NEUROSCIENCE
RESEARCH ARTICLE

A. Luque-Casado et al. / Neuroscience 443 (2020) 120–130

An Integrative Clustering Approach to tDCS Individual Response Variability in Cognitive Performance: Beyond a Null Effect on Working Memory

Antonio Luque-Casado, [a†] **Rocío Rodríguez-Freiría,** [b†] **Noa Fogelson,** [a] **Eliseo Iglesias-Soler** [b] **and Miguel Fernández-del-Olmo** [a*]

[a] *Area of Sport Sciences, Faculty of Sports Sciences and Physical Education, Center for Sport Studies, King Juan Carlos University, Madrid, Spain*

[b] *Department of Physical Education and Sport, Faculty of Sports Sciences and Physical Education, University of A Coruna, Performance and Health Group, A Coruña, Spain*

Abstract—Despite the growing interest in the use of transcranial direct current stimulation (tDCS) for the modulation of human cognitive function, there are contradictory findings regarding the cognitive benefits of this technique. Inter-individual response variability to tDCS may play a significant role. We explored the effects of anodal versus sham tDCS over the left prefrontal cortex (LPFC) on working memory performance, taking into account the inter-individual variability. Twenty-nine healthy volunteers received an 'offline' anodal tDCS (1.5 mA, 15 min) to the left prefrontal cortex (F3 electrode site) in an intra-individual, cross-over, sham-controlled experimental design. n-back and Sternberg task performance was assessed before (baseline), immediately after tDCS administration (T1) and 5 min post-T1 (T2). We applied an integrative clustering approach to characterize both the group and individual responses to tDCS, as well as identifying naturally occurring subgroups that may be present within the total sample. Anodal tDCS failed to improve working memory performance in the total sample. Cluster analysis identified a subgroup of 'responders' who significantly improved their performance after anodal (vs. sham) stimulation, although not to a greater extent than the best baseline or sham condition. The proportion of 'responders' ranged from 15% to 59% across task conditions and behavioral outputs. Our findings show a high inter-individual variability of the tDCS response, suggesting that the use of tCDS may not be an effective tool to improve working memory performance in healthy subjects. We propose that the use of clustering methods is more suitable in identifying 'responders' and for evaluating the efficacy of this technique. **© 2020 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: non-invasive brain stimulation, transcranial direct current stimulation, inter-individual variability, cluster analysis, cognition, working memory.

INTRODUCTION

Working memory provides the ability to temporary store and manipulate information over a short period of time, representing a critically relevant function in our daily activities (Weng et al., 2019). Working memory function supports a wide range of higher order cognitive skills (Johnson et al., 2013), and its dysfunction is a core feature in a range of neuropsychiatric disorders including depression (Baune et al., 2014), schizophrenia (Potkin et al., 2009), Parkinson's disease (Fallon et al., 2017) as well as in post-chemotheraphy cognitive deficits (Wang et al., 2016). Therefore, investigation into methods that may boost working memory ability may be of clinical relevance.

Transcranial direct current stimulation (tDCS) is an emerging form of non-invasive techniques of brain stimulation, that has come to the fore in recent years, as a promising tool for modulating spontaneous cortical activity (Nitsche and Paulus, 2000). It has been proposed that tDCS shifts target cortical areas excitability in a polarity-dependent manner, i.e., anodal stimulation induces hypopolarization, while cathodal induces hyperpolarization (Nitsche and Paulus, 2001; Zaghi et al., 2010). However, others suggest that both the outcomes and mechanisms of tDCS are far more complex (Giordano et al., 2017; Jamil et al., 2017; Kronberg et al., 2017). For a more detailed description of the mechanisms of tDCS see (To et al., 2016; Stagg et al., 2018).

*Corresponding author. Address: Rey Juan Carlos University. Departamental II, Campus Alcorcón, Avenida de Atenas s/n, 28922 Alcorcón, Madrid, Spain.
E-mail address: miguel.delolmo@urjc.es (M. Fernández-del-Olmo).
† Contributed equally to this manuscript.
Abbreviations: DLPFC, dorsolateral prefrontal cortex; LPFC, left prefrontal cortex; LTP, long-term potentiation; tDCS, transcranial direct current stimulation.

https://doi.org/10.1016/j.neuroscience.2020.07.036
0306-4522/© 2020 IBRO. Published by Elsevier Ltd. All rights reserved.

Animal and human studies suggest that anodal tDCS modifies the threshold of action potentials and induces long-term potentiation (LTP)-like mechanisms (Fritsch et al., 2010). Thus, the use of tDCS as a potential therapeutic or enhancing tool for the modulation of motor or cognitive performance has grown exponentially. Indeed, a number of studies have been published over the last years, claiming that anodal tDCS over the dorsolateral prefrontal cortex (DLPFC) is able to benefit working memory performance in both healthy (Fregni et al., 2005; Hoy et al., 2013) and neuropsychiatric populations (Loo et al., 2012; Hoy et al., 2014). Nonetheless, the true enhancement potential of tDCS for working memory remains uncertain, with recent meta-analyses showing that the effects are reliable though small (Hill et al., 2016), limited to response times but not accuracy (Brunoni and Vanderhasselt, 2014; Dedoncker et al., 2016), or nonexistent (Horvath et al., 2015; Medina and Cason, 2017).

Several methodological aspects may contribute to the inconclusive findings in this field. The heterogeneity of the tDCS protocols that are employed is one important source of variability in the efficacy of this technique (Dedoncker et al., 2016). Thus, tDCS protocols utilize different variables for the stimulus duration, current intensity, electrode size, position and polarity. Another key element is the use of a small sample size (average $n = 18$ in within-subjects designs), resulting in low statistical power (approximately 14% for the cognitive studies in general, and 5% for the working memory studies in particular), and a small proportion of studies that have been replicated (Medina and Cason, 2017). This is especially critical when considering the influence of a wide variety of other individual factors such as age, gender, hormones, cognitive ability or differences in head anatomy, which are also likely to modulate the effects of tDCS (Wurzman et al., 2016). In fact, recent tDCS studies have shown that only around 50% of individuals demonstrate the typical polarity-specific modulation of motor cortex excitability (López-Alonso et al., 2014; Wiethoff et al., 2014). Thus, due to publication bias (Medina and Cason, 2017), effect sizes in single studies with small samples might be substantially overestimated (Minarik et al., 2016), while meta-analyses may underestimate the efficacy of tDCS by including underpowered studies reporting null effects (Héroux et al., 2017).

New methodological approaches are required in order to account for the high variability in the responsiveness to tDCS stimulation (Parkin et al., 2015; Filmer et al., 2020). A potentially useful approach to address this issue would be the systematic use of formally agreed sample stratification methods. A recent study has used this methodological approach to explore the effects of tDCS over the DLPFC on working memory in healthy subjects (Luque-Casado et al., 2019). The study showed that a single session of 'offline' anodal tDCS stimulation failed to improve the memory span performance for the total sample of participants. Crucially, a clustering procedure identified that only 47% of the subjects exhibited an increase in performance after anodal tDCS, although it did not significantly exceed the baseline performance. These findings highlighted the importance for the use of clustering methods in order to determine tDCS-linked cognitive outcomes. However, some factors limited the representativeness of this preliminary finding. Working memory was assessed using the digit span task, a simple task that depends on the total number of remembered items, mainly involving memory storage, with only limited demands on other working memory processes. Consequently, a ceiling effect may have limited the tDCS effects from emerging in this study (Tseng et al., 2012; Mancuso et al., 2016). In addition, the possibility that the efficacy of the technique was selectively linked to more complex executive abilities (i.e., updating, shifting and inhibition) (Wu et al., 2014; Nejati et al., 2017; Imburgio and Orr, 2018) or different types of behavioral output (Brunoni and Vanderhasselt, 2014; Dedoncker et al., 2016), still remain to be clarified.

