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Research report

Electrode montage dependent effects of transcranial direct current stimulation on semantic fluency

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HIGHLIGHTS

- TDCS effects on a semantic fluency task were evaluated.
- The comparative effectiveness of different electrode montages was tested.
- None of the different montages improved fluency immediately after the stimulation.
- Yet, left frontal anodal stimulation improved fluency ~18 min after tDCS end.
- These findings add new information on tDCS spatial and temporal dynamics.

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ABSTRACT

Transcranial direct current stimulation (tDCS) has proved to be valuable in improving many language processes. However, its influence on verbal fluency still needs to be fully proved. In the present study, we explored the effects of different electrode montages on a semantic fluency task, aimed at comparing their effectiveness in affecting language production. Ninety healthy, right-handed volunteers were randomly assigned to receive one of the following stimulation protocols: (1) anode over the left frontal cortex/cathode over the right supraorbital (rSO) area, (2) anode over the left fronto-temporal (lFT) cortex/cathode over the rSO area, (3) anode over the lFT cortex/cathode over the right FT cortex, (4) anode over the lFT cortex/big-size cathode over the rSO area, (5) sham. In the active stimulation conditions, 2 mA current was delivered for 20 min. Participants performed the semantic fluency task before the stimulation, immediately after it, and 15 min after the first post-stimulation task. Although none of the different protocols improved language production immediately after the stimulation, anodal stimulation over the left frontal cortex (standard-size cathode over the rSO area) improved fluency at the second post-stimulation task. This proved that small differences in either active electrode positioning, or reference positioning/size can impact tDCS behavioral effects also in the cognitive domain. These findings, which can be sometimes missed when tested immediately after the stimulation only, add new information on tDCS spatial and temporal features, thus providing new indications to increase the effectiveness of stimulation protocols.

1. Introduction

Transcranial direct current stimulation (tDCS) has been shown to facilitate linguistic processing in a growing number of studies on both healthy individuals and patients with some kind of language deficit [8,21]. However, whereas some tasks have been widely investigated (e.g., picture naming), other tasks, like verbal production driven by phonemic or semantic cues, have received little attention, despite their relevance for both basic and clinical research. In this regard, the few studies addressing tDCS effects on either phonemic or semantic fluency (typically assessed by requiring to produce exemplars beginning with a given letter or belonging to a given semantic category, respectively) showed partially incongruent results. Specifically, a significant increase of healthy individuals' phonemic fluency was first reported by Iyer et al. [13]. This effect resulted from 20 min of 2 mA anodic stimulation over the left prefrontal cortex (in correspondence of F3 electrode of 10–20 International EEG System), with cathode over

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the contralateral supraorbital area. Remarkably, neither a stimulation of 1 mA with the same electrode set-up, nor the reverse electrode positioning, with a stimulation of 2 mA, was found to facilitate phonemic fluency. A subsequent study [12] tested the effects of an identical tDCS protocol (except for a stimulation duration of 40 min) on a sample of patients with fronto-temporal dementia and reported no phonemic fluency improvement immediately after the stimulation. More recently, Cattaneo et al. [5] investigated the effects of tDCS on both phonemic and semantic fluency. They used the same duration and intensity parameters used by Iyer et al. [13], but moved the active electrode from prefrontal to frontal cortex (i.e., intersection between T3-Fz and F7-Cz, which the authors identified with the inferior frontal gyrus, IFG). Using this protocol, they found an improvement of both semantic and phonemic fluency when anode was over the left cortex, but not when it was over the right cortex (control condition). In a subsequent study, Vannorsdal et al. [31] found no improvement of phonemic fluency and only a marginally significant improvement of semantic fluency following anodal stimulation of left dorsolateral prefrontal cortex (active electrode in F3). As this last study differed in many tDCS parameters with respect to those illustrated above (i.e., 30 min of stimulation, 1 mA of current intensity, reference electrode in Cz), incongruent findings may depend just from one or more of these parameters. Finally, two other studies tested tDCS effects on verbal fluency using functional magnetic resonance imaging (fMRI) to explore the neural underpinning of stimulation [19,26]. In detail, Pereira et al. [26] found an improvement of phonemic, but not semantic, fluency, in patients with Parkinson's disease, after 20 min of 2 mA anodic stimulation in F3. Instead, Meinzer et al. [19] found an improvement of semantic fluency (phonemic fluency was not assessed) in healthy volunteers, during 1 mA anodic stimulation of a part of frontal cortex approximately corresponding to the crossing point between T3–F3 and F7–C3, which also these authors identified with the IFG.

