).

The 2x2x2 ANOVA conducted on the N2 component only revealed a significant main effect of ROI ( $F(1, 29) = 97.848$ ;  $p < 0.001$ ;  $\eta_p^2 = 0.77$ ) explained by higher negative values over the Anterior as compared to Central ROI (Anterior ROI:  $-2.57 \pm 0.60 \mu\text{V}$ ; Central ROI:  $0.95 \pm 0.57 \mu\text{V}$ ). There were no other significant main effects or interactions (all  $ps > 0.328$ ).

In summary, ERP results revealed that the amplitude of the N1 and the P2 components were modulated by the repetitions of the visuo-spatial contexts both in the group receiving Real- and Sham-tDCS. Furthermore, Real-tDCS produced an enhancement of the P2 component regardless of the type of stimulus presented.

#### 2.4. TMS-evoked potentials

Separate 2x3 mixed design ANOVAs with the between-subject factor Stimulation (Real-tDCS and Sham-tDCS) and the within-subject factor Time (TEP1, TEP2 and TEP3) were performed on mean amplitude values of each identified TEP component and each identified pool of electrodes (see [Supplementary Materials](#) for an in-depth description of identified TEP components). The analyses showed no significant main effect of Stimulation (all  $ps > 0.09$ ). There was a significant main effect of Time in the time windows between 95 and 125 ms over fronto-central electrode pool (N100; FCZ, FZ, FC2 and F2;  $F(2, 60) = 17.601$ ;  $p < 0.001$ ;  $\eta_p^2 = 0.37$ ) and left-occipital electrode pool (P100; O1 and PO7;  $F(2, 60) = 4.612$ ;  $p = 0.014$ ;  $\eta_p^2 = 0.13$ ), suggesting that amplitude decreases over time, regardless of the Stimulation protocol employed. TEP Components around 100 ms could be a mix of cortical activation generated by the magnetic field and the auditory response to the sound produced by TMS

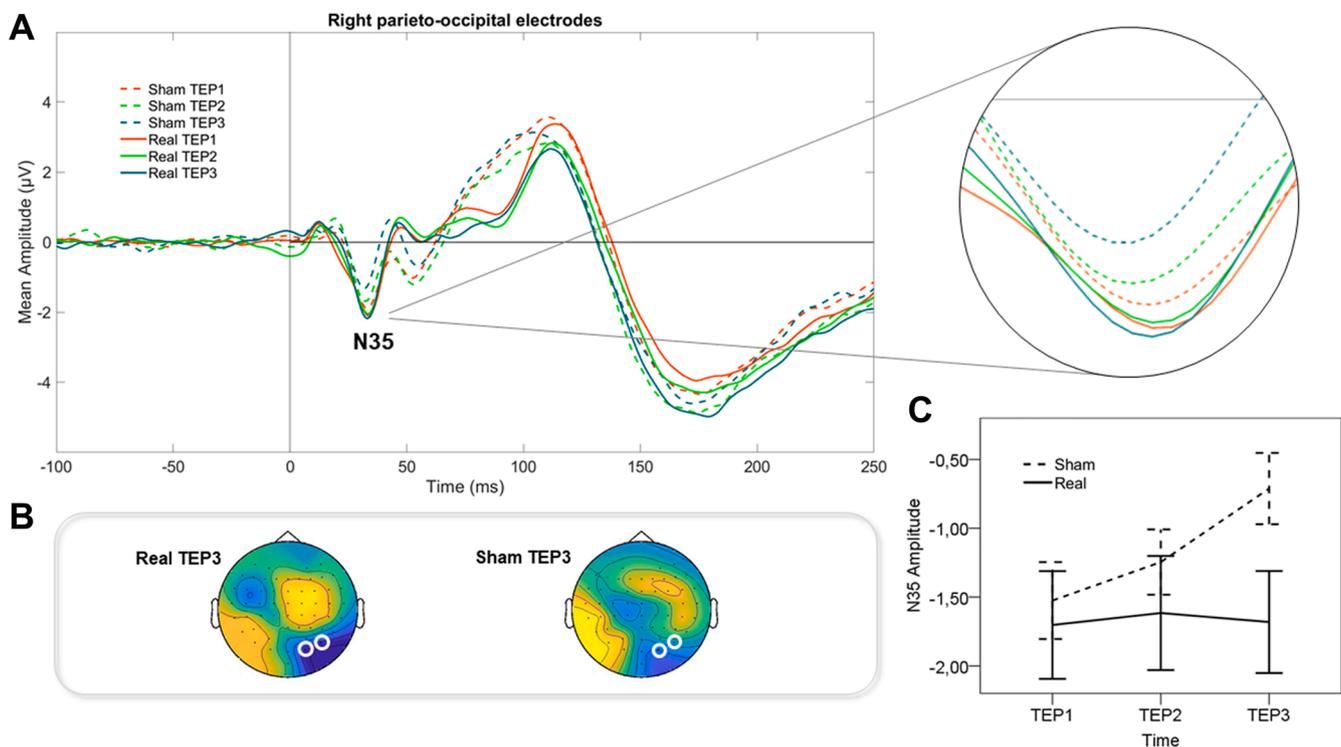
click ([ter Braack et al., 2015](#)).