In the current study, we proposed an intra-individual, cross-over, sham-controlled experimental design aimed to test the hypothesis that anodal tDCS over the left prefrontal cortex (LPFC) has a performance enhancing effect on working memory in healthy subjects. To this end, we used a two-step methodological approach by exploring the behavioral response to tDCS both in the whole sample size and an enriched sample obtained through a cluster analysis procedure. This approach allowed us to stratifies the sample and identify the subgroup of subjects in which the effect of the anodal tDCS was significantly different from the effect of sham tDCS ('responders'), and whether this effect differs from 'non-responders'. We used the n-back task and Sternberg task, two paradigms frequently employed as indexes of working memory function in tDCS research in which inconclusive results have been reported (Hill et al., 2016). Importantly, in order to guarantee a homogeneous range of variation in performance avoiding floor or ceiling effects to detect the influence of stimulation (Tseng et al., 2012; Luque-Casado et al., 2019), we individually assigned the level of task difficulty based on a previous load-adaptive training session.

EXPERIMENTAL PROCEDURES

Participants

A total of 29 Caucasian healthy subjects (4 women), aged between 18 and 25 years (mean age ± SD: 19.8 ± 2.0), participated in the study from an initial sample of 31 subjects. Two subjects were excluded after not completing all the experimental sessions of the study. Subjects with neurological, psychiatric, including a past medical history of head injury or seizures, or other contraindications to undergo the tDCS were excluded from the study. This study was conducted in full compliance with the Declaration of Helsinki 1964 (updated in Fortaleza, 2013) and approved by the Local Ethics Committee of the University of A Coruña. All participants signed a contraindication form and gave written informed consent before the experimental sessions.

General procedure

We used a double-blind, cross-over, sham controlled experimental design. The experiment was carried out in

five separate experimental sessions. First, a familiarization session was performed in which participants initially practiced the two cognitive tasks (n-back and Sternberg). This session also allowed us to evaluate the level of task execution in order to individually adapt the task difficulty in the subsequent experimental sessions. Then, each participant performed four separate blind sessions corresponding to each stimulation condition and cognitive task (anodal tDCS and sham tDCS for the n-back and Sternberg task). The order of the sessions was counterbalanced and a 'wash-out' period was implemented in order to minimize carry-over effects for stimulation condition and task interference. In all the subjects we implemented a 'wash-out' period of exactly 15 days between each of the three first sessions, and a seven days 'wash-out' period prior to the fourth session. Thus, there was no variation in the duration of the 'wash-out' period across the participants. Each experimental session began with a familiarization period in which participants initially completed the cognitive task (n-back or Sternberg) as a practice trial. Then, after a 5-minute rest interval, cognitive testing commenced with a baseline trial of the task, prior to the stimulation period. Stimulation (anodal tDCS or sham tDCS) was carried out 'offline' and lasted a total of 15 min. The participants remained seated and were instructed to avoid unnecessary movements during the stimulation period in order to minimize any possible interference. Immediately after the stimulation, two trials (T1 and T2) of the task were performed, separated by a 5-minute rest interval.

Cognitive tasks

We used two different paradigms in order to evaluate working memory performance, the n-back and the Sternberg task. In both cases, the level of difficulty was adapted to the execution of each subject by considering an acceptable level of performance while allowing some range of improvement (Fregni et al., 2005; Martin et al., 2013). To this aim, we established a level of difficulty in which participants reached and maintained a percentage of response accuracy between 80% and 85%. This procedure allowed us to homogenize the likelihood to detect any impairment or improvement following tDCS by standardizing the baseline cognitive performance.

n-back task

We used a MATLAB pre-programmed application (Matlab 2010, Mathworks Inc.) to control the stimulus presentation and response collection. We tested the performance in the n-back task, and the maximum level of difficulty achieved by each subject (from a minimum 'n' of 2-back to a maximum 'n' of 7-back). In order to individually adjust the difficulty, we established a response accuracy output between 80% and 85%.

The task comprised a total of 40 trials. Each trial began with the presentation of an uppercase letter in white color randomly generated from a set of 21 consonants. Each letter stimulus was presented for 500 ms in the middle of the screen on a gray background. Participants had to compare each new letter with that presented 'n' times before, and respond whether they matched or not by pressing two different keys on the PC Keyboard. The maximum time to give a response was 2000 ms and trials without response within this time period were considered as errors. The inter-stimulus interval lasted 1000 ms.

Sternberg task

We used the *Psychology Experiment Building Language* (PEBL) software (Mueller and Piper, 2014) to control the stimulus presentation and response collection. The task comprised a total of 75 trials divided into 3 blocks (25 trials per block). Each block included the presentation of a memory set containing 'n' letters, which the participants memorized for 15 s before the letters disappeared. In each trial, participants were then presented with a probe letter and where instructed to use a button press to indicate whether the probe was present or absent in the memory set. The probe stimulus was presented at the center of the screen in white color on a black background and was randomly generated from a set of 21 uppercase consonant letters.

We tested the individual task performance in the familiarization session in order to individually adjust the task difficulty level. The maximum level of difficulty achieved by each participant ranged from a minimum of 8 to a maximum of 14 letters. Thus, since the task was divided into 3 blocks of increasing difficulty, two different sequences of difficulty increase were established in order to maintain a response accuracy output between 80% and 85%. Each sequence was composed of 3 blocks of increasing difficulty as follows: Level 1 (memory set of 8, 10 and 12 letters for each block, respectively) and Level 2 (memory set of 10, 12 and 14 letters for each block, respectively). Participants were encouraged to respond as quickly and accurately as possible. Since the time until response was not limited, only the response accuracy output was taken into consideration in this task.

For both, n-back and Sternberg tasks 80–85% accuracy rate was chosen since it has been shown from pilot data to be adequate for keeping the subjects motivated during the performance of the utilized tasks, in comparison with lower accuracy rates. In addition, although a ceiling effect may not be completely ruled out, a previous study showed no ceiling effects on behavior using similar accuracy rates (Corbin and Marquer, 2013).

tDCS

Anodal tDCS was delivered for 15-minutes at 1.5 mA through a pair of saline-soaked sponge surface electrodes of 35 cm^2 (current density of 0.04 mA/cm^2) connected to an aDC stimulator (neuroConn). The stimulation parameters were chosen according to two meta-analysis studies, which indicated that a current density greater than 0.029 mA/cm^2 for stimulation periods greater than 10 min are more likely to have a

significant effect in cognitive tasks (Dedoncker et al., 2016; Hill et al., 2016).

The active electrode (anode) was placed over the left PFC and the reference electrode (cathode) was placed over the contralateral supraorbital region (F3 and Fp2 electrode position according to the International 10-20 EEG system, respectively). Current was faded in and out for 10 s in both the anodal and sham stimulation conditions. For the sham tDCS condition the current of the anodal tDCS was switched off after 60 s of stimulation.

Statistical analysis

All statistical analyses were performed using SPSS software (SPSS, Chicago, IL). Two different statistical approaches were employed in order to explore the effect of the tDCS: a traditional analysis of variance (ANOVA) of repeated measures and a cluster analysis. In order to control for any difference in baseline performance, all analyses were carried out using the standardized units both in RT and response accuracy (Δ = [(T1 or T2 − baseline)/baseline]). The normal distribution of data was corroborated by means of the Shapiro-Wilk test and Q-Q plot inspection.