In light of the reviewed results, it is clear that findings in literature are somewhat inconsistent, and the possibility that tDCS can improve verbal fluency is still not fully proved. Nonetheless, in those cases in which stimulation successfully enhanced fluency, improvements have been found with anodal stimulation over the left pre-frontal [13,26], or inferior frontal [5,19] areas, whereas other relevant stimulation control-conditions (i.e. left pre-frontal cathodal [13], right inferior frontal anodal [5], left temporo-parietal anodal [26] stimulations) appeared to produce no effects. More specifically, pre-frontal stimulations seem to improve (although not in all cases) phonological fluency [13,26], but not semantic fluency [26,31], whereas inferior frontal stimulations seem to improve both kinds of fluency [5,19]. In this regard, however, it has to be mentioned that the only two studies [5,19] reporting positive tDCS effects on fluency, by stimulating what they both identified with the IFG, placed the active electrode sponges in largely overlapping, though not perfectly equivalent, areas (the area stimulated by Meinzer et al. [19], being slightly shifted down with respect to that stimulated by Cattaneo et al. [5], included also a little portion of temporal lobe). Such little differences in electrode positioning could be relevant especially for semantic fluency, given that neuropsychological and neuroimaging evidence attested an involvement of left frontal cortex in both fluency tasks, but a stronger involvement of left temporal cortex in semantic than in phonemic fluency [1,9]. Hence, specifically for semantic fluency, a stimulation involving both frontal and temporal areas [19] could be, in principle, more effective than a stimulation involving only the frontal areas [5] (starting from the hypothesis that the activation of a greater portion of a neural network subserving a given process could facilitate that process more than the activation of a smaller portion of the same network). In this regard, a direct comparison (i.e., within the same study) between the effects of these two montages on semantic fluency is still missing. Further, the qualitative comparison between the available data [5,19] is limited by the fact that Meinzer at al. [19] considerably modified the structure of their semantic fluency task due to the requirements of fMRI research protocols. Therefore, we addressed this issue in the present study, which directly compared the use of two slightly different stimulation placements in correspondence of the left IFG [5,19], aimed at testing whether they can modulate semantic fluency to a different extent. The outcomes could potentially enrich the knowledge on both neural networks involved in standard semantic fluency tasks, and the spatial resolution feature of tDCS. Remarkably, this kind of methodological investigations are still needed, because, at least in the cognitive domain, the behavioral effects of little variations in electrode positioning have not yet been fully investigated for the more standard tDCS protocols (i.e., those using 16–35 cm^2 electrodes). Therefore, we cannot rule out that such small differences play a relevant role in determining variations of results across different studies. As regards our fluency task, on the basis of the above described tDCS studies [5,19] we expected a facilitation of production for both stimulation sites; however, based on neuropsychological and neuroimaging evidence [1,9] the montage in which the active electrode covered also a little portion of temporal cortex [19] (hereafter referred as "fronto-temporal") should induce a bigger facilitation compared with that covering only the frontal cortex [5] (hereafter referred as "frontal"). Starting from this hypothesis we compared the fronto-temporal montage to other two montages, each differing in only one feature. Specifically these differences were related to the reference electrode, whose position and size can affect the current path through the brain from the active electrode [3].

We compared the fronto-temporal montage, with a less used montage (hereafter referred as "bilateral"), only different in the position of the reference electrode: whereas in the former the reference electrode was placed over the supraorbital region contralateral to the active electrode, in the latter the reference was placed over the homologue contralateral area (i.e., the right fronto-temporal cortex). Bilateral (or bi-hemispheric) stimulations of homologue areas simultaneously modulate such areas in opposite directions (i.e., enhancing excitability under the anode and, at the same time, diminishing excitability under the cathode), and therefore could potentially be used to improve tDCS efficacy [23]. Further, since bilateral montages enhance hemispheric lateralization (i.e., by greatly facilitating the anodally stimulated hemisphere, at the expense of the other), they can be particularly useful to assess the specific contribution of each hemisphere to the processes under investigation. Recent data showed the selective influence of bilateral modulation not only in motor [32], but also in various cognitive [16,25,27] and linguistic [28,30,33] domains. As regard the last research field, although most linguistic processes are highly left-lateralized, we cannot ignore the right hemisphere involvement in lexical and semantic processing [4,14,17]. However, the relevance of the right hemisphere contribution to these processes has not been fully clarified yet. In this view, we tested the effects of the bilateral montage to determine whether increasing cortical excitability in the left hemisphere, while simultaneously decreasing excitability in the right hemisphere, could improve semantic fluency.