There was a trend towards a Stimulation  $\times$  Time interaction in the 30–40 ms time windows ( $F(2, 60) = 2.896$ ;  $p = 0.063$ ;  $\eta_p^2 = 0.08$ ). For exploratory purposes, we contrasted the amplitude of the N35 between Real and Sham-tDCS groups at TEP1, TEP2 and TEP3 using uncorrected two-tailed t-tests. Results showed a significant reduction of the N35 component at TEP3 in the group receiving Sham as compared to Real-tDCS (Sham-tDCS:  $-0.711 \pm 0.26 \mu\text{V}$ ; Real-tDCS:  $-1.681 \pm 0.37 \mu\text{V}$ ;  $p = 0.04$ ). There were no significant differences in TEP1 and TEP2 (all  $ps > 0.445$ ) (Fig. 2).

In summary, TMS-EEG data did not reveal any significant difference in patterns of cortical excitability between Real and Sham-tDCS groups though a trend towards a sustained early cortical response only after AtDCS and task execution (i.e., TEP3) was evident in the Real-tDCS group.

### 3. Discussion

In the present study, we appraised the neurophysiological effects caused by the interaction of AtDCS and VSCL. In a previous work we showed that 3 mA AtDCS applied on PPC before the execution of a VSCL paradigm reduced the participants' ability to learn visuo-spatial configurations of stimuli ([Grasso et al., 2020](#)). Behavioural data from the present study confirmed the AtDCS-mediated reduction of VSCL. Both HIT-VSCL and IES-VSCL data showed a significant decrease, suggesting that offline AtDCS on left PPC had a detrimental effect on learning. However, unlike our previous work, the present analysis shows that the reduction in RT-VSCL was not statistically significant. Previously we showed that the AtDCS-mediated reduction in RT-VSCL was less prominent in those participants with higher accuracy scores on *new* trials as expressed by a significant direct relationship between the two variables. Therefore, we believe that the RT-VSCL discrepancy could be mainly ascribed to the higher level of participants task performance (as



**Fig. 2.** A. TEPs resulting from condition-average of O2 and PO8. Continuous lines depict TEPs from the group receiving Real-tDCS, dashed lines TEPs from the group receiving Sham-tDCS, red lines TEPs at baseline (TEP1), green lines TEPs after Real or Sham tDCS (TEP2) and blue lines after VSCL task (TEP3). B. Topographic distribution of N35 after Real or Sham tDCS and VSCL task (TEP3). White circles highlight the electrodes of interest. C. Mean amplitude values of the TEPs in the specified conditions.

measured by accuracy scores on the sole *new* trials) in the AtDCS group of the present study ( $\sim 0.84$ ) as compared to AtDCS group of our previous work ( $\sim 0.79$ ).

ERP measures collected during the execution of the task showed a significant increase in amplitude of the N1 component elicited by the presentation of repeated *old* trials as compared to non-repeated *new* trials. The increase was present in both groups (i.e., Real- and Sham-tDCS), suggesting that this component was not affected by the tDCS but was rather modulated by the repetition of visuo-spatial configurations. Previous evidence shows that the amplitude of the N1 is modulated by the allocation of visuo-spatial attention (e.g., Hillyard et al., 1998; Mangun, 1995) and is related to perceptual discrimination processes (Grasso et al., 2016; Martínez et al., 1999; Vogel & Luck, 2000). A more recent work also shows that the N1 component is reduced in strong crowding regimes revealing an association with mechanisms of distractors suppression (Ronconi et al., 2016). Thus, the here reported N1 amplitude increase could indicate that repeated trials were associated to higher levels of visuo-spatial attention, leading participants to a more efficient target-distractors segregation mirrored by enhanced discrimination processes. This result is in line with previous evidence on the same task showing that the N1 was the first component of the ERPs found to be sensitive to the repetition of the visual contexts (Schankin & Schubö, 2010).

