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ABSTRACT

Background: The Stroop test is a well-known model to denote the decline in performance under the incongruent condition, which requires selective attention and control of competitive responses. Functional near-infrared spectroscopy can identify activated brain regions associated with the Stroop interference effect.

Objective: This research aims to identify the neural correlates associated with the Stroop tasks within the brain activated regions.

Materials and Methods: In this cross sectional study, twelve right-handed healthy controls were investigated by means of a multi-channels fNIRS unit during the execution of the Stroop test. Effective connectivity changes in the prefrontal cortex between Stroop attentional conflict and rest states were calculated using DCM approach to investigate (1) areas known for selective attention and (2) analyze inter-network functional connectivity strength (FCS) by selecting several brain functional networks.

Results: The results indicated that an increased activity was recorded in the LDLPFC during incongruent condition, while under neutral condition, the increase in activity was even more pronounced in those areas. Effect of Stroop interference associated with significant consistent causes an increase in the RDLPFC to DMPFC, LDLPFC to DMPFC and LDLPFC to RPFC effective connectivity strengths.

Conclusion: This study showed the use of DCM algorithm for fNIRS data with respect to fMRI has provided additional information about the directional connectivity and causal interactions in LPFC networks during a conflict processing. Eventually, high temporal resolution fNIRS can be a promising tool for monitoring functional brain activation under the cognitive paradigms in neurological research and psychotherapy applications.

Keywords
Stroop Test; Fnirs; Connectivity; Prefrontal Cortex; Attention

Introduction
The exploration of brain neural correlates in executive functioning has always been the subject of discussion in cognitive neuroscience [1]. One of the most common cognitive tests examining the
field of the executive functions is the Stroop Color and Word test, which is specifically related to the semantic conflict processing [2]. As a basic principle, this test demonstrates that reading the ink-color of the words independently of the written color name (incongruent condition) is always more difficult than simply reading the names of color alone (congruent condition) [3, 4]. This phenomenon is called Stroop interference, requiring the inhibitions of competitive responses [5]. Hence, some attentional executive function mechanisms, such as interference resolution, response inhibition and individual’s processing speed can be investigated with Stroop test [6].

As a result of advanced brain mapping techniques, recently, significant advancements have been made in understanding the relationship between the activation of brain regions and cognition. Therefore, cognitive scientists have been able to study the human brain in a wide range of actions, from perception to higher mental activities [7, 8]. A wide variety of existing brain functional imaging techniques can provide different measurements of the neural connections in cognitive processes, for example, magnetoencephalography (MEG) and electroencephalography (EEG) methods record signals related to brain neural activity. While other methods, such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) measure regional cerebral blood flow and blood-oxygenation level dependent (BOLD) signal [9-11]. These advanced approaches are applied in different research domains of cognitive neuroscience for exploring brain functions such as attention and concentration. Although above imaging methods have determined neural connections of the Stroop interference and reported activity in the regions of relevant cerebral cortex, there have been still some disadvantages, such as the low temporal resolution (fMRI, PET), low signal-to-noise ratios (EEG), lack of appropriate spatial resolution (PET, EEG) and high sensitivity to motion artifacts (fMRI, EEG), non-portability (MEG, FMRI and PET) [12, 13].

Over the past two decades, the use of functional near-infrared spectroscopy (fNIRS) in the fields of cognitive neuroscience and psychological studies has created new opportunities for investigating oxygenation changes and the hemodynamics of cerebral activated regions during the execution of cognitive functions [14, 15].

Some fNIRS investigations focus on identifying the brain areas that are specifically activated during the Stroop interface condition in healthy adults. For instance, it was shown that the interference during the incongruent condition of a color–word matching Stroop test leads to bilateral brain activity in the prefrontal cortex (PFC) [16]. Furthermore, the increase of oxy-hemoglobin and the decrease of deoxy-hemoglobin concentrations are occurred at positions over the lateral prefrontal cortex, which are significantly higher in the incongruent compared to neutral conditions [17]. Another study indicates significant oxy-hemoglobin signal increases association with the Stroop interference in the lateral prefrontal cortex (LPFC), including the left frontopolar area (FPA), anterior ventromedial prefrontal cortex (VLPFC), and bilateral DLPFC [18]. Besides, a greater motor cortex activation was reported in the hemisphere ipsilateral regarding to the response hand in the Stroop interference effect during the hemodynamic response [19].

With brain fNIRS, cognitive researchers started to not only locate brain-activated areas for a specific cognitive function, but also consider the relationship between neural activation patterns of anatomically separated regions during a mental activity. According to the above definition, functional connectivity (FC) as a statistical concept determines the regions belonging to the same functional network, only when their activation time course is correlated with each other [20]. However, FC assessments do not provide information...
about the directionality and causality underlying brain functional interactions. It has been suggested that an effective connectivity (EC) approach can provide a more meaningful interpretation of information flow through neural network, but the causal influences of one neural unit over another has been still unknown [21]. A more precise method to estimate EC is proposed by Dynamic Causal Modeling (DCM), which describes the dynamic nature of interactions between hidden neuronal network nodes [22].