Furthermore, we also tested a montage identical to the fronto-temporal one, except for having a big-size cathode. Given that an increase of electrode size reduces its efficacy (via a reduction of its current density), using a big-size electrode as reference can help to increase the focality of stimulation, by minimizing unwanted effects of reference on the underlying cortex (for this reason we called this montage as "unilateral"). This has been shown in the motor domain [22], where increasing the size of the reference electrode threefold in relation to the active electrode made its

Table 1
Absolute values of performance in the fluency and simple reaction time tasks as a function of stimulation condition and session.

Stimulation condition	Sample features	Fluency task			SRT task	
	Gender Age (yrs)	Pre-stim (word numb.)	Post-stim 1 (word numb.)	Post-stim 2 (word numb.)	Pre-stim (RT)	Post-stim 1 (RT)
Frontal	11f,7 m 23.2 ± 0.2	20.83 ± 1.40	21.28 ± 1.44	24.61 ± 1.48	290.73 ± 6.64	290.78 ± 8.02
Fronto-temporal	11f,7 m 20.7 ± 0.5	25.53 ± 1.44	25.24 ± 1.48	25.12 ± 1.52	296.50 ± 6.56	278.82 ± 4.27
Bilateral	11f,7 m 21.7 ± 0.6	23.72 ± 1.40	24.94 ± 1.44	24.44 ± 1.48	290.73 ± 6.63	277.60 ± 6.15
Unilateral	11f,7 m 21.4 ± 0.5	21.17 ± 1.40	21.06 ± 1.44	22.44 ± 1.48	283.25 ± 4.79	283.58 ± 5.86
Sham	11f,7 m 21.0 ± 0.5	22.78 ± 1.40	22.67 ± 1.44	22.17 ± 1.48	286.48 ± 8.81	268.25 ± 3.68

Notes: SRT: simple reaction time; yrs: years; RT: reaction times; f: females; m: males; values represent mean ± SE of the variables indicated in brackets in the second table row.

stimulation functionally inefficient without compromising the effects under the active electrode. Among the reported tDCS studies on verbal fluency, only one used a big-size cathode as reference [19], but the effects of this montage were not directly compared with those achievable with an identical montage except for a standard-size reference electrode. We investigated this issue in the present study, in which we hypothesized that reference electrode size could be relevant in affecting neuro-modulation under the active electrode, and therefore we expected different behavioral effects for the two montages differing in this feature only.

Finally, a further strength of the present study was the administration of the fluency task not only immediately after the stimulation, but also after a short interval from the stimulation end (~18 min, that is 15 min after the first assessment). Follow-up tests are quite common in the clinical domain to evaluate potential long-term effects of repeated sessions of stimulation. However, very few studies on healthy individuals have planned a re-administration of the same tasks following the first one. A recent review on tDCS application in the linguistic domain [21] confirmed that only two out of ten studies on healthy volunteers included some kind of follow-up measure [18,29], and none of the quoted tDCS studies on linguistic fluency included such a form of delayed retests. Given that little is still known about the time-extension of the effects induced by single-tDCS sessions on healthy individuals' cognitive processing, in the present research we included both an immediate and a delayed assessment of stimulation effects.

In summary, to the best of our knowledge, the present study investigated for the first time the immediate and short-term behavioral effects induced on semantic fluency by different tDCS protocols (some of which already tested in previous studies, some others not yet tested for semantic fluency). Their relative effectiveness was compared with a critical focus on both methodological and applicative implications. From a methodological perspective, we were interested in collecting new information on the spatial and temporal features of this technique of brain modulation. This aim is particularly relevant when considering that tDCS potential and limitations still need to be fully estimated, and relatively "simple" tasks (like the one we used) can be particularly suitable for this kind of methodological research. From an applicative point of view, detecting the montage more effective in ameliorating semantic fluency pursued the clinical goal of improving this function in patients with deficits in language production.