A similar, though reversed, relationship was evident at later latencies as indexed by a positive deflection peaking around 200–250 ms post stimulus onset, likely reflecting the P2 component. In this case, trial repetition induced a reduction of the amplitude, and this likely reflects a fluid processing of the stored visuo-spatial arrays (Giesbrecht et al., 2013; Manelis & Reder, 2012). More interestingly, a main effect revealing a selective influence of AtDCS on P2 amplitude was present. In the Real-tDCS group, P2 was larger in all four ROIs, while in the Sham-tDCS group P2 was dim over fronto-central ROIs and almost absent over posterior ROIs. This component was found to be affected by various cognitive processes including spatial attention and working memory (Anillo-Vento & Hillyard, 1996; Mecklinger & Müller, 1996) and its amplitude is thought to represent a correlate of top-down processes involved in the selection of relevant information and amount of cognitive demands allocated to the task (Freunberger et al., 2007). For instance, previous evidence showed that P2 has an inverse relationship with saliency related task difficulty as revealed by larger amplitude values in contexts of low stimulus saliency (Straube & Fahle, 2010). In light of this finding, the here reported increase of P2 could have been caused by a stimulation-dependent reduction of perceived saliency leading to enhanced cognitive processing demands and increased excitability of those areas implicated in top-down attentional control during visual search. The selection of relevant information in a crowded environment generally involves an interaction between bottom-up perceptual processing and top-down processing of contextual information by working memory (e.g., Corbetta & Shulman, 2002). However, despite the importance of contextual memory-guided attention in visual search, it has been suggested that an excessive increase could reduce the formation of implicit forms of learning. This idea has been supported by TMS studies showing that inhibition of the dorsolateral prefrontal cortex improved implicit learning (Lee et al., 2013; Rosero Pahi et al., 2020) and promoted the formation of spontaneous associations (Limb and Braun, 2008; Liu et al., 2012). To note, one of these studies applied continuous theta-burst stimulation on dorsolateral prefrontal cortex before the execution of the same visuo-spatial learning task here employed. The results revealed a significant improvement of VSCL behavioural performance associated with a reduction of beta band oscillatory activity over fronto-central channels at latencies around 140 and 370 ms post-stimulus onset (Rosero Pahi et al., 2020). Here, we speculate that the selective increase of the P2 component observed in our study for the group receiving AtDCS could be a marker of a disproportionate top-down control exerted by higher order fronto-parietal areas. Indeed, source localizing studies showed that P2 likely

originates from a distributed cortical network involving both frontal and parietal regions (Maeno et al., 2004).

Considering previous evidence which highlights the crucial role of timing in tDCS-learning interactions (Fricke et al., 2011; Pirulli et al., 2013; Roth-Alpermann et al., 2006), it is unlikely that the P2 increase and VSCL decrease that we observed are mere by-products of the stimulation alone, but rather reflect the interaction between offline 3 mA AtDCS induced effects and the following task-related cortical activation. This interpretation is supported by results obtained in our previous work where we showed that online (rather than offline) application of the same AtDCS protocol did not produce such a detrimental effect on behaviour (Grasso et al., 2020).

TMS-EEG results did not find significant stimulation-mediated changes in cortical excitability measured at rest, though a trend towards a sustained early cortical response after both stimulation and task execution (i.e., TEP3) was evident in the Real-tDCS group, suggesting that a coupling between AtDCS and VSCL was necessary for changes in cortical excitability to occur.

The N100 component of TEPs was significantly reduced over time, regardless of the stimulation protocol employed. In our study, this component could reflect auditory habituation to the TMS click. Specifically, it may reflect a mix of cortical activation generated by the magnetic field and the auditory response to the TMS click, despite the use of earplugs (Conde et al., 2019).

An improvement of the TMS-EEG protocol in future studies, including identification of the target on individual MRI and orientation of the TMS coil according to gyri and sulci shapes (Gomez-Tames et al., 2018), may minimize the possibility that the lack of significant results is due to interindividual anatomic variability of the stimulation target. Similarly, the development of a method to establish TMS intensity based on the induced electric field in the stimulated cortex may also reduce inter-subject variability.

Differences between AtDCS-mediated changes of cortical responses obtained from ERP and TEP could be due to the intrinsic differences in the two type of measures. One possibility is that ERP, as an index of cortical response, is more suitable to highlight online changes associated with the interaction of tDCS- and task-mediated activation. This could be due to the very different regional interactions occurring when the brain is at rest (i.e., during TEP) as compared to when it is explicitly involved in the task execution (i.e., ERP in this work).