Initially, DCM has been developed and widely used in the field of fMRI, but recently the DCM approach has also extended to fNIRS modality [23, 24]. In this study, fNIRS is used to acquire cortical hemodynamic signals from healthy controls to investigate effective connectivity patterns among cortex activated areas involved in Stroop interference effect. This work is mainly based on the use of DCM algorithm to evaluate fNIRS data through interactions between hidden neural states, whenever a person has been trying to focus on selective attention in Stroop conflict processing. The results of this research can help to improve the understanding of the brain in the response conflict caused by Stroop interference effect.

Material and Methods

Participants

In this cross sectional study, twelve healthy subjects, including five women (21–35 years), and seven men (20–30 years), from Iranian National Brain Mapping Lab participated in this study. The following criteria were considered for all participants: (1) native speaker of Persian (2) normal vision or corrected-to-normal vision (3) normal color vision (4) without any history of neurological or psychiatric disorders (5) not taking any medication at the time of testing. The ethics committee of Tehran University of Medical Sciences (Iran) reviewed and approved the research protocol (Ethical Committee Approval Code: IR. TUMS .MEDICINE.REC.1395.1018). The protocol was in accordance to the Declaration of Helsinki. Before participating in the study, all participants provided written informed consent after explaining experimental procedures.

fNIRS Data acquisition

A multi-channel, continuous wave, fNIRS system (Oxymoron Artinis) was utilized to monitor prefrontal hemodynamic responses during the performance of Stroop task. This device consists of NIR light emitting diodes (760 nm and 850 nm) and photodiode detectors, measuring fNIRS signals with a sampling rate of 10 Hz. In our experiment, an 8-channel symmetrically configured setup was applied with 4 channels for each forehead side. Each side consists of two light emitters and signal detectors that provided four emitters-detectors pairs with 30 mm distance between them. Penetration depth into the cerebral cortex is approximately 2 cm. fNIRS sensor arrays locations in which were defined on the basis of both the Brodmann and Montreal Neurological Institute (MNI) atlases displayed in Figure 1. The MNI coordinates of recording electrodes during Stroop task are also illustrated in Table 1.

Stroop task

The classical Stroop color and word task were conducted in three conditions, which were generated in the middle of a 19-inch computer screen 70 cm in front of the subject. The computer also controlled displaying the tasks and recording the responses of the participants. Subjects were asked to have a response by pressing right and left arrow keys with the ring (Yes-response) and index (No-response) fingers of the right hand. In congruent trial, participants were presented as the series of four Persian color names in a black background (mean “red”, “yellow”, “blue” and “green”, respectively) and they were asked to read the names of the written color independent of the ink (e.g. reading a “red” written
with a color other than red). In incongruent trial, conflict colors-words was used as stimuli, so that the participants were asked to check and agree/disagree if the ink color, which is independent of the written color name, is same as the white written word below or not, which led to color name and color word interference. During neutral trial, two rows of printed letters were displayed. In the top row, “XXXX” was printed in blue, red, green and yellow and in the bottom row, the white printed of color words, including (blue)’, (red)’, (green)’ and (yellow)’ were considered (Figure 2). Prior to each task conditions, there was a baseline or resting period of 20 seconds (black screen).

The Stroop task procedure consists of 15 blocks of stimulation-and-resting conditions sequentially presented on the computer screen. Within each block, 10 random Stroop trials were presented to the participants in 40 seconds (Figure 3). Before fNIRS measurements, all participants practiced a few trials of both congruent and incongruent tasks that the experiment organizers verified their correct understanding of the paradigm. Throughout the performance of the Stroop task, fNIRS data were recorded continuously.

**fNIRS Data Analysis**

Initially, fNIRS raw data were pre-processed using a low-pass filter at a cut-off frequency of 0.15 Hz to reduce the effects of noise produced by either movements or physiological factors (e.g. cardiac pulsation and respiratory fluctuations). Then the brain areas with significant hemodynamic alterations were identified during the course of the task and their related time series were extracted. Therefore, the concentration changes of hemodynamic parameters [oxy-Hb, deoxy-Hb, and total Hb] relative to the baseline according to the modified Beer-Lambert Law were determined. The mean sig-