2. Methods and materials

2.1. Participants

Ninety healthy university students (55 females, 35 males, mean ± SE age: 21.59 ± 0.23 years, see Table 1 for the demographic features of each experimental group) participated in the study. All volunteers were right-handed [24] native Italian-speakers, naïve as to the purpose of the study. They reported no history of neurologic/psychiatric disorder, seizure, head trauma, and declared the absence of metal in the head and implanted electrical devices. Participants gave their written informed consent to take part in the study in accordance with the principles of the Declaration of Helsinki. The local ethical committee approved the study, which followed the safety procedures of non-invasive brain stimulation [2].

2.2. Tasks and procedure

A single-blind, sham-controlled, between-groups design was used: participants were randomly assigned to one of five stimulation conditions (one consisting of the sham protocol), without being informed about the kind of stimulation they received. Therefore, the participants (18 in each stimulation group: 11 females) underwent a single experimental session lasting about one hour (see Fig. 1A for the experimental procedure timeline).

The participants were asked to perform a semantic fluency task (i.e. to produce as many exemplars as possible belonging to a given category within one minute) three times: before the stimulation (*pre-stimulation test*, considered as a baseline measure), immediately after the stimulation (*1st post-stimulation test*), and 15 min after the end of the first post-stimulation test (*2nd post-stimulation test*, i.e. approximately 18 min after the end of stimulation). Given the scarcity in literature on short-term retest sessions (also referred to as "follow-ups"), our 2nd post-stimulation test was aimed to better understand the time-course of stimulation-dependent effects.

In each test session, the participants were required to perform the fluency task for two different categories. In order to avoid uncontrolled effects due to the

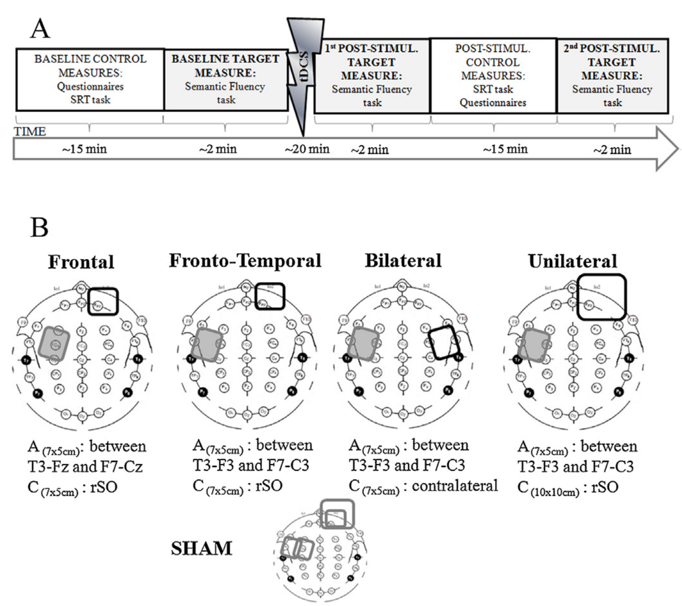

Fig. 1. (A) Timeline of experimental procedure, (B) Stimulation conditions corresponding to the different electrode montages. Notes: montages are schematically represented (active electrode in gray, reference electrode transparent) in the extended EEG system, where T3/4 electrodes are renamed in T7/8; A = anode, C = cathode, rSO = right supraorbital area.

semantic categories, those we used were selected, from a total of 15 categories, through a preliminary study on 57 volunteers not participating in the tDCS study. The category selection procedure was aimed to obtain triplets of categories matched for the mean number of produced exemplars in one minute and free from gender biases. In this way, we identified two triplets having the described features: "tools"–"furniture"–"vegetables", and "clothes"–"musical instruments"–"vehicles". Each category of a triplet was associated to a category of the other triplet to form category pairs. These pairs were randomly assigned in a counterbalanced order across participants, so that each participant performed the task using each of the six categories, and each category pair was used the same number of times in the three test sessions (pre-stimulation, 1st post-stimulation, 2nd post-stimulation) and in the five stimulation groups.

Besides the target fluency task, we included a simple reaction time (SRT) task, in which participants were required to press a button, with the left hand, as quickly as possible, when a single stimulus (an "X") appeared, in the center of a computer screen, at different delays from the previous response (delay range: 250–2500 ms). The SRT task was used as a control task in order to evaluate the presence of unspecific effects of stimulation on the level of general alertness. This task, which consisted of 100 trials, lasted approximately five minutes, and, as shown in Fig. 1A, was administered before (baseline measure) and after the stimulation. Given that the SRT task had the only purpose of controlling for alternative interpretations of results, we did not administer it a second time after the stimulation. For the same reason, the administration order of target and control tasks was kept fixed in both pre- and post-stimulation tests.