Analysis of variance

Repeated-measures ANOVA with intra-subject factors Condition (anodal tDCS vs. sham tDCS) and Trial (T1 and T2) or Condition (anodal tDCS vs. sham tDCS), Trial (T1 and T2) and Set (Set 1, Set 2 and Set 3) were conducted in order to compare the performance in the n-back task and the Sternberg task, respectively. We explored the potential order effect by introducing Order (anodal-sham or sham-anodal) as the between-subject factor. The interaction of the Order factor with any variable of interest (i.e., Condition, Trial or Set) was reported when applicable. Post-hoc analyses were conducted using Bonferroni corrections when necessary. Sphericity was tested by means of the Mauchley sphericity test and the Green-House Geisser correction was applied when violation of this assumption occurred.

Cluster analysis

In order to address the high inter-individual variability in response to non-invasive brain stimulation protocols (López-Alonso et al., 2014; Luque-Casado et al., 2019), a follow-up cluster analysis on the whole sample was applied to better characterize the subjects' response to tDCS, and to identify naturally occurring subgroups that may be present. Cluster analysis was carried out in two stages.

First, we used a hierarchical cluster analysis with the Ward clustering method and squared Euclidean distance as dissimilarity measure. We used this analysis in order to determine the number of clusters and the centroid for each of them and to identify outliers. To this end, a hierarchical relationship between the cases and/or clusters was represented in a standardized scale dendogram from 0 to 25, where the longer the distance, the greater the dissimilarity was between the clusters. In the present study, clusters were identified if the distance was lower than 7. We considered a case as an outlier if it had a greater dissimilarity compared to the rest of the cases without joining any of the identified clusters (Clatworthy et al., 2005), or if the distance was higher than 15.

Second, we performed a k-means cluster analysis using the number of clusters and the centroids identified in the first stage. The number of clusters was considered appropriate if there was a maximum of two changes in the cluster assignment of the subjects from the first analysis and if the distance between the centroids of the clusters was always greater than the distance of any individual case to the centroid within each cluster.

We assumed that some subjects would respond to the anodal tDCS at T1 and others at T2, due to possible different time courses of the tDCS. Therefore, we carried out two separate cluster analyses for T1 and for T2 using the normalized units of task performance (ΔT1 or ΔT2). Cluster analysis 1 was performed for T1 in order to identify a subgroup that showed significant improvement in performance immediately after anodal tDCS vs. the sham condition. Cluster analysis 2 was performed for T2 in order to identify a subgroup that showed significant improvement in performance in the subsequent 5 min after anodal tDCS vs. the sham condition.

The definition of the clusters was based on the value of the centroids. A subgroup was identified as 'responder' when subjects improved their performance after the anodal tDCS (but not sham), or improved their performance to a greater extent after the anodal vs. the sham condition. When the cluster analysis revealed a responsive subgroup, we proceed to test whether the subjects in that group improved significantly their performance for the anodal in comparison with the sham stimulation. Thus, we conducted a separate repeated-measures ANOVA only for the 'responders' subgroup with Condition (anodal tDCS vs. sham tDCS) and Trial (baseline, T1 or T2) as intra-subject factors in the n-back task and Condition (anodal tDCS vs. sham tDCS), Trial (baseline, T1 and T2) and Set (Set 1, Set 2 and Set 3) for the Sternberg task. Note that this analysis was performed on the absolute values of task performance considering the baseline as a level of the Trial factor. This allowed us to identify whether performance in T1 or T2 was significantly different in absolute terms from baseline performance regardless of the magnitude of the change. Post-hoc analyses using Bonferroni corrections were conducted when necessary. Finally, we also performed paired samples *t*-tests of the normalized units of task performance (ΔT1 or ΔT2) in order to corroborate any tDCS effects after controlling for baseline differences.

RESULTS

A total of 116 tDCS sessions (both anodal and sham) were performed. During the anodal tDCS sessions 18

subjects (62%) occasionally experienced mild and transient adverse effects during stimulation, such as 'itching', 'burning' or 'discomfort' but these did not disrupt the stimulation sessions. During the anodal and sham tDCS sessions 53% and 52% of the subjects believed that they were being stimulated, respectively, confirming the success of the blinding procedure.

n-Back

The repeated-measures ANOVA comparing RT showed no significant effects for Condition, Trial, or the Condition*Trial interaction. Similarly, the response accuracy did not show any statistically significant main effects or interactions (Fig. 1).

The factor Order did not show any significant interaction with the variables of interest, except for the Condition*Trial*Order interaction of the response accuracy ($F_{1,27}$ = 6.66 p = .02). Further post-hoc analyses indicated a greater increase of the response accuracy from baseline in the anodal (vs. sham condition) at T2, only when anodal session was carried out first (t_{12} = 2.40 p = .03). Importantly, the response accuracy achieved in the anodal condition at T2 for the group that performed the anodal condition first, did not significantly improve the baseline performance across groups and conditions. None of the remaining post-hoc comparisons reached statistical significance.

