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## Original article

Potential impact of bifrontal transcranial random noise stimulation (tRNS) on the semantic Stroop effect and its resting-state EEG correlates

Clément Dondé\*<sup>1-3</sup>, Charlotte Brevet-Aeby\*<sup>1,2,5</sup>, Emmanuel Poulet<sup>1,2,4</sup>, Marine Mondino<sup>1-3</sup>, Jérôme Brunelin<sup>1-3</sup>

\*Both authors contributed equally to this manuscript

1. INSERM, U1028; CNRS, UMR5292; Lyon Neuroscience Research Center, Psychiatric Disorders: from Resistance to Response Team, Lyon, F-69000, France;
2. University Lyon 1, Villeurbanne, F-69000, France;
3. Centre Hospitalier Le Vinatier, Bron, France;
4. Department of Psychiatry, University Hospital Edouard Herriot, Hospices civils de Lyon, Lyon, France.
5. Department of Psychiatry, University Hospital Lyon Sud, Hospices civils de Lyon, Lyon, France.

### Author correspondence to:

Clément Dondé, M.D.  
CH Le Vinatier, Batiment 416, 95 boulevard Pinel,  
BP 300 39; 69 678 BRON cedex, FRANCE  
e-mail: [clement.dondecoquelet@gmail.com](mailto:clement.dondecoquelet@gmail.com) ; tel: +33 43 79 15 49 5

## ABSTRACT

**Objective:** The Stroop effect performance reflects cognitive resistance to interference. We aimed to investigate the effect of a single transcranial random noise stimulation session (tRNS) applied over the dorsolateral prefrontal cortex (DLPFC) on the semantic Stroop effect and its resting electroencephalography (EEG) correlates ( $\beta/\alpha$  ratio).

**Methods:** In a randomized, double-blind study, healthy volunteers were allocated to receive either one session of active tRNS (N=8) or one session of sham tRNS (N=11). The anode pad was placed on the scalp over the right-DLPFC and the cathode pad was placed over the left-DLPFC. A computerized adaptation of the French Stroop Color-Word Test (Victoria version) and a resting-state continuous EEG recording were administered before and after the tRNS.

**Results:** No significant difference were observed for either Stroop Interference/Congruent ( $F_{(1,15)}=0.5$ ,  $P=.5$ ,  $BF=.19$ ) or Interference/Cross ( $F_{(1,14)}=3.2$ ,  $P=.1$ ,  $BF=0.25$ ) ratios. No significant effect of tRNS was observed on EEG  $\beta/\alpha$  ratios across electrodes ( $F_{(5,95)}=0.6$ ,  $P=.7$ ,  $BF=0.59e^{-05}$ ). Under active stimulation, Pearson's tests showed significant correlations with moderate evidence between post-pre differences of Stroop Interference/Congruent and Fz- $\beta/\alpha$  ratios ( $r=0.88$ ,  $P=.02$ ,  $BF=4.05$ ), and Stroop Interference/Crosses and Cz- $\beta/\alpha$  ratios ( $r=0.89$ ,  $P=.008$ ,  $BF=8.25$ ), while the same correlations did not reach significance under sham conditions.

**Discussion:** We observed no significant changes in either semantic Stroop task reaction time or its EEG correlates after tRNS. However, we provide the original finding that fronto-central  $\beta/\alpha$  activity becomes related to cognitive resistance to interference when the DLPFC is stimulated with random noise current. The results suggest a potential resynchronization of relevant brain frequency patterns into Stroop-related cortical involvement.

**Keywords:** Dorsolateral prefrontal cortex; inhibitory control; noninvasive brain stimulation; resting-EEG; semantic Stroop task; Stroop effect; transcranial random noise stimulation

**Running title:** Prefrontal tRNS on semantic Stroop task

## INTRODUCTION

The Stroop task that elicits the Stroop effect is one of the best-established psychological experiments to measure executive function, and more specifically the ability to inhibit an automatic response in favor of an unusual one [15]. In its semantic version, the Stroop task requires participants to successively name the ink color of congruent (symbols or words) and incongruent (color-words displayed in a different color than the written name) stimuli. The Stroop effect relates to the slower response latency and errors in naming incongruent stimuli relative to congruent. Hence, it measures the interference between automatic processing of the color-word meaning and the required response (ink color) when meaning and color are incongruent.

A large body of neuroimaging evidence locates generators of the Stroop effect in the left dorsolateral prefrontal cortex (DLPFC), although the specific lateralized involvement of this brain region remains controversial [25]. The DLPFC is associated with executive control in general and particularly with solving a distracting interference [1]. With regards to EEG correlates at rest, increased  $\beta/\alpha$  ratio, which reflects cortical engagement in attentional processing, has been shown to correlate with improved performance in a semantic Stroop task [2].