As a further control to rule out alternative accounts of tDCS effects, a self-report questionnaire measuring mood and arousal, through few items rated on a 10-point-scale, was administered both before and after the stimulation.

Finally, in order to detect possible differences in the sensations experienced during the different stimulation conditions, participants were asked to complete a questionnaire assessing the intensity with which participants felt several sensations (i.e. itching, pain, burning, heat, pinching, iron taste, fatigue, effect on performance) through a 5-point-scale [7].

2.3. Transcranial direct current stimulation

Transcranial direct current was delivered through a battery-driven constant current stimulator (Eldith DC-Stimulator, NeuroConn GmbH, Germany), using a pair of surface saline-soaked sponge electrodes. A constant current of 2 mA was applied for 20 min (including 1 min at the beginning and 1 min at the end of treatment in which current was ramped up and down, respectively) in all of the active stimulation conditions. These were as follows (see Fig. 1B):

- "*Frontal*" montage: anode over the crossing point between T3–Fz and F7–Cz sensors of 10–20 EEG system (i.e., centered on a region of left frontal cortex previously stimulated in other language studies [5,6,20], and cathode over the right supraorbital (rSO) area.
- "*Fronto-temporal*" montage: anode over the crossing point between T3–F3 and F7–C3, (i.e., centered on a region of left frontal cortex previously stimulated in other studies [10,19]), and cathode over the rSO area. Notably, in this condition, electrode positioning was largely overlapping to that of the Frontal montage, although, being shifted down, it also included a portion of temporal lobe.
- "*Bilateral*" montage: anode as in fronto-temporal montage and cathode over the right homologue area.
- "*Unilateral*" montage: anode as in fronto-temporal montage and cathode of big size (100 cm^2) over the rSO area. Unlike our former montages, in which only 35 cm^2 electrodes were used, in the present one we used a 100 cm^2 electrode for cathode. To note, this montage was exactly the same (i.e., same electrode positioning, and also same size of the reference electrode) successfully used by Meinzer et al. [19] in their fMRI study.

In addition, a sham condition was also included, in which a 2 mA current was applied for 30 s by means of electrodes (of both 35 and 100 cm^2) placed to cover the same positions of the previous described active stimulation montages.

2.4. Data analyses

Statistical analyses of the fluency task were performed by using the mean number of words produced by participants as a function of both test session and stimulation condition, as the dependent variable. Given the high variability in the fluency task performance even in the pre-stimulation condition (see the absolute values in Table 1), we used normalized values. Namely, for each participant we calculated the difference between the number of words produced in a given post-stimulation session and the number of words produced in the pre-stimulation session, then we divided this difference by the number of words produced in the pre-stimulation session. When multiplied by 100, such normalized value indicates the percentages of increase (positive values), or decrease (negative values), of a given post-stimulation session with respect to the baseline performance. To note, whereas many of the produced words were easily categorized as correct exemplars (or incorrect intrusions) of a given category, few other words left doubts about their categorization, since they could be judged as a member of a category or not depending on the strictness of inclusion criteria (for example, "paraglider" or "skates" for the category "vehicles"; "oven" or "vase" for "furniture", etc.). Therefore, we performed analyses by using both more stringent (i.e., on the items unambiguously rated as members of a given category) and less stringent (i.e., on the items unambiguously rated as members of a given category plus items rated as more peripheral members) inclusion criteria. For these scorings, performed by three independent raters blind to the stimulation condition, an analysis of variance (ANOVA) was performed with *test session* as a within-group factor (2 levels: 1st post-stimulation vs. 2nd post-stimulation) and *stimulation condition* as a between-group factor (5 levels: frontal vs. fronto-temporal vs. bilateral vs. unilateral vs. sham). For significant ANOVA results, Fisher's least significant difference method was used as post hoc test.

For the SRT task a one-way ANOVA with *stimulation condition* as a between group factor was performed only on normalized values (i.e., (post-stimulation-pre-stimulation)/pre-stimulation) of reaction times (RTs), because accuracy rate reached the ceiling level for all stimulation conditions (see Table 1 for RT absolute values). Analog one-way ANOVAs were performed for the items included in the self-report questionnaires assessing mood/arousal and tDCS-induced sensations.