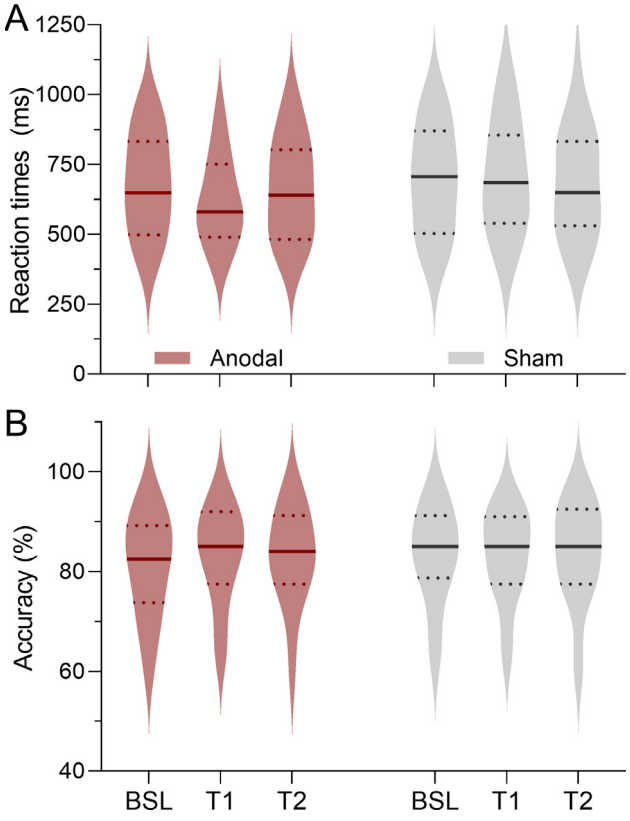

Fig. 1. Median (horizontal line) and interquartile range (horizontal dotted line) reaction times **(A)** and response accuracy **(B)** in the total sample as a function of Condition (anodal tDCS [red] and sham tDCS [gray]) and Trial (baseline [BSL], trial 1 [T1] and trial 2 [T2]) in the n-back task. The shaded area (violin) illustrates kernel density estimation to show the distribution shape of the data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The cluster analysis of the RT normalized values identified a subgroup of 'responders' of 12 (41%) and 13 (45%) subjects at T1 and T2, respectively (Fig. 2). The clustering procedure did not report any outliers. The repeated-measures ANOVA comparing RT for the subgroup of 'responders' at T1 showed a significant main effect for Trial ($F_{1,11}$ = 6.68 p = .025), a non-significant effect for Condition and a significant Condition*Trial interaction ($F_{1,11}$ = 12.21 p < .005). A paired samples t-test of the T1 baseline-normalized scores confirmed a significantly greater improvement in performance in anodal tDCS compared with sham tDCS (t_{11} = −3.45 p = .005). Further post-hoc analysis showed faster RT at T1 (vs. baseline) after anodal tDCS (t_{11} = 4.06 p = .002) but not after sham tDCS. However, the RT performance at T1 after anodal tDCS was not significantly superior to baseline or T1 values during the sham tDCS (Table 1). The ANOVA for 'responders' at T2 showed non-significant main effect for Condition or Trial, but a significant Condition*Trial interaction ($F_{1,12}$ = 4.92 p = .047). A paired samples t-test of the T2 baseline-normalized scores confirmed a significant improvement in performance after anodal tDCS with respect to sham tDCS (t_{12} = −2.41 p = .033). Post-hoc analysis showed a non-significant decrease in RT at T2 (vs. baseline) after anodal tDCS, accompanied by a significant increase in the sham condition tDCS (t_{12} = −2.59 p = .02). Importantly, the RT performance at T2 after anodal tDCS was not superior to baseline or T2 values in the sham tDCS (Table 1).

The cluster analysis of the response accuracy normalized scores did not show an appropriate consistency between clustering methods (hierarchical Ward and k-means) and thus did not identify a subgroup of 'responders' at T1 or T2 (Fig. 2).

Sternberg task

The repeated-measures ANOVA comparing response accuracy showed non-significant effects for Condition, Trial, Set, and interactions (Fig. 3). The factor Order did not show any significant interaction with the variables of interest.

The cluster analysis of the response accuracy normalized scores identified the following subgroups of 'responders': 12 subjects (46%) at T1 (3 outliers) and 4 subjects (15%) at T2 (2 outliers) for Set 1; 17 subjects (59%) at T1 (no outliers) and 16 subjects (57%) at T2 (1 outlier) for Set 2; 6 subjects (21%) at T1 (no outliers) and 9 subjects (32%) at T2 (1 outlier) for Set 3 of the Sternberg task (Fig. 4).

The repeated-measures ANOVA comparing response accuracy for the subgroup of 'responders' showed non-significant effects for Condition and Trial in all the cases, except for Set 3 at T1 (Condition, $F_{1,5}$ = 25.80 p = .004; Trial, $F_{1,5}$ = 30.61 p = .003). There was a significant interaction between Condition and Trial in all the cases: Set1-T1 ($F_{1,11}$ = 12.08 p = .005), Set1-T2

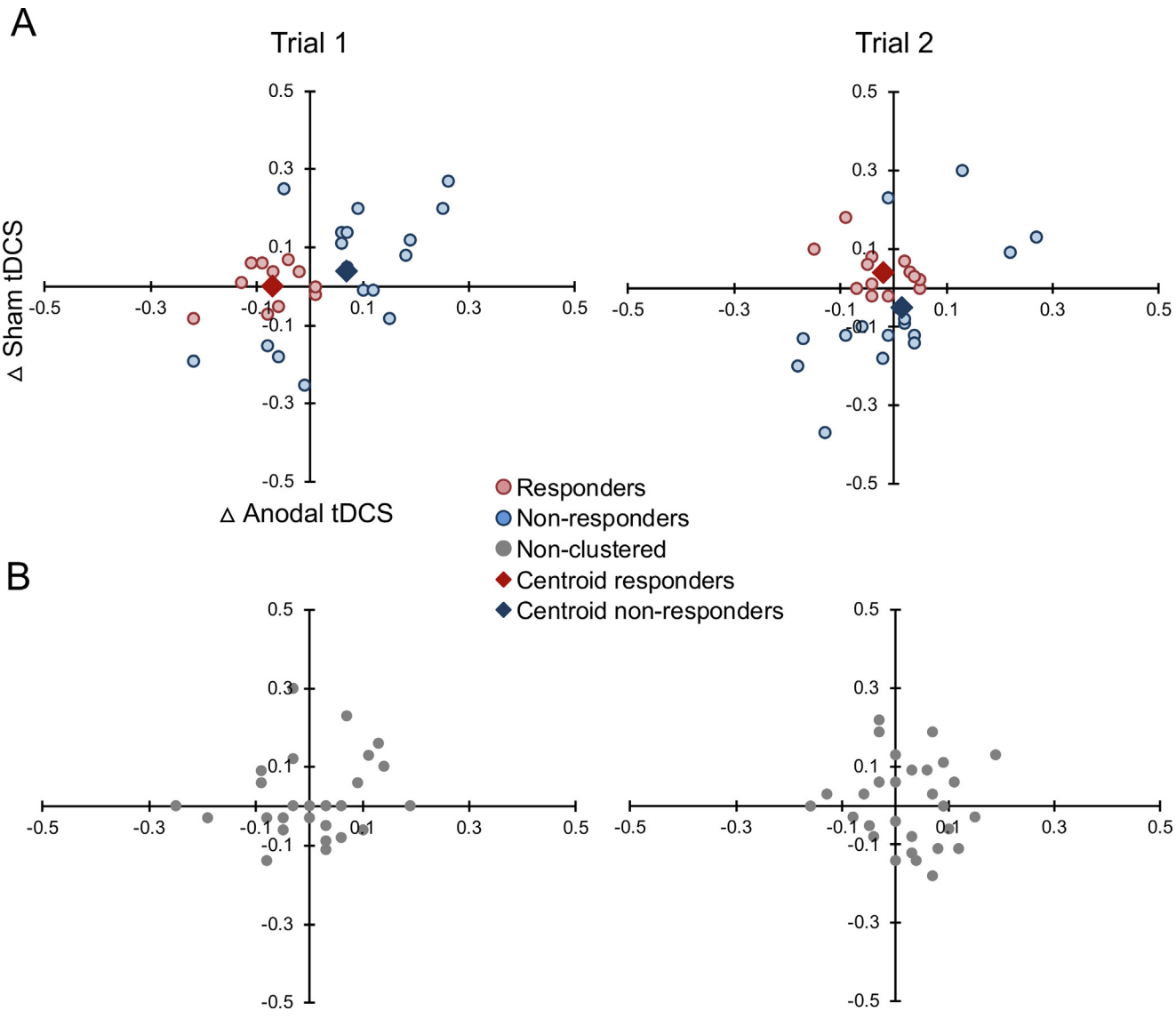

Fig. 2. Cluster representation for the 'responders' subgroup in n-back task using the normalized reaction times **(A)** and response accuracy **(B)** for anodal and sham tDCS condition at Trial 1 and 2 (i.e., Δ = [(T1 or T2 – baseline)/baseline]). The definition of the 'responders' clusters was based on the value of the centroids (represented when applicable). The remaining identified clusters are merged as a 'non-responders' subgroup for representation purposes. Cluster analysis on response accuracy did not report sufficient consistency between clustering methods (hierarchical Ward and *k*-means) for the identification of homogeneous subgroups ('non-clustered' cases).