Relationships between the Stroop effect and the brain have also been explored with transcranial electrical stimulation testing. These approaches allow a weak current to circulate between an anode and a cathode pad overlying the scalp, which modulates the targeted cortical areas, their inter-connected brain regions and their related behavioral measures, with distinct cognitive effects depending on electrode montage (rev. in [18]). However, transcranial stimulation with direct current (tDCS) showed no effect on Stroop effect reaction times (RTs), when either the left DLPFC or the right DLPFC were targeted by the anode [4, 12]. A more recent model of non-invasive transcranial electrical stimulation proposed

displaying current with a random noise frequency pattern (so-called tRNS: transcranial random noise stimulation), which is suggested to increase the effect of displayed current on neural excitability and plasticity [17]. One proposed mechanism is stochastic resonance, whereby the induced random activity potentiates task-related neuronal activity [16, 22]. In regard to executive control, we recently reported that tRNS (2mA with a +1mA offset) with the anode placed over the right-DLPFC and the cathode over the left-DLPFC modulates cognitive inhibition by decreasing reaction time in a Go/No-Go task [5]. However, against the background of current literature, it is still unclear whether tRNS can have an impact on Stroop performance.

Here, we propose an original approach by measuring the impact of tRNS applied over the DLPFC on the semantic Stroop effect. We first posit that active stimulation over the right-DLPFC will decrease Stroop interference RTs compared to sham. To investigate the role of the DLPFC in controlling Stroop interference, we used a tRNS electrode montage with the anode placed over the right and the cathode over the left DLPFC [5]. We measured quantitative resting EEG before and after stimulation. As a second hypothesis, we predicted that  $\beta/\alpha$  ratio would show a correlation pattern with Stroop performance and that it would be modulated by tRNS stimulation.

## **METHODS**

In a randomized, double-blind, 2-arm study, 24 healthy volunteers were allocated to receive either one session of active tRNS (20 minutes, 2mA) or one session of sham tRNS. A computerized adaptation of the French Stroop Color-Word Test-Victoria version was administered before and after the tRNS. Additionally, a resting-state continuous EEG recording was collected for each participant before and after the tRNS. The experiment was approved by the local ethics committee (CPP Sud Est 6, AU1222) and the study was part of a

larger clinical trial recorded on the clinicaltrials.gov database (NCT02717260).

## **Participants**

Participants had to be free of any physical or psychiatric condition and all reported normal or corrected-to normal vision. All were right-handed according to the Edinburgh Handedness Inventory. They also had to be naïve to transcranial electrical stimulation. Behavioural and EEG outcomes were measured independently before and after the tRNS session.

## **Procedure**

### *Behavioral*

The experiment used a computerized adaptation of the French Stroop Color-Word Test (Victoria version) [3]. The task uses three conditions which consisted of naming the ink color of (i) crosses ('XXXX'), (ii) congruent color-words (BLEU– blue, ROUGE– red, VERT– green, and JAUNE–yellow) displayed in the same color as the written name, and (iii) interfering color-words displayed in a different color from the written name (e.g., "red" written in blue ink). The Crosses and Congruent conditions are used to control for naming of non-semantic and semantic features, respectively. In the Interference condition, the participant has to inhibit the displayed word in order to correctly name its written color. In each condition respectively, participants were asked to name as quickly as possible the color of the crosses (Crosses condition) and the color of the written words (Congruent and Interference conditions) in a microphone attached to a voice-operated relay, which was connected to the computer. Conditions contained 30 randomized items each and were presented in the following order: (i) Crosses; (ii) Congruent; (iii) Interference. Stroop interference RTs were measured in milliseconds for correct responses from the onset of the

stimuli (cross or word) to when the subject's answer onset was detected by the computer. To specifically examine semantic interference and thus control for naming of nonverbal features, we used Stroop interference RTs ratios as outcomes: (i) Interference/Crosses and (ii) Interference/Congruent. Task stimuli are pictured Figure 1A.