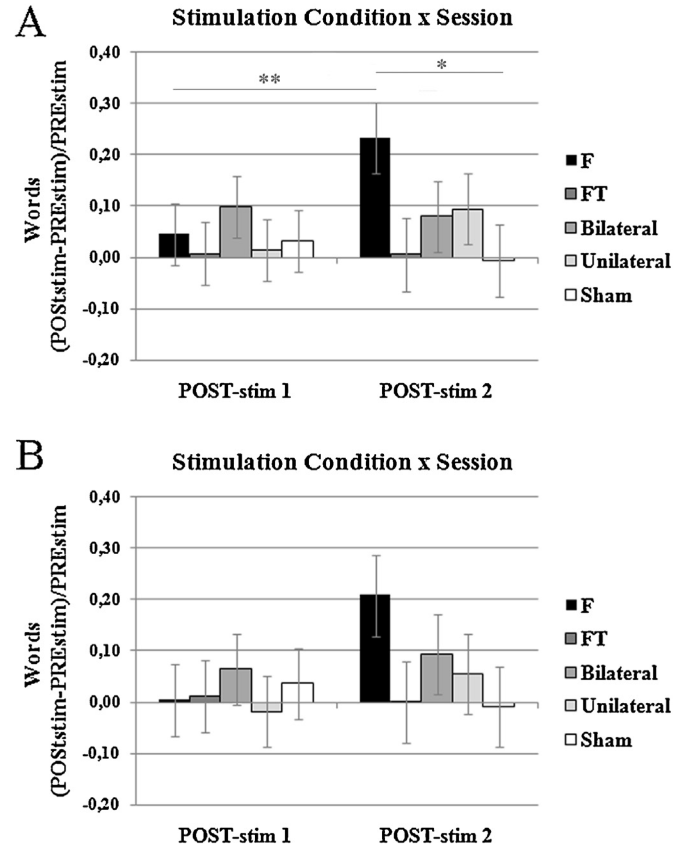

Fig. 2. Stimulation condition × time session interaction in the semantic fluency task for the normalized number of produced words using (A) more or (B) less stringent item inclusion criteria for the scoring. Notes: F = frontal, FT = fronto-temporal, bars denote SE, asterisks denote significant post hoc tests.

3. Results

Visual inspection of the graphs related to the Semantic Fluency task (Fig. 2) suggests that performance in the frontal montage condition was better than performance in all the other montages in the second post-stimulation test. In addition, always on a qualitative level, it can be seen that bilateral condition seemed the most effective at the first post-stimulation test, and also the only condition whose effects extended to the second post-stimulation test. Statistical analyses gave only a partial confirmation of such remarks. The first ANOVA performed using highly stringent inclusion criteria to score the produced items did not reveal any main effect of

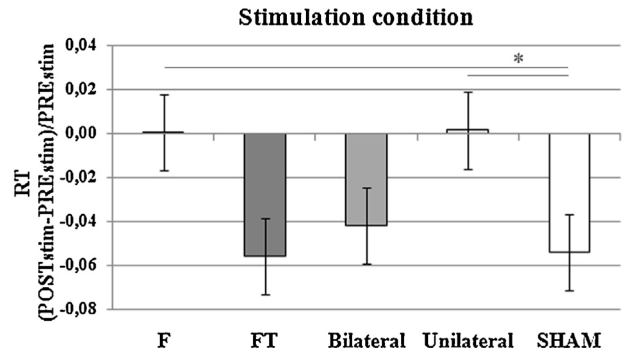

Fig. 3. Stimulation condition effect for the normalized RT of the simple reaction time task. Notes: F = frontal, FT = fronto-temporal, bars denote SE, asterisks denote significant post hoc tests.

the factors test session ($F_{(1,84)} = 2.72$, $\eta_p^2 = 0.03$, n.s.) and stimulation condition ($F_{(4,84)} = 0.88$, $\eta_p^2 = 0.04$, n.s.). Remarkably, the test session × stimulation condition interaction turned out to be significant ($F_{(4,84)} = 2.69$, $\eta_p^2 = 0.11$, $p = 0.036$). As shown in Fig. 2A, the condition that clearly appeared to facilitate the verbal production, although only in the second post-stimulation test, was the frontal stimulation. Post hoc tests confirmed that this stimulation condition produced an increase, with respect to the baseline, significantly higher than that it produced in the first post-stimulation test ($p < 0.001$) and, more crucial, significantly higher than that produced by the sham condition in the second post-stimulation test ($p < 0.05$). In the second test session, frontal stimulation seemed to be superior to the fronto-temporal stimulation, although this difference only approached statistical significance ($p = 0.06$).