In conclusion, behavioural results from the present work confirmed that 3 mA AtDCS delivered over the left PPC reduced participants' ability to implicitly store visuo-spatial regularities associated with the task. The ERP data collected during the execution of the VSCL task revealed that this reduction could be associated to an AtDCS-mediated increase in top-down control exerted by higher order cortical areas as revealed by an augmented ERP response in the group receiving AtDCS. Overall, our results suggest that offline AtDCS can increase cortical responses to "sensory" stimulation (i.e., ERPs), but this increase is not necessarily associated with a concurrent learning enhancement. Our results do not necessarily imply that the application of offline AtDCS is always detrimental at the behavioural level. For instance, other studies showed that offline AtDCS could be even more effective than online AtDCS in enhancing low-level perceptual learning (e.g., Pirulli et al., 2013). However, it is possible that the combination of a relatively high current intensity (i.e., 3 mA) together with the execution of a learning task which involves a distributed network of cortical areas, has produced an imbalance between excitatory and inhibitory network mechanisms necessary for contextual learning to occur. Taken together our results suggest that the direction of tDCS induced effects are regulated within a physiological range that might not be always functional to task execution.

## 4. Experimental procedure

### 4.1. Participants

Thirty-two healthy participants took part in the study after being screened to exclude any contraindication to the use of tDCS and TMS (Antal et al., 2017; Rossi et al., 2009). Because of technical problems with EEG recording during task execution, one participant was excluded from the sole ERP analysis. This resulted in thirty-one participants included in ERP analysis (mean age: 24.5 years, SD: 4.0 years; 15 males) and thirty-two in behavioural and TEP analyses (mean age: 24.5 years, SD: 3.9 years; 16 males). All participants were naïve to the purpose of the study, were right handed (as assessed by the Edinburgh Handedness Inventory; Oldfield, 1971), had normal or corrected to normal visual acuity and were paid for their participation. Before taking part to the experiment, all participants were informed about the procedures of the study and provided written informed consent in accordance with the Declaration of Helsinki. The study was approved by the University of Trento Human Research Ethics Committee and was carried out in accordance with approved guidelines.

### 4.2. Experimental apparatus and design

The whole experiment was conducted in a dimly lit and sound attenuated room with participants seated in front of an LED monitor (24 in., refresh rate: 60 Hz, 1920x1200-pixel resolution).

Participants first completed a session of training blocks of the behavioural task (see *Visuo-Spatial contextual cueing task* section below for further details about the task). Then we proceeded with tDCS/EEG preparation and resting motor threshold (RMT) calculation (see *Transcranial magnetic stimulation* section below for further details). This was followed by the first session of TEPs acquisition (TEP1; ~5 min, i.e., pre-tDCS session). Participants were assigned to one of the two electrical stimulation protocols chosen for the present study (see *Transcranial direct current stimulation* section below) each comprising 16 participants (8 males). Therefore, participants received either Real or Sham-tDCS while listening to an audiobook (15 min) and afterwards the second session of TEP acquisition (TEP2; ~5 min, i.e., post-tDCS session) was performed. The VSCL task was then performed while EEG was recorded, and subsequently the last session of TEP (TEP3; ~5 min, i.e., post-VSCL) was administered. The entire design, including preparation time, took ~3 h for each participant (Fig. 3).

### 4.3. EEG and transcranial magnetic stimulation-EEG recording

EEG was recorded with a TMS-compatible equipment (BrainAmp, Brain Products GmbH, Munich, Germany) from 59 (FP1, FPz, FP2, AF7, AF3, AF4, AF8, F7, F5, F3, F1, Fz, F2, F4, F6, F8, FT7, FC5, FC3, FC1, FCz, FC2, FC4, FC6, FT8, T7, C5, C3, C1, Cz, C2, C4, C6, T8, TP7, CP5, CP3, CP1, CP2, CP4, CP6, TP8, P7, P5, Pz, P2, P4, P6, P8, PO7, PO3, POz, PO4, PO8, O1, Oz, O2, Iz) sintered, TMS-compatible, Ag/AgCl electrodes (EasyCap, Brain Products GmbH, Germany). Electrodes P1 and P3, corresponding to the area covered by the tDCS anode electrode, were removed from the cap. The signal was referenced online to the

right mastoid and AFz as ground electrode. Impedances between the skin and EEG electrodes were always below 5 k $\Omega$ . Vertical and horizontal electrooculogram (EOG) signals, were recorded from above and below the right eye and from the outer canthi of both eyes. A continuous recording mode, without the use of any sample-and-hold circuit, was adopted. The signal was recorded with a band-pass filter of 0.01–1000 Hz and digitized at a sampling rate of 5000 Hz (Veniero et al., 2009).