### *Resting-state EEG*

Resting-state continuous EEG recordings were performed for each participant during a 5-minute period at rest with eyes open. EEG was acquired using the Starstim (NeuroElectrics, Barcelona, Spain) amplifier system with 6 scalp electrodes placed according to the international 10/20 system (Fp1, Fp2, Fz, Cz, P3, P4), plus two CMS/DRL reference electrodes placed over the left mastoid. The sampling rate was 500 samples per second. Electrode impedance were kept under  $<10\text{ k}\Omega$ . All data were bandpass filtered from 0.5 to 80 Hz and notch filtered at 50 Hz. Data were re-referenced to average-reference and analyzed offline using Matlab software, version 2017a (MathWorks). EEG signals were segmented into non-overlapping epochs of 1.024 ms duration, in order to facilitate fast Fourier transform. Epochs with eye-movements and blinks were rejected off-line by visual inspection. An artifact criterion of  $\pm 500\text{ }\mu\text{V}$  was used at all other scalp sites to reject trials with excessive EMG or other noise transients. The average EEG epoch acceptance rate was 78.2% and did not differ between groups.

To represent a better quantitative measure of trait (i.e., resting-state related) brain activity, we chose to use the  $\beta/\alpha$  power ratio measure (dB) ( $\alpha$ -range 8-12 Hz,  $\beta$ -range 13-30 Hz), instead of the relative power in separate frequency segments of the EEG spectrum. Extensive data show that such a ratio better reflects increased cortical engagement and subsequent attentional investment in information processing [2].

### *Transcranial random noise stimulation (tRNS)*

As previously described [5], tRNS was administered using a battery-driven (Starstim device, NeuroElectrics, Barcelona, Spain) high-frequency oscillatory direct current with random noise frequency (100–500 Hz). The current was set at 2 mA intensity with a +1 mA direct current offset that avoids polarity change between the anode and cathode. The current was delivered for 20 min with a 30-second ramp up/ramp down period. The offset was chosen as an augmentation strategy, since tRNS with an offset has been shown to enhance cortical excitability in comparison to tRNS with no offset [11]. The tRNS used two (7x5cm) electrodes encased in saline-soaked sponges (NaCl 0.9% solution). The anode pad was placed on the scalp over the right-DLPFC (F4, according to the 10/20 electrode placement EEG-system) and the cathode pad was placed contralaterally, over the left-DLPFC (F3). To avoid misinterpretation of the results, a particular attention was given to the sham condition [9]. In sham condition, the electrodes were placed in the same positions as in the active condition; however, the stimulator was only active for initial and final ramp up/ramp down periods of current, in order to mimic the sensation of stimulation. Blinding integrity was assessed by asking participants to guess the nature of the received condition (active or sham) after the tRNS session. Additionally, stimulation side effects that could have hampered the blinding procedure or interfered as distractors were systematically assessed. Participants were also asked after the tRNS session if they felt a slight itching sensation during the initial/final ramps of stimulation. tRNS set-up and modelization are pictured in Fig. 1BC.

### **Data analyses**

Statistical analyses were performed using JASP version 0.9.1. (<https://jasp-stats.org>). Quantitative outliers were excluded on the basis 95% confidence interval. Significant threshold was defined at  $p < 0.05$ . Bayesian Factor referred to level of evidence for the alternative hypothesis as  $<1$ =no evidence,  $1-3$ =anecdotal,  $3-10$ =moderate and  $>10$ =strong.



Normality of the distributions was investigated with Shapiro-Wilk tests. Between-group comparisons were performed with Mann-Whitney z-tests and two-sample t-tests for non-normal and normal continuous outcomes, respectively. Categorical data was compared with  $\chi^2$  tests.

To assess for between-group differences in patterns of Stroop interference RTs ratios, we used separate repeated measure ANOVAs considering factors of time (pre-tRNS, post-tRNS) and group (active, sham). Regarding EEG measures, same ANOVAs mixed-models were used with  $\beta/\alpha$  power ratio as the dependent measure. Time, group and electrode site (Fp1, Fp2, Fz, Cz, P3, P4) were entered as independent variables.

In addition, Pearson correlations analyses were conducted to measure associations between behavioral and EEG data. For correlations, baseline corrected differences (i.e., post – pre) in Stroop RTs ratios and  $\beta/\alpha$  power ratio were used.

## **RESULTS**

The analyses were conducted on 8 participants in the active group, and 11 in the sham group (9 females, 10 males). Other participants were excluded on the basis of excessive artefacts on the EEG records (4 in the active group, one in the sham group). The mean age was  $25.7 \pm$  standard deviation 4.1 years old, the highest grade achieved  $15.6 \pm 2.5$  years and the French adaptation of the National Adult Reading Test (fNART) mean score  $31.2 \pm 2.9$ . No significant difference between groups regarding age, gender, highest grade achieved and fNART was observed (Table 1). tRNS was well tolerated, as none of the participants reported adverse effects during and after the tRNS session. Regarding blinding, only 2/19 participants (both in the active group) correctly guessed their stimulation, which indicates a satisfactory blinding. In addition, all subjects described a slight itching sensation under both electrodes during the first 30-second of stimulation.