The second ANOVA performed using less stringent inclusion criteria for item scoring did not disclose any effect of the factors test session ($F_{(1,84)} = 2.80$, $\eta_p^2 = 0.03$, n.s.), and Stimulation Condition ($F_{(4,84)} = 0.07$, $\eta_p^2 = 0.02$, n.s.). Notably, the test session × stimulation condition interaction, which was significant in the previous ANOVA, only approached significance ($F_{(4,84)} = 2.12$, $\eta_p^2 = 0.09$, n.s., Fig. 2B), although the pattern of results was comparable to that of the previous analyses.

As regards the SRT task, which controlled for possible unspecific tDCS effects on general alertness, the ANOVA performed on normalized RTs disclosed a significant effect of Stimulation Condition ($F_{(4,84)} = 2.77$, $\eta_p^2 = 0.12$, $p = 0.032$). As shown in Fig. 3, in the control sham condition (like in the fronto-temporal and bilateral conditions) RTs decreased immediately after the stimulation with respect to the baseline. In contrast, responses in both frontal and unilateral conditions did not show an analog response facilitation after the stimulation ($p < 0.05$ with respect to sham condition). Since the sham condition (which must always be considered as a baseline measure of performance) showed a RT decrease at the second test, likely for a mere effect of practice, the fact that this effect is missing for frontal and unilateral conditions suggests they somehow interfered with RT improvements.

Analyses of the self-report questionnaire measuring mood and arousal revealed no significant differences among stimulation conditions for any of the items (all $ps > 0.05$).

With regard to the self-report questionnaire assessing the sensations experienced during the stimulation, participants generally reported having tolerated the stimulation without discomfort. Sensations induced by sham and active protocols were hardly discernible as confirmed by the fact that we only found a significant difference among stimulation conditions for the "itching" sensation ($F_{(4,85)} = 4.10$, $\eta_p^2 = 0.16$, $p = 0.004$), sham protocol showing, on this item, a rating significantly lower than ratings of all the other conditions ($p < 0.05$).

4. Discussion

In the present study we tested the effect of different tDCS electrode montages on a semantic fluency task. Although none of these montages increased language production immediately after the stimulation, the left frontal anodal stimulation significantly improved fluency at the second post-stimulation test.

The first unexpected result, in contrast with some previous reports [5,19], was the absence of significant effects on verbal production immediately after the stimulation. Indeed, despite the bilateral montage improved the fluency (∼10%) compared to baseline, such enhancement was not statistically different from that of the sham condition. The inconsistency of the findings concerning the effects immediately following the stimulation could depend on the unsuitability of the tDCS parameters tested up to now to improve verbal fluency. However, the present study suggests an alternative explanation: against similar result patterns, the statistical significance can change as a function of item scoring (as proved by the two analyses we performed using more or less stringent criteria in defining item membership for a given category). Therefore, when considering tasks like the one implemented in the present experiment, whose scoring involves not completely objective criteria, the result discrepancy in literature may depend on the criteria used, rather than on task insensitivity to experimental manipulations.

Remarkably, tDCS was found to significantly facilitate semantic fluency in the test session performed at about 18 min after the end of stimulation. Specifically, the frontal stimulation improved the fluency compared to baseline (∼25%) significantly more than the sham condition. Crucially, since the results of the control task suggested that frontal stimulation did not improve alertness levels (if anything, the effects were in the opposite direction), we can reasonably rule out the possibility that the positive effects of anodal left frontal stimulation on verbal production were mediated by unspecific tDCS effects on general alertness. Similarly, given that this stimulation condition did not change the self-report levels of mood and arousal, we can exclude that changes in such dimensions could mediate linguistic improvements.

This finding provides new information on both the spatial and temporal features of tDCS, thus giving notable cues for planning future tDCS protocols.