### 4.4. Transcranial magnetic stimulation

TMS pulses were delivered during a resting-state condition, while participants were sitting in a comfortable position and looked ahead toward a fixation point. All participants wore ear plugs. TMS was delivered with a Super Rapid<sup>2</sup> (Magstim, Whitland, UK) stimulator through a standard 70 mm figure-of-eight coil. Individual RMT was determined for each participant by stimulating the left primary motor cortex with the EEG cap in place, the coil placed tangentially on the scalp, and the handle pointing backward at about 45° angle from the mid-sagittal axis of the participant's head. This was done to ensure the right intensity even with the spacing of the EEG cap. The RMT was defined as the minimum intensity at which 5 out of 10 Motor-Evoked Potentials (MEPs) of at least 50  $\mu$ V (Rossini et al., 2015) could be reliably induced in the relaxed right first dorsal interosseus. The mean RMT was 66% of the maximal stimulator output (SD = 7.0). To stimulate the left posterior parietal cortex (PPC), the coil was placed tangentially to the scalp with the handle at 45° from the midline and targeting P3 (according to the International 10–20 EEG System; Herwig et al., 2003). The tDCS anode electrode was positioned beneath the EEG cap in correspondence of electrodes P3 and P1, which were removed from the EEG cap (see below *Transcranial direct current stimulation* section for further details). This procedure allowed maintaining roughly the same coil-scalp distance during both RMT calculation and TEP sessions. The use of the neuronavigation system (Softaxic Optic EMS, Bologna, Italy) allowed for a constant control of the stability of coil position throughout the session and for a high, between sessions, spatial consistency. One hundred and thirty TMS pulses for each time (TEP1, TEP2 and TEP3) were delivered over P3 at random intervals of 2–3.5 s and with stimulus intensity of 110% of the RMT.

### 4.5. Transcranial direct current stimulation

tDCS was delivered by a battery-driven DC stimulator (BrainStim EMS, Bologna, Italy) via two rubber electrodes. The 4x4 cm anode electrode was placed over the left PPC, targeting left PPC (i.e., P3). Although VSCL has been associated with bilateral activation of the PPC, there tends to be a lateralization towards the left hemisphere (Giesbrecht et al., 2013; Greene et al., 2007). As such, we chose to target left rather than right PPC. Electro-conductive paste (Ten20, Weaver and Company) was applied under the anode electrode and the EEG cap was worn above. The cathode was a 6x7 cm electrode inserted in a saline-soaked sponge placed in the upper part of the right arm, kept in place with the use of an elastic band. An extracephalic “reference/return electrode” has been used to minimize the confounding effects due to a bipolar cephalic montage, where the electrodes location and orientation

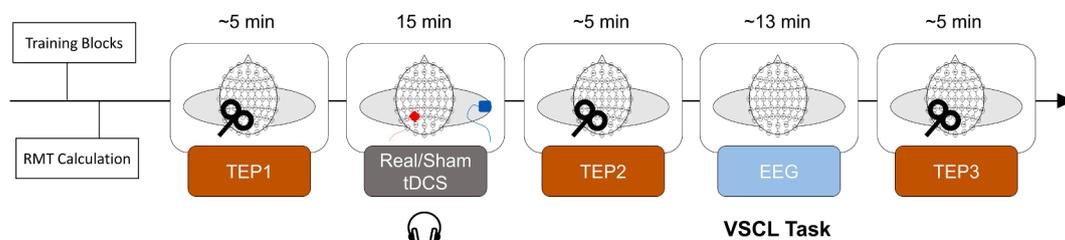


Fig. 3. Timeline of the study.

has a much greater influence on the distribution of the currents over the scalp (Im et al., 2012).

Impedance levels were below 5 k $\Omega$ . In Real-tDCS condition, current was ramped up and down over the first and last 10 s of stimulation and was applied for 15 min at an intensity of 3 mA (anode current density: 0.187 mA/cm<sup>2</sup>; cathode current density: 0.071 mA/cm<sup>2</sup>). In the control Sham-tDCS, the stimulation was applied only in the first and last 20 s (i.e., 10 s ramp up and 10 s ramp down) to mask the stimulation manipulation to participants.