The repeated measure ANOVAs showed no significant difference for both Interference/Congruent ( $F_{(1,15)} = 0.5$ ,  $P = .5$ ,  $BF = .19$ ) and Interference/Cross ( $F_{(1,14)} = 3.2$ ,  $P = .1$ ,  $BF = 0.25$ ) ratios. Independent sample mean comparison tests with post – pre differences were conducted as post-hoc. A non-significant trend for Interference/Cross ratio reduction was observed after active tRNS but not sham (active =  $-0.08 \pm 0.14$ ; sham =  $0.04 \pm 0.12$ ;  $t_{(1,14)}=0.19$ ,  $P=.85$ ).

No significant effect of tRNS was observed on  $\beta/\alpha$  ratios across electrodes after repeated measure ANOVAs ( $F_{(5,95)} = 0.6$ ,  $P = .7$ ,  $BF= 0.59e^{-05}$ ). Similar post-hoc analyses comparing post – pre differences of  $\beta/\alpha$  ratios under active vs. sham for each electrode separately were all non-significant.

Pearson's tests showed a significant correlation with moderate evidence between post – pre differences of Stroop Interference/Congruent and Fz- $\beta/\alpha$  ratios under active stimulation ( $r = 0.88$ ,  $P = .02$ ,  $BF = 4.05$ ), while the same correlation did not reach significance under sham ( $r = -0.33$ ,  $P = .39$ ,  $BF = .57$ ; Fig.1D). Similarly, Stroop Interference/Crosses and Cz- $\beta/\alpha$  ratios were significantly correlated with moderate evidence under active ( $r = 0.89$ ,  $P = .008$ ,  $BF = 8.25$ ) and not under sham ( $r = 0.51$ ,  $P = .16$ ,  $BF = .96$ ; Fig.1E).

## DISCUSSION

Here, we compared the modulation of the Stroop interference RTs after tRNS applied over bilateral DLPFC. Additionally, we evaluated the neuronal electrical activity changes using spectral power analysis after and before tRNS. This is to the best of our knowledge the first study exploring behavioural and neural effects of tRNS on the Stroop effect performance and brain oscillations respectively.

Conversely to our main hypotheses, we observed no significant changes in both semantic Stroop task reaction time and its EEG correlates after tRNS. This is consistent with

previous experiments where RT in semantic Stroop interference were not modulated by direct current stimulation (tDCS) [7, 8, 10, 14] and high-frequency rTMS [23] over the right-DLPFC. This can be explained by a ceiling effect reached by the healthy young participants involved in our study. Also, our analyses were conducted on a small sample of subjects.

However, in regard to EEG correlates, we reproduced a previous report demonstrating that  $\beta/\alpha$  frequency ratio is significantly correlated with Stroop interference RT performance [2]. Moreover, we provide the original finding that  $\beta/\alpha$  ratio increase, as detected by fronto-central electrodes, is strongly correlated with interference Stroop RT improvement under active tRNS, while not under sham conditions. This suggests that fronto-central  $\beta/\alpha$  activity becomes related to cognitive resistance to interference when the DLPFC is stimulated with random noise current. Knowing that beta frequencies in a given cortical area increase with its BOLD activation [13, 19], we posit that active tRNS induced a shift in the electrophysiological spectral profile toward increased attention for a task by which the DLPFC was repeatedly elicited. This result is also consistent with the stochastic resonance effect, wherein the cortical networks that are most effective during a specific task are enhanced when adding noise to the brain [16, 22]. An important limitation is that caffeine and tobacco intake were not controlled in our sample. These substances have been shown to modulate executive control and Stroop performance [20, 24], as well as transcranial electrical stimulation after-effects [6, 21], which could have biased our results.

In summary, while tRNS over the DLPFC showed no effect on Stroop performance and its EEG correlates, the results suggest a potential resynchronization of relevant brain frequency patterns into Stroop-related cortical involvement, perhaps limited by a ceiling phenomenon in our healthy sample. Larger studies are warranted to replicate these findings in healthy participants.

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**Disclosure of interest:** The authors declare that they have no competing interest.

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## FIGURE CAPTIONS

### Figure 1:

(A) Semantic Stroop-task conditions.