($F_{1,3}$ = 12.36 p = .039), Set2-T1 ($F_{1,16}$ = 20.65 p < .001), Set2-T2 ($F_{1,15}$ = 13.09 p = .003), Set3-T1 ($F_{1,5}$ = 22.47 p = .005) and Set3-T2 ($F_{1,8}$ = 38.89 p = < .001). Additionally, paired samples *t*-tests on T1 and T2 change scores confirmed a significantly greater improvement in performance in anodal tDCS (vs. sham tDCS) after baseline normalization in all the cases: Set1-T1 (t_{11} = 3.39 p = .006), Set1-T2 (t_3 = 3.30 p = .046), Set2-T1 (t_{16} = 4.27 p = .001), Set2-T2 (t_{15} = 3.74 p = .002), Set3-T1 (t_5 = 5.66 p = .002) and Set3-T2 (t_8 = 7.52 p < .001). Further post-hoc analyzes reported a significant increase in performance after anodal tDCS (vs. baseline) in Set 1-T2 (t_3 = −5.00 p = .015), Set 3-T1 (t_5 = −8.43 p < .001) and Set 3-T2 (t_8 = −4.99 p = .001), but not after sham tDCS; and a significant impairment of performance after sham tDCS (vs. baseline) in Set1-T1 (t_{11} = 2.61 p = .024), Set 2-T1 (t_{16} = 3.27 p = .005) and Set 2-T2 (t_{15} = 2.98 p = .01), but not after anodal tDCS. However, the response accuracy after anodal tDCS at T1 or T2 was not significantly superior to the performance in sham tDCS in any case (Table 1).

DISCUSSION

In the present study, we aimed to explore the effects of anodal vs. sham tDCS over the LPFC on the working memory performance, by considering inter-individual variability in healthy subjects. To this end, we used a two-step methodological approach by exploring the behavioral response to tDCS in two working memory task paradigms, both in the whole sample size and in an

Table 1. Mean (±standard deviation) for the n-back and Sternberg task output as a function of Condition (anodal tDCS vs. sham tDCS) and Trial (Baseline, T1 or T2) in 'responders'

n-back (reaction times [ms])				
	'Responders' T1 (n = 12)		**'Responders' T2 (n = 13)**	
	Baseline	**T1**	**Baseline**	**T2**
Anodal tDCS	753.6 (178.0)	699.3 (171.2)*	741.8 (230.4)	719.8 (216.1)
Sham tDCS	752.5 (148.4)	755.6 (167.2)	707.9 (211.4)	739.0 (228.0)*
Sternberg task (response accuracy [%])				
Set 1	**'Responders' T1 (n = 12)**		**'Responders' T2 (n = 4)**	
	Baseline	**T1**	**Baseline**	**T2**
Anodal tDCS	87.33 (9.47)	90.00 (6.66)	84.00 (9.09)	91.50 (6.81)*
Sham tDCS	90.67 (6.79)	88.17 (7.31)*	87.50 (8.85)	84.50 (11.82)
Set 2	**'Responders' T1 (n = 17)**		**'Responders' T2 (n = 16)**	
	Baseline	**T1**	**Baseline**	**T2**
Anodal tDCS	74.59 (8.49)	79.41 (7.90)	73.75 (9.52)	78.00 (7.16)
Sham tDCS	80.94 (8.40) #	75.29 (9.08)*	80.25 (9.03) #	73.88 (10.39)*
Set 3	**'Responders' T1 (n = 6)**		**'Responders' T2 (n = 9)**	
	Baseline	**T1**	**Baseline**	**T2**
Anodal tDCS	65.00 (5.48)	81.67 (7.84)*	68.22 (8.63)	80.67 (8.12)*
Sham tDCS	77.33 (8.45) #	80.67 (7.55)	76.22 (8.15) #	66.67 (10.39)

*Indicates statistical significant differences with respect to baseline ($p < .05$); #Indicates statistical significant differences between anodal tDCS and sham tDCS at baseline ($p < .05$).

enriched sample obtained through a cluster analysis procedure. The results showed that a single session of 'offline' anodal tDCS stimulation did not improve the working memory performance for the total sample of participants. The cluster analysis identified a subgroup of 'responders' who significantly improved their performance after anodal (vs. sham) stimulation. However, this tDCS induced improvement in task performance still did not significantly exceed those observed during the baseline and sham conditions.

The absence of any effect of anodal tDCS on the n-back or the Sternberg task performance in the total sample is in agreement with previous evidence (Marshall et al., 2005; Teo et al., 2011; Lally et al., 2013; Nilsson et al., 2015) and seems to challenge studies demonstrating positive after-effects of tDCS on behavioral performance using the same tasks (Ohn et al., 2008; Keeser et al., 2011; Mulquiney et al., 2011; Berryhill and Jones, 2012; Gladwin et al., 2012; Hoy et al., 2013). Differences in the stimulation parameters, specifically of the current density and density charge, stand out in the literature as the main contributing factors to inconsistent results across studies (Dedoncker et al., 2016). Thus, the stimulation parameters that we used (0.043 mA/cm^2 for 15 min) may not have been sufficient to elicit a performance modulation. However, mixed results have been reported using current and charge density values both higher (Keeser et al., 2011; Nilsson et al., 2015) and lower (Mulquiney et al., 2011; Lally et al., 2013) than those used here, which reduces this probability and points to the presence of other mediating factors that may account for the inconclusive results.

All the studies that are methodologically comparable to ours and that have reported a beneficial effect of tDCS, used a relatively small numbers of subjects (average of n = 13, ranging from 10 to 18) resulting in low statistical power (Medina and Cason, 2017). By contrast, the studies that have used larger sample sizes (average of n = 25, ranging from 21 to 30), reported null tDCS after-effects on working memory performance (Lally et al., 2013; Nilsson et al., 2015), or positive effects that were limited to a proportion of the total sample (Berryhill and Jones, 2012). Thus, it seems that only a subset of individuals may experience real enhancement effects (Ziemann and Siebner, 2015) and these may be overrepresented in small sample sizes leading to inflated effect sizes (Minarik et al., 2016). Furthermore, using large heterogeneous samples may mask a real effect in a subgroup of subjects, resulting in overall null or small effect sizes. These are relevant methodological issues that affect the reliability of tDCS studies.

We propose that a cluster analysis is one methodological approach that may account for the inter-individual variability often observed in tDCS studies, by identifying the presence of a subgroup of 'responders' in the total sample. These 'responders' are subjects that either improved their performance after the anodal (but not sham) tDCS condition, or improved their performance to a greater extent after the anodal vs. the sham condition. The average proportion of 'responders' in our total sample was about 40%, which is in line with reports of 47% of subjects showing positive tDCS after-effects on memory span performance (Luque-Casado et al., 2019) and with the proportion of subjects having