At the end of the experiment, participants completed a questionnaire about the sensations experienced during tDCS in which they were asked to provide a score from 0 (none) to 4 (strong) to rate their own perception of tDCS-induced irritation, pain, burning, heat, itch, iron taste and fatigue (Antal et al., 2017; Fertonani et al., 2015).

#### 4.6. Visuo-spatial contextual learning task

The VSCL task used in the present experiment is an adapted version of the spatial contextual cueing paradigm elaborated by Chun and Jiang (Chun & Jiang, 1998). During task execution and ERP acquisitions participants had their head placed on a chinrest at 60 cm from the monitor. The task consisted of searching for a rotated “T” (90° clockwise or 90° counter clockwise) target stimulus amongst eleven heterogeneously rotated “L” distractors and reporting target stimulus orientation (i.e. the side of the long leg) by pressing “-” (counter clockwise) or “z” (clockwise) keys on the keyboard. Visual stimuli could appear within an invisible array of 8x6 grids (27°x20.5° of visual angle), were heterogeneously coloured (red, blue, green and yellow) and were presented amongst a black background. Search arrays could be either “new” or “old” (12 new arrays and 12 old arrays per block). In new arrays, the position, the colour and the rotation of the items were pseudo-randomly assigned. Target stimulus could appear at the centre of one of twelve predetermined grids, could have a random rotation (90° clockwise or counter-clockwise) and a random colour (red, green, blue or yellow). Each distractor could appear in one of the remaining grids (jittered position within grid’s possible coordinates), could have a random rotation (0°, 0°-horizontally flipped, 90°, 90°-horizontally flipped, 180°, 180°-horizontally flipped, 270°, 270°-horizontally flipped) and a pseudo-random colour. In old arrays, the position, the colour and the orientation of items were a priori defined within a set of twelve predetermined arrangements that were repeated across blocks. Only rotation of the target stimulus (90° clockwise or 90° counter-clockwise) was randomly varied. In both new and old trials, an equal number of red, blue, green and yellow items was presented for each search array and an equal number of targets was presented for each colour at the end of each block. Furthermore, in order to control for any effect of proximity to the fixation cross, the eccentricities of target locations were balanced across new and old arrays.

Each trial started with a fixation cross at the centre of the screen (jittered time between 500 and 800 ms) followed by the presentation of the search array (either new or old). Participants had to report the

orientation of the target stimulus within 1500 ms. The response was followed by an auditory feedback (200 ms; hit: 800 Hz; miss: 500 Hz; incorrect: 200 Hz). The search array disappeared whenever participants provided a response and was followed by a blank black screen (variable duration) ensuring a constant trial duration regardless of individual RTs. If no response was provided within 1500 ms, a new trial began (see Fig. 4 for task design and timeline).

Participants performed 5 training blocks at the beginning of the experimental design which included only new trials and served to get participants familiarized with the task, followed by 11 experimental blocks each including the presentation of 24 trials (12 new and 12 old).

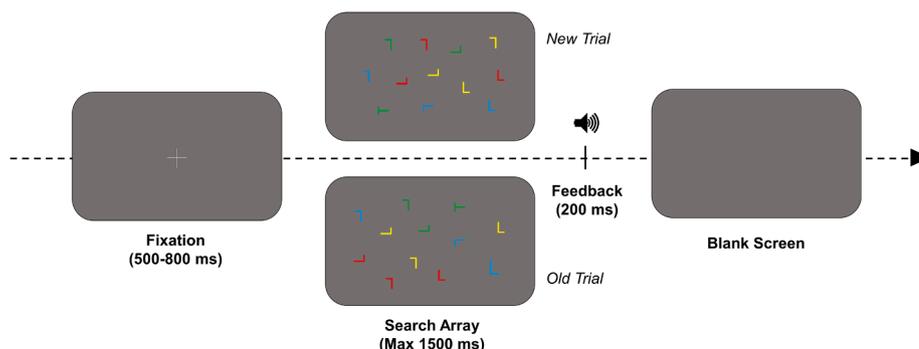
#### 4.7. Behavioural analysis

In all the employed measures, Block 1 was excluded from the analysis as contexts repetitions were de facto spanning from Block 2 to Block 11 (10 experimental blocks). Only hit trials and trials within 4 SD from the individual mean were considered (mean excluded trials - Real tDCS group: 16.4%; Sham tDCS group: 15.9%). Data were analysed using separate mixed design analyses of variance (ANOVA) with Blocks (from 2 to 11) as the within-subjects variable, and Stimulation (Real-tDCS, Sham-tDCS) as the between-subjects variable. Further analyses comprised mixed design ANOVAs on Training Blocks as well as mixed design ANOVAs on RTs, Hits and IES on the sole new trials which were performed for control purposes. To compensate for violations of sphericity, Greenhouse-Geisser corrections were applied whenever appropriate (Greenhouse & Geisser, 1959). Data from the sensations induced by tDCS were analysed using appropriate non-parametric statistics. A p-value < 0.05 was considered significant for all statistical analyses and post-hoc comparisons were corrected with Bonferroni correction for multiple comparisons.