(B) Anodal tRNS (blue, F3) delivered to the right-DLPFC and Cathodal tRNS (red, F4) delivered to the left-DLPFC and the 6 electrodes used for EEG; Electrical current waveform associated with tRNS

(C) Modelization of current strength pattern associated with the bifrontal tRNS montage (HDExplore™, Soterix Medical, New York, NY, USA)

(D) Scatter plot showing correlations between Stroop Interference/Congruent and  $\beta/\alpha$ -Cz ratios under active and sham tRNS. \* $P < 0.05$

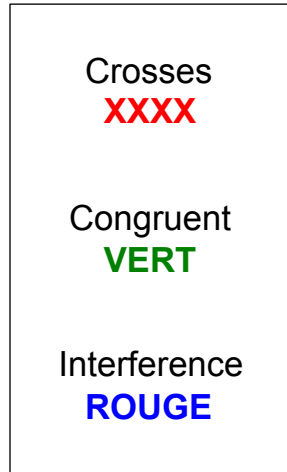
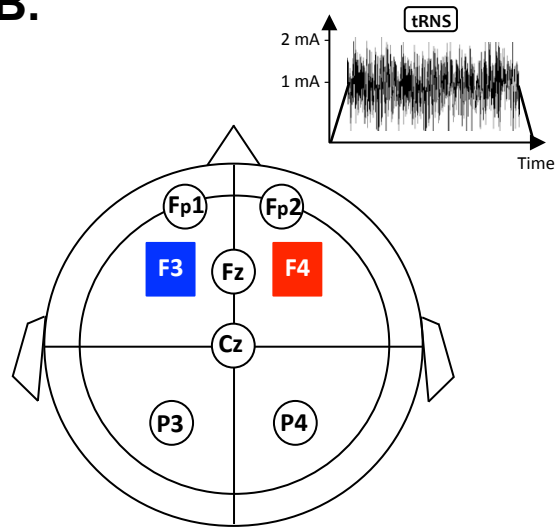
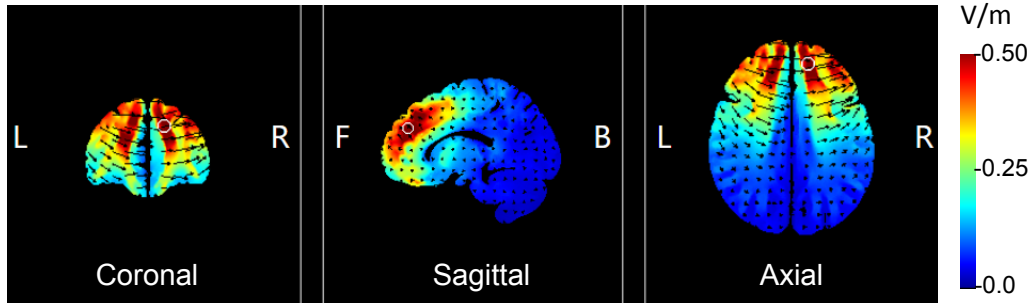
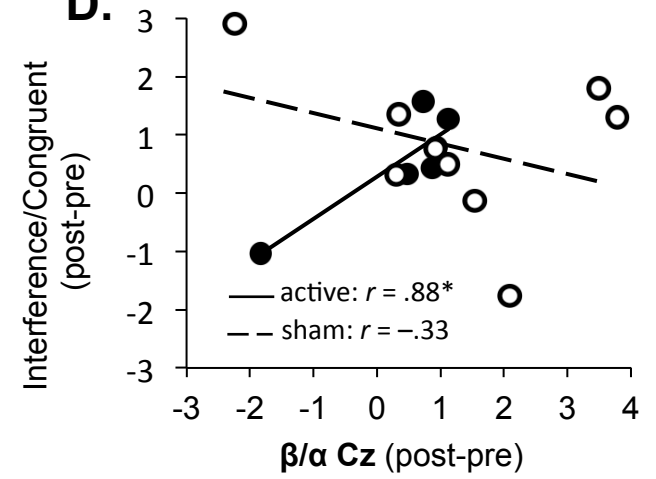
(E) Scatter plot showing correlation between Stroop Interference/Crosses and  $\beta/\alpha$ -Fz ratios under active and sham tRNS. \* $P < 0.05$

## TABLE

**Table 1:** Group characteristics

Variable	Group		Statistics		
	Active ( $N = 8$ )	Sham ( $N = 11$ )	test	stat	p-value
Age (years)	23.8 $\pm$ 1.8	27.1 $\pm$ 4.8	$t$	1.84	n.s. (0.08)
Gender (M/F)	4/4	6/5	$\chi^2$	0.04	n.s. (0.84)
fNART	31.9 $\pm$ 2.9	30.7 $\pm$ 2.9	$t$	0.85	n.s. (0.41)
Highest grade achieved	15.0 $\pm$ 2.9	16.0 $\pm$ 1.8	$z$	0.59	n.s. (0.55)

Continuous data is presented as mean  $\pm$  SD; n.s.: non-significant

**A.****B.****C.****D.****E.**