As regard the spatial issue, based on neuro-imaging studies attesting an involvement of both frontal and temporal areas in semantic fluency [1,9], we hypothesized a stronger verbal facilitation following left fronto-temporal stimulation than following left frontal stimulation. The present results, however, showed an effect in the opposite direction. This pattern seems to suggest that, despite temporal lobe being strictly involved in word retrieval constrained by semantic cues [1,9], category fluency has more benefit from a stimulation involving a larger portion of the frontal cortex compared to a stimulation involving both frontal and temporal areas. From the present study we are not able to establish whether this result was due to a general superiority of frontal stimulations on temporal stimulations in facilitating semantic fluency, or rather to a superiority of our frontal montage with respect to our fronto-temporal montage. Indeed, it may well be that stimulation of other portions of temporal lobe (like Wernicke's area) do improve semantic fluency more than frontal or fronto-temporal stimulations. However, the noteworthy point is that, despite our frontal and fronto-temporal montages were considerably overlapping, they induced quite different effects. Although this sounds trivial, it is a detail often underestimated when comparing studies which, despite stimulating the same cortical area, do not place the electrodes exactly in correspondence of the same sites. Therefore, as highlighted by our findings, these little variations in active electrodes positioning could play a major role in determining the somewhat inconsistent results reported in the literature. Turning to the qualitative comparisons between the various other tested montages, some effects are worth discussing, although they were not statistically significant. First, we found that bilateral stimulation was the only montage to show a constant improvement across the two testing sessions, being the only condition to show fluency facilitation immediately after the stimulation. Since bilateral montage differed from fronto-temporal montage only for the reference electrode positioning (in the former being over the contralateral cortex with respect to the active electrode, in the latter being over the contralateral supraorbital area), this result seems to suggest that the bilateral montage results in a more effective stimulation than the corresponding more traditional non-bilateral montages [15]. With regard to the contribution of the two hemispheres to the investigated process, this finding may indicate an interfering effect of the right fronto-temporal cortex in performing semantic fluency (given that driving the lateralization to the left hemisphere, through the bilateral stimulation, seems to improve the performance). Second, always on a qualitative level, ~18 min after stimulation end, unilateral montage was the second most effective condition. To note, unilateral stimulation differed from fronto-temporal stimulation only for the larger size of the reference electrode (corresponding to a lower current density), nevertheless, it induced quite different behavioral effects, not only in the fluency task, but also in the control SRT task. Indeed, by reducing the influence of the reference electrode under the supraorbital area we observed an improvement of language production and a reduction of alertness levels. Although the nature of such effects remains unclear, and deserves further investigations, it is relevant to remark that, in line with findings reported for the motor domain [3], our results further confirm that the reference electrode can exert an influence on the underlying area (and on the current path), which needs to be carefully considered, especially when this area can affect fundamental functions, like attention, arousal, and executive processes.

As regards tDCS temporal features, the considerable effect of stimulation, found ~18 min after its cessation, but not immediately after it, suggests that there might be variations in the time the stimulation needs to produce effects detectable at the behavioral level. Although direct comparisons between different brain stimulation techniques should be made with caution, some reports using repetitive transcranial magnetic stimulation are in line with this hypothesis, showing that short theta burst stimulation over the motor cortex can affect response RT at different intervals from the stimulation (i.e., 30 min, but not 10, after the stimulation, or vice versa), depending on the response hand [11]. We speculate that these variations in the time the stimulation needs to produce detectable effects could likely depend on the type of processes underlying the observed behavioral measures, more high-level processes presumably requiring a longer interval to make the stimulation effects noticeable. In this regard, we are incline to believe that the lack of data on delayed tDCS effects can reflect biases in the literature more than the lack of the effects themselves. Indeed, a recent review on tDCS application in linguistic domain [21] confirmed that only two out of ten studies on healthy volunteers included some kind of follow-up measure [18,29]. Therefore we cannot a priori exclude the presence of delayed tDCS effects in those studies (the majority) in which their assessment was not performed. For this reason, our findings suggest that it is highly advisable to include one or more short-term retests also following single-session stimulations, in order to avoid losing important information on the time-course of tDCS effects.

To conclude, although the present study had some limitations (i.e., it is a single-blind, between-groups design) and some results need further investigations, our findings attest that differences in either the active electrode positioning (even if small), or the positioning/size of reference electrode can impact the behavioral effects also in the cognitive domain. In addition, such effects can be sometimes detected after a short time-interval from the stimulation end. These cues on spatial and temporal features of tDCS can give notable advices for planning increasingly effective stimulation paradigms, not exclusively within the linguistic domain.

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