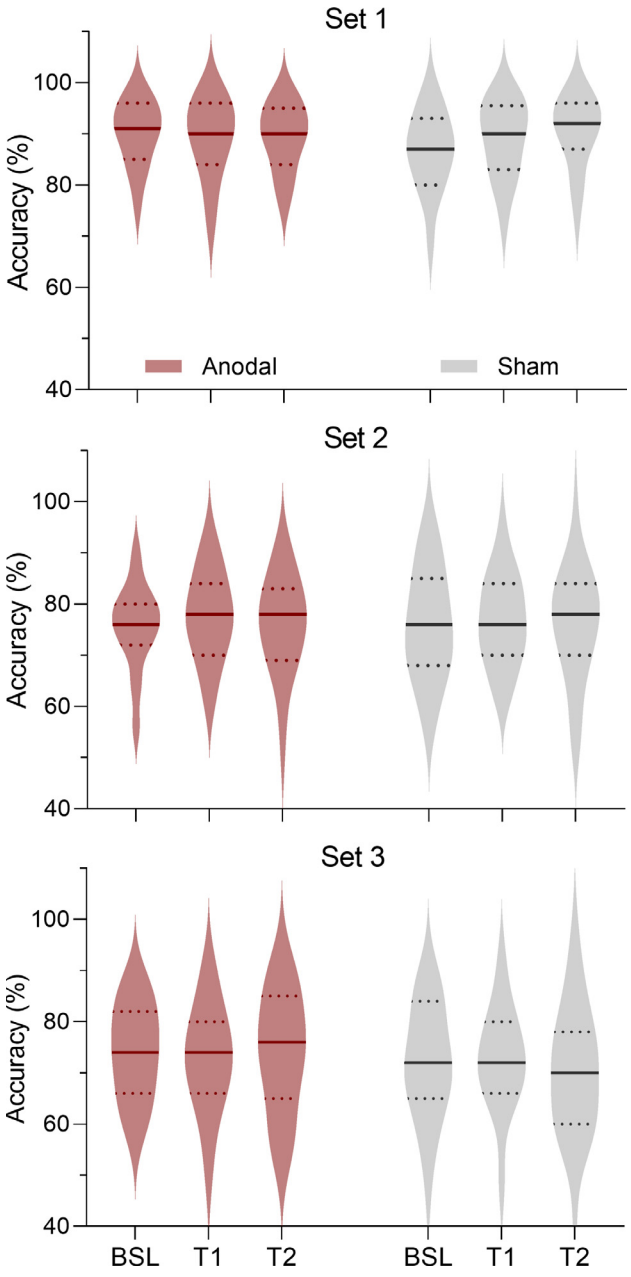

Fig. 3. Median (horizontal line) and interquartile range (horizontal dotted line) response accuracy in the total sample as a function of Condition (anodal tDCS [red] and sham tDCS [gray]), Trial (baseline [BSL], trial 1 [T1] and trial 2 [T2]) and Set (1, 2 and 3) in the Sternberg task. The shaded area (violin) illustrates kernel density estimation to show the distribution shape of the data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the typical polarity-specific modulation in the excitability of the motor cortex (López-Alonso et al., 2014; Wiethoff et al., 2014). However, our results showed that the proportion of 'responders' ranged from 15% to 59% and we were unable to identify 'responders' when we used the accuracy of response index in the n-back task. These findings highlight the high variability and instability in the individual response patterns, and that these may also vary across tasks and behavioral outputs.

Overall, our findings together with those of a recent study (Luque-Casado et al., 2019) showed that even in the 'responders' subgroup, the effect of anodal tDCS on working memory did not exceed the baseline or sham condition performance. We demonstrated these findings across different cognitive tasks (i.e., digit span backwards, n-back and Sternberg), involving different processes (i.e., storage, retrieval, updating, shifting and inhibition), and type of behavioral output (RT and response accuracy). Thus, our results do not support the hypothesis that a single session of 'offline' anodal tDCS over the left DLPFC is an effective means to improve working memory performance. It has been shown that the effects of tDCS on cognitive measures are less robust and less predictable compared with the more consistent effects on motor outcomes, especially when using single-session tDCS designs in which there are small effects amid high variability confounded by individual differences (Berryhill and Martin, 2018). The mechanisms of non-invasive brain stimulation of the motor system are understood to a greater extent, showing greater correspondence between input and output (Fricke et al., 2011). However, in the case of tDCS of the DLPFC, the relationship between the input and the behavioral outcomes is far more complex, since the tDCS induced-changes in cortical excitability do not necessarily translate into behavioral effects (Hill et al., 2017, 2019). Furthermore, the mechanisms linking stimulation-induced changes in cortical excitability with complex cognitive processes remain largely unknown (De Berker et al., 2013; Bestmann et al., 2015; Bonaiuto and Bestmann, 2015).

Our findings support the growing consensus that variability in response to non-invasive brain stimulation is a consistent and significant issue in the field of tDCS and cognition research (Parkin et al., 2015; Guerra et al., 2017). So far, the assessment of the real effect of tDCS has probably been hindered (at least in part) by a possible publication bias (Medina and Cason, 2017), leading to over-reporting of a non-natural predominance of 'responders' among small sample sizes, contributing to the poor replicability in this research area (Héroux et al., 2017). Thus more systematic studies need to be performed in order to maximize consistent and replicable findings, especially when utilizing long term stimulation protocols in which more consistent broader cognitive benefits seem to emerge (Berryhill and Martin, 2018). A potentially useful approach is to analyze individual differences rather than averaging across a group, when comparing variables that may affect stimulation efficacy. Further investigating the factors contributing to the variability of tDCS effects on behavior may potentially facilitate the design of more effective tailored interventions of neuromodulation techniques (Ziemann and Siebner, 2015).

To summarize, our results suggest that a single session of 'offline' anodal tCDS over the LPFC is not an effective means to improve working memory performance in healthy subjects and confirms the existence of heterogeneous patterns of behavioral effects across participants. These findings highlight the