#### 4.8. Event related potential analysis

One participant assigned to the Sham-tDCS group was excluded because of technical problems in EEG recording during the task. In line with behavioural analysis, Block 1 was excluded also from the ERP analysis.

ERP data were analysed using custom routines in MATLAB (R2018b The MathWorks, Inc, Natick, Massachusetts USA) and EEGLAB v14.1.2 (Delorme & Makeig, 2004). EEG signal was offline re-referenced to the average of both mastoids, down sampled (from 5000 to 1000 Hz; anti-aliasing filter cut-off: 0.9) and band-pass filtered from 1 to 40 Hz (type: FIR; cut-off frequency: -6 dB; 0.5 40.5 Hz; order: 3300). Epochs from -500 to 1500 ms were extracted from the continuous EEG and baseline corrected from -100 to 0 ms. Epochs with incorrect or missed responses were rejected (Real-tDCS group: 15.9%; Sham-tDCS group: 15.7%). Epochs containing muscular artifacts, horizontal eye-movements in the baseline period or blinks during stimulus presentation were discarded by visual inspection (Real-tDCS group: 4%; Sham-tDCS group: 4.6%). Subsequently, infomax independent component



**Fig. 4.** Experimental task. After varying fixation time, participants were asked to search for a rotated target “T” stimulus amongst eleven randomly rotated “L” distractors and to report the direction of the target stimulus. Half of the visuo-spatial arrays were repeated across blocks (Old Trials) while the other half was randomly generated (New Trials). A feedback sound informed about the correctness of their response followed by a blank screen (variable duration) ensuring a constant trial duration regardless of individual RTs. For graphical purposes the background is here reported as dark grey though it was black.

analysis (ICA) algorithm was run and components corresponding to the residual horizontal or vertical eye-movements were removed. After ICA correction, epochs still contaminated with artifacts were again removed by visual inspection (Real-tDCS group: 0.4%; Sham-tDCS group: 0.3%) while missing channels (P1 and P3) were interpolated (therefore data from these two electrodes should be considered not an index of direct recording). On average, the following number of epochs per conditions were considered: Real-tDCS – *new* trials: 94.1 epochs, *old* trials: 99.2 epochs, Sham-tDCS – *new* trials: 90.7 epochs, *old* trials: 102.4 epochs).

Electrodes were grouped into four separate ROIs each containing six channels: Anterior ROI (FC1, FCz, FC2, C1, Cz, C2), Central ROI (CP1, CPz, CP2, P1, Pz, P2), Posterior ROI (PO3, POz, PO4, O1, Oz, O2) and Lateral ROI (P7, P5, PO7, P6, P8, PO8). Grand-average waveforms were separately extracted from each ROI and each condition (Fig. 1B). Given the expected variability on peak latencies obtained from the different ROIs here selected, time windows for the ERP analysis were identified after a visual inspection of the ERP peaks which was conducted separately on each ROI. A shorter time window was selected for the earlier narrower components (50 ms length for both P1 and N1) while longer time windows were chosen for the later wider components of the ERP (70 ms length for P2 and 80 ms length for N2). This procedure led to the selection of the following time windows: P1 component (Posterior ROI: 70–120 ms; Lateral ROI: 80–130 ms), N1 component (Posterior ROI: 130–180 ms; Lateral ROI: 135–185 ms), P2 component (Anterior ROI: 170–240 ms, Central ROI: 180–250 ms, Posterior and Lateral ROI: 205–275 ms) and N2 component (Anterior ROI: 260–340 ms, Central ROI: 270–350 ms).

Mean amplitudes values in the specified time windows and ROIs were analysed with mixed design ANOVAs with Stimulus Type (2 levels: New Trials, Old Trials) and ROI (2 to 4 levels: Anterior, Central, Posterior and Lateral) as within-subjects variables and Stimulation (2 levels: Real-tDCS, Sham-tDCS) as between-subjects variable. Separate ANOVAs were performed for each identified ERP component and post-hoc comparisons were performed using Bonferroni corrections. To compensate for violations of sphericity, Greenhouse-Geisser corrections were applied whenever appropriate (Greenhouse & Geisser, 1959). A p-value < 0.05 was considered significant for all statistical analyses.