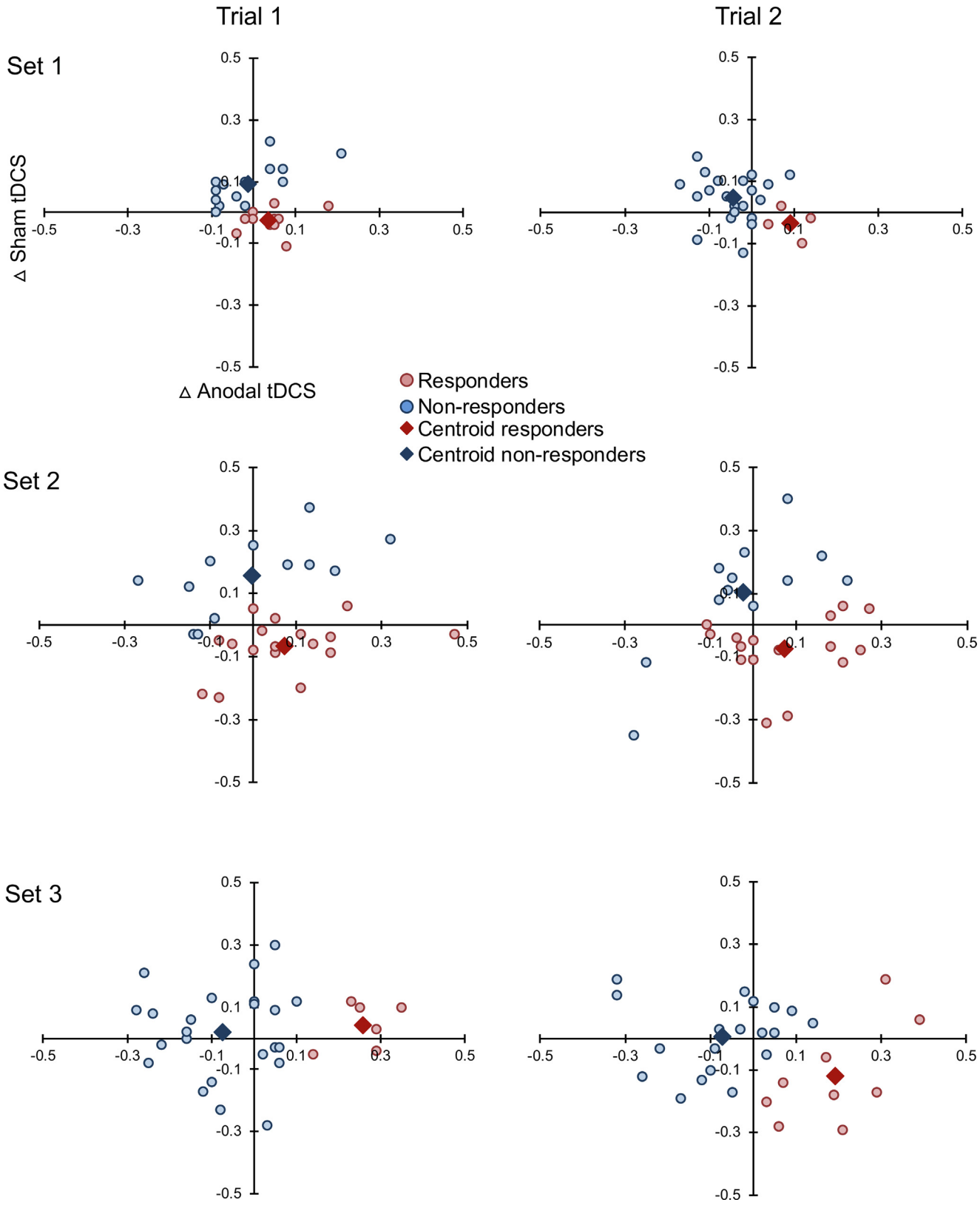

Fig. 4. Cluster representation for the 'responders' subgroup as a function of Set (1, 2 and 3) in Sternberg task using the normalized response accuracy for anodal and sham tDCS condition at Trial 1 and 2 (i.e., Δ = [(T1 or T2 – baseline)/baseline]). The definition of the 'responders' clusters was based on the value of the represented centroids. The remaining identified clusters are merged as a 'non-responders' subgroup for representation purposes.

need to optimize methodological approaches in order to account for individual variability and appropriate sample size selection. This will ensure a greater transparency and representativeness of the findings by providing further insight in to tDCS-linked cognitive outcomes.

ACKNOWLEDGEMENTS

This work was supported by a 'Juan de la Cierva' postdoctoral grant from the Spanish 'Ministerio de Ciencia, Innovación y Universidades' (FJCI-2016-28405) to Antonio Luque-Casado, 'Ramón y Cajal' national fellowship program to Noa Fogelson and a project grant funded by 'Rey Juan Carlos' University (REtDCS) to Miguel Fernández-del-Olmo, Noa Fogelson and Antonio Luque-Casado. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

Baune BT, Fuhr M, Air T, Hering C (2014) Neuropsychological functioning in adolescents and young adults with major depressive disorder–a review. Psychiatry Res 218:261–271.

Berryhill ME, Jones KT (2012) tDCS selectively improves working memory in older adults with more education. Neurosci Lett 521:148–151.

Berryhill ME, Martin D (2018) Cognitive effects of transcranial direct current stimulation in healthy and clinical populations: an overview. J ECT 34:e25–e35.

Bestmann S, de Berker AO, Bonaiuto J (2015) Understanding the behavioural consequences of noninvasive brain stimulation. Trends Cognitive Sci 19:13–20.

Bonaiuto JJ, Bestmann S (2015) Understanding the nonlinear physiological and behavioral effects of tDCS through computational neurostimulation. In: Bestmann S, editor. Computational neurostimulation - progress in brain research. Elsevier: Amsterdam. p. 75–103.

Brunoni AR, Vanderhasselt M-A (2014) Working memory improvement with non-invasive brain stimulation of the dorsolateral prefrontal cortex: a systematic review and meta-analysis. Brain Cogn 86:1–9.

Clatworthy J, Buick D, Hankins M, Weinman J, Horne R (2005) The use and reporting of cluster analysis in health psychology: a review. Br J Health Psychol 10:329–358.

Corbin L, Marquer J (2013) Is Sternberg's memory scanning task really a short-term memory task? Swiss J Psychol 72:181–196.

De Berker AO, Bikson M, Bestmann S (2013) Predicting the behavioral impact of transcranial direct current stimulation: issues and limitations. Front Hum Neurosci 7:613.

Dedoncker J, Brunoni AR, Baeken C, Vanderhasselt M-A (2016) A systematic review and meta-analysis of the effects of transcranial direct current stimulation (tDCS) over the dorsolateral prefrontal cortex in healthy and neuropsychiatric samples: influence of stimulation parameters. Brain Stimulation 9:501–517.

Fallon SJ, Mattiesing RM, Muhammed K, Manohar S, Husain M (2017) Fractionating the neurocognitive mechanisms underlying working memory: independent effects of dopamine and parkinson's disease. Cereb Cortex 27:5727–5738.

Filmer HL, Mattingley JB, Dux PE (2020) Modulating brain activity and behaviour with tDCS: Rumours of its death have been greatly exaggerated. Cortex 123:141–151.

Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, Marcolin MA, Rigonatti SP, et al. (2005) Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res 166:23–30.

Fricke K, Seeber AA, Thirugnanasambandam N, Paulus W, Nitsche MA, Rothwell JC (2011) Time course of the induction of homeostatic plasticity generated by repeated transcranial direct current stimulation of the human motor cortex. J Neurophysiol 105:1141–1149.

Fritsch B, Reis J, Martinowich K, Schambra HM, Ji Y, Cohen LG, Lu B (2010) Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. Neuron 66:198–204.

Giordano J, Bikson M, Kappenman ES, Clark VP, Coslett HB, Hamblin MR, Hamilton R, Jankord R, et al. (2017) Mechanisms and effects of transcranial direct current stimulation. Dose Response 15. 1559325816685467.

Gladwin TE, den Uyl TE, Fregni FF, Wiers RW (2012) Enhancement of selective attention by tDCS: interaction with interference in a Sternberg task. Neurosci Lett 512:33–37.

Guerra A, López-Alonso V, Cheeran B, Suppa A (2017) Variability in non-invasive brain stimulation studies: reasons and results. Neurosci Lett. 133330.

Héroux ME, Loo CK, Taylor JL, Gandevia SC (2017) Questionable science and reproducibility in electrical brain stimulation research. PLoS ONE 12 e0175635.

Hill AT, Fitzgerald PB, Hoy KE (2016) Effects of anodal transcranial direct current stimulation on working memory: a systematic review and meta-analysis of findings from healthy and neuropsychiatric populations. Brain Stimul 9:197–208.

Hill AT, Rogasch NC, Fitzgerald PB, Hoy KE (2017) Effects of prefrontal bipolar and high-definition transcranial direct current stimulation on cortical reactivity and working memory in healthy adults. NeuroImage 152:142–157.

Hill AT, Rogasch NC, Fitzgerald PB, Hoy KE (2019) Impact of concurrent task performance on transcranial direct current stimulation (tDCS)-induced changes in cortical physiology and working memory. Cortex 113:37–57.

Horvath JC, Forte JD, Carter O (2015) Quantitative review finds no evidence of cognitive effects in healthy populations from single-session transcranial direct current stimulation (tDCS). Brain Stimul 8:535–550.

Hoy KE, Arnold SL, Emonson MRL, Daskalakis ZJ, Fitzgerald PB (2014) An investigation into the effects of tDCS dose on cognitive performance over time in patients with schizophrenia. Schizophr Res 155:96–100.

Hoy KE, Emonson MRL, Arnold SL, Thomson RH, Daskalakis ZJ, Fitzgerald PB (2013) Testing the limits: investigating the effect of tDCS dose on working memory enhancement in healthy controls. Neuropsychologia 51:1777–1784.

Imburgio MJ, Orr JM (2018) Effects of prefrontal tDCS on executive function: methodological considerations revealed by meta-analysis. Neuropsychologia 117:156–166.

Jamil A, Batsikadze G, Kuo H-I, Labruna L, Hasan A, Paulus W, Nitsche MA (2017) Systematic evaluation of the impact of stimulation intensity on neuroplastic after-effects induced by transcranial direct current stimulation. J Physiol 595:1273–1288.

Johnson MK, McMahon RP, Robinson BM, Harvey AN, Hahn B, Leonard CJ, Luck SJ, Gold JM (2013) The relationship between working memory capacity and broad measures of cognitive ability in healthy adults and people with schizophrenia. Neuropsychology 27:220–229.

Keeser D, Padberg F, Reisinger E, Pogarell O, Kirsch V, Palm U, Karch S, Möller H-J, et al. (2011) Prefrontal direct current stimulation modulates resting EEG and event-related potentials in healthy subjects: a standardized low resolution tomography (sLORETA) study. NeuroImage 55:644–657.

Kronberg G, Bridi M, Abel T, Bikson M, Parra LC (2017) Direct current stimulation modulates LTP and LTD: activity dependence and dendritic effects. Brain Stimul 10:51–58.

Lally N, Nord CL, Walsh V, Roiser JP (2013) Does excitatory fronto-extracerebral tDCS lead to improved working memory performance? F1000Res 2:219.

Loo CK, Alonzo A, Martin D, Mitchell PB, Galvez V, Sachdev P (2012) Transcranial direct current stimulation for depression: 3-week, randomised, sham-controlled trial. Br J Psychiatry 200:52–59.
López-Alonso V, Cheeran B, Río-Rodríguez D, Fernández-Del-Olmo M (2014) Inter-individual variability in response to non-invasive brain stimulation paradigms. Brain Stimul 7:372–380.
Luque-Casado A, Fogelson N, Iglesias-Soler E, Fernandez-Del-Olmo M (2019) Exploring the effects of transcranial direct current stimulation over the prefrontal cortex on working memory: a cluster analysis approach. Behav Brain Res 375 112144.
Mancuso LE, Ilieva IP, Hamilton RH, Farah MJ (2016) Does transcranial direct current stimulation improve healthy working memory?: A meta-analytic review. J Cognit Neurosci 28:1063–1089.
Marshall L, Mölle M, Siebner HR, Born J (2005) Bifrontal transcranial direct current stimulation slows reaction time in a working memory task. BMC Neurosci 6:23.
Martin DM, Liu R, Alonzo A, Green M, Player MJ, Sachdev P, Loo CK (2013) Can transcranial direct current stimulation enhance outcomes from cognitive training? A randomized controlled trial in healthy participants. Int J Neuropsychopharmacol 16:1927–1936.
Medina J, Cason S (2017) No evidential value in samples of transcranial direct current stimulation (tDCS) studies of cognition and working memory in healthy populations. Cortex 94:131–141.
Minarik T, Berger B, Althaus L, Bader V, Biebl B, Brotzeller F, Fusban T, Hegemann J, et al. (2016) The importance of sample size for reproducibility of tDCS effects. Front Hum Neurosci 10:453.
Mueller ST, Piper BJ (2014) The psychology experiment building language (PEBL) and PEBL test battery. J Neurosci Methods 222:250–259.
Mulquiney PG, Hoy KE, Daskalakis ZJ, Fitzgerald PB (2011) Improving working memory: exploring the effect of transcranial random noise stimulation and transcranial direct current stimulation on the dorsolateral prefrontal cortex. Clin Neurophysiol 122:2384–2389.
Nejati V, Salehinejad MA, Nitsche MA, Najian A, Javadi A-H (2017) Transcranial direct current stimulation improves executive dysfunctions in ADHD: implications for inhibitory control, interference control, working memory, and cognitive flexibility. J Atten Disord. 1087054717730611.
Nilsson J, Lebedev AV, Lövdén M (2015) No significant effect of prefrontal tDCS on working memory performance in older adults. Front Aging Neurosci 7:230.
Nitsche MA, Paulus W (2000) Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 527:633–639.
Nitsche MA, Paulus W (2001) Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. Neurology 57:1899–1901.
Ohn SH, Park C-I, Yoo W-K, Ko M-H, Choi KP, Kim G-M, Lee YT, Kim Y-H (2008) Time-dependent effect of transcranial direct current stimulation on the enhancement of working memory. NeuroReport 19:43–47.
Parkin BL, Ekhtiari H, Walsh VF (2015) Non-invasive human brain stimulation in cognitive neuroscience: a primer. Neuron 87:932–945.
Potkin SG, Turner JA, Brown GG, McCarthy G, Greve DN, Glover GH, Manoach DS, Belger A, et al. (2009) Working memory and DLPFC inefficiency in schizophrenia: the FBIRN study. Schizophr Bull 35:19–31.
Stagg CJ, Antal A, Nitsche MA (2018) Physiology of transcranial direct current stimulation. J ECT 34:144–152.
Teo F, Hoy KE, Daskalakis ZJ, Fitzgerald PB (2011) Investigating the role of current strength in tDCS modulation of working memory performance in healthy controls. Front Psychiatry 2:45.
To WT, Hart J, Ridder DD, Vanneste S (2016) Considering the influence of stimulation parameters on the effect of conventional and high-definition transcranial direct current stimulation. Expert Rev Med Devices 13:391–404.
Tseng P, Hsu T-Y, Chang C-F, Tzeng OJL, Hung DL, Muggleton NG, Walsh V, Liang W-K, et al. (2012) Unleashing potential: transcranial direct current stimulation over the right posterior parietal cortex improves change detection in low-performing individuals. J Neurosci 32:10554–10561.
Wang L, Apple AC, Schroeder MP, Ryals AJ, Voss JL, Gitelman D, Sweet JJ, Butt ZA, et al. (2016) Reduced prefrontal activation during working and long-term memory tasks and impaired patient-reported cognition among cancer survivors postchemotherapy compared with healthy controls. Cancer 122:258–268.
Weng W, Liang J, Xue J, Zhu T, Jiang Y, Wang J, Chen S (2019) The transfer effects of cognitive training on working memory among chinese older adults with mild cognitive impairment: a randomized controlled trial. Front Aging Neurosci 11:212.
Wiethoff S, Hamada M, Rothwell JC (2014) Variability in response to transcranial direct current stimulation of the motor cortex. Brain Stimul 7:468–475.
Wu Y-J, Tseng P, Chang C-F, Pai M-C, Hsu K-S, Lin C-C, Juan C-H (2014) Modulating the interference effect on spatial working memory by applying transcranial direct current stimulation over the right dorsolateral prefrontal cortex. Brain Cogn 91:87–94.
Wurzman R, Hamilton RH, Pascual-Leone A, Fox MD (2016) An open letter concerning do-it-yourself users of transcranial direct current stimulation. Ann Neurol 80:1–4.
Zaghi S, Acar M, Hultgren B, Boggio PS, Fregni F (2010) Noninvasive brain stimulation with low-intensity electrical currents: putative mechanisms of action for direct and alternating current stimulation. Neuroscientist 16:285–307.
Ziemann U, Siebner HR (2015) Inter-subject and Inter-session variability of plasticity induction by non-invasive brain stimulation: boon or bane? Brain Stimul 8:662–663.

(Received 25 March 2020, Accepted 20 July 2020)
(Available online 28 July 2020)