#### 4.9. Transcranial magnetic stimulation-EEG analysis

The EEG signals were pre-processed offline using custom scripts on MATLAB (R2019a, The MathWorks, Inc, Natick, Massachusetts, USA), and functions from EEGLAB v14.1.2 (Delorme & Makeig, 2004) and Fieldtrip (Oostenveld et al., 2011). Continuous data were linearly interpolated between –1 to 6 ms from TMS pulse, down sampled from 5000 to 1024 Hz, and filtered with a 0.1 Hz high pass filter (type: FIR; cut-off frequency: –6dB, 0.1 Hz; order: 4224). Epochs around the TMS pulse were cut from –100 ms to 400 ms post stimulus. Signal components corresponding to ocular artifacts (blinks and eye movements) were rejected using infomax ICA (up to 3 components were removed).

Then, further measurement noise was reduced by applying the source-estimate-utilizing noise-discarding algorithm (the SOUND algorithm; Mutanen et al., 2018). The same spherical 3-layer model and the same regularization parameter ( $\lambda = 0.01$ ) as applied in the original work (Mutanen et al., 2018) were applied. To account for possible non-stationarity in TMS-EEG data, SOUND was applied on 5-point nonoverlapping windows as in previous works (Bagattini et al., 2019). To reduce TMS-evoked muscle artifacts, a method combining signal-space projection and source-informed reconstruction (SSP-SIR; Mutanen et al., 2016) was applied to project out artifacts during the first 50 ms after the TMS pulse. The muscle artefact components were identified based on each component's time-frequency behaviour and the corresponding signal power (Mutanen et al., 2016). Muscular components were removed as follows: out of 96 datasets, i.e., 3 datasets by 32 subjects, one component was removed in 27 datasets, two components were removed in four datasets and three components were removed in one

dataset. The number of components removed in remaining datasets is 0. Finally, epochs were filtered with a 70-Hz low-pass filter, rejected upon visual inspection if containing residual artifacts, re-referenced to the average of all EEG channels and then baseline-corrected on the interval from –100 ms to –2 ms. The Iz electrode was excluded from analysis due to high level of residual noise.

We did not have an a priori hypothesis regarding the spatial and temporal distributions of the effects on the TEPs. Therefore, we applied the collapsed localizer strategy (Luck, 2014). More specifically, we collapsed all conditions and all electrodes in order to identify TEP components. Amplitude was measured in a time window around the peak and on a pool of electrodes showing the maximum amplitude. For each identified component we defined a time window around the peak and we visually selected from scalp distribution the pool of electrodes showing the maximum amplitude (Luck & Gaspelin, 2017). This procedure led to defining the following components: at 13–23 ms, a positive peak over O1, PO3 and PO7; at 20–30 ms, a positive peak over CPZ, CP2, CZ and C2 and a negative peak over FPZ, FP1 and FP2; at 30–40 ms, a negative peak over O2 and PO8; at 40–60 ms, a positive peak over CPZ and CZ, a positive peak over O1, PO3, PO7 and P5, and a negative peak over FPZ, FP1 and FP2; at 70–90 ms, a negative peak over CZ and FCZ, and a positive peak over O1, PO7 and P7; at 95–125 ms, a negative peak over FCZ, FC2, FZ and F2, and a positive peak over O1 and PO7; at 165–195 ms, a positive peak over CZ and FCZ.

Mean amplitude values in the specified time windows and electrode pool were analysed with mixed design ANOVAs with Time (TEP1, TEP2 and TEP3) as the within-subjects variable and Stimulation (Real-tDCS, Sham-tDCS) as the between-subjects variable. To compensate for violations of sphericity, Greenhouse-Geisser corrections were applied whenever appropriate (Greenhouse & Geisser, 1959). A p-value < 0.05 was considered significant for all statistical analyses. For exploratory purposes a limited number of uncorrected two-tailed t-tests was employed on specific TEP component of interest in order to compare Real- and Sham-tDCS effects on TEP1, TEP2 and TEP3.

#### CRedit authorship contribution statement

**Paolo A. Grasso:** Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration. **Elena Tonolli:** Software, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Project administration. **Marta Borletto:** Software, Formal analysis, Writing - original draft, Writing - review & editing. **Carlo Miniussi:** Conceptualization, Funding acquisition, Methodology, Writing - original draft, Writing - review & editing, Supervision.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.brainres.2020.147227>.

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