**Table 1: MNI coordinates of recording electrodes during the performance of Stroop task.**

<table>
<thead>
<tr>
<th>Position in MNI ATLAS (x, y, z)</th>
<th>Optode Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 50.34 40.19 48.04</td>
<td>Rx2 - Tx1</td>
</tr>
<tr>
<td>2 33.74 36.92 67.17</td>
<td>Rx2 - Tx2</td>
</tr>
<tr>
<td>3 35.2 63.15 39.44</td>
<td>Rx1 - Tx1</td>
</tr>
<tr>
<td>4 17.12 59.31 57.98</td>
<td>Rx1 - Tx2</td>
</tr>
<tr>
<td>5 -52.98 27.57 58.07</td>
<td>Rx1 - Tx3</td>
</tr>
<tr>
<td>6 -63.76 30.82 38.37</td>
<td>Rx1 - Tx4</td>
</tr>
<tr>
<td>7 -42.51 46.65 53.51</td>
<td>Rx2 - Tx3</td>
</tr>
<tr>
<td>8 -53.39 52 31.01</td>
<td>Rx2 - Tx4</td>
</tr>
</tbody>
</table>

**Figure 1:** Data acquisition and 3D topography of optode positions of prefrontal cortex related regions: (a) Superior view, (b) Coronal view, (c-d) Sagittal view of brain cortex.
The Stroop Task Effective Connectivity by fNIRS

Signal intensity for the both baseline (20 s before the task) and vascular response (task period) were calculated for each subject and task. Differences between the mean signal intensities of the baseline and the vascular response were considered as the task related to activation and compared between the neutral, congruent and incongruent conditions. The paired Student’s t-tests were carried out to determine the cortical regions with significant activation for the three kinds of Stroop tasks. The significance level for statistical analysis of results was selected at p-value=0.05.

Spatial mapping of cerebral hemodynamic activities, during the neutral and Stroop tasks, was created based on a standard statistical model, known as general linear model (GLM) [25, 26]. The GLM analysis has expanded to the fNIRS studies to identify cortical regions that are significantly activated during a given task. The GLM uses a hemodynamic response function (HRF) as a predictor of changes in the oxy-Hb and deoxy-Hb signals due to stimulation of work. The GLM analysis was performed in two levels: At the first level, matrices of canonical HRF and its temporal and elements of dispersion derivatives were designed and at the second level, contrast im-

Figure 2: Samples of the three different conditions for Persian version of Stroop test: (a) neutral condition, (b) congruent condition and (c) incongruent condition.

Figure 3: Stroop task timing sequence: the task includes 15 blocks and each block has 10 event related trial.
ages indicating congruent, incongruent and neutral versus baseline conditions were produced based on a random effects GLM and a one-sample t-test ($p \leq 0.05$). All acquired data were analyzed using the SPM-fNIRS and SMP8 toolboxes using Matlab software.

**Dynamic Causal Modeling (DCM)**

Dynamic causal modeling (DCM) was applied to fNIRS data in order to investigate the effective connectivity among brain regions involved in the processing of Stroop interference effect. DCM provides a framework for implementing differential equation models for brain imaging data based on the Bayesian hypothesis and describes the interaction between nerve populations as a generative model [27]. In this study, first, the source regions of the cerebral cortex were selected; second, the DCM models were specified and estimated. The main equations for hemodynamic modeling of DCM-fNIRS analysis were as follows:

The HbT changes rate $p_j$ was modeled according to Eq. 1:

$$
\tau_j p_j = \left( f_{j,in} - f_{j,out} \right) \frac{p_j}{v_j}
$$  

Where $j$ denotes the region of cortical source, $p_j$ is the total hemoglobin variations, $f_{j,in}$ is input flow, $f_{j,out}$ is output flow, $v_j$ is blood volume, and $\tau_j$ is the transit time.

Optical density changes and hemodynamic sources related to each other are described through an optical model (Eq. 2). In this equation $y_i(\lambda)$ is optical density changes of wavelength $\lambda$ in the $i^{th}$ channel. We can see linear combination of light absorption changes due to hemoglobin oxygenation describe $y_i(\lambda)$.

$$
y_i(\lambda) = \frac{1}{w_{i,j}} \sum_{j=1}^{k} S_{j,i}(\lambda) \epsilon_{H,j}(\lambda) \Delta H_{j,c} + \frac{1}{w_{i,j}} \sum_{j=1}^{k} S_{j,i}(\lambda) \epsilon_{Q,j}(\lambda) \Delta Q_{j,c}
$$

where $\Delta H_{j,c}$ and $\Delta Q_{j,c}$ are the HbO and HbR changes in the cortical source region $j$, $S(\lambda)$ is the sensitivity matrix at wavelength $\lambda$, $\epsilon$ and $\Delta H$ and $\Delta Q$ are the extinction coefficients for HbO and HbR, $\omega$ = cortical / (cortical+pial) is a factor for correcting the effect of pial veins oxygenation changes on fNIRS measurements.

In order to specify and estimate the DCM, the first step is producing a connectivity model. Using this model, one can determine how interactions between hidden neural states lead to fNIRS data generation. For this purpose, the following notes should be considered: (i) stimulation input areas (ii) connected areas in the absence of an empirical input and (iii) connections that are adjusted by input. Finally, the best model is produced compared to the Bayesian model, in which task input leads to regional activities associated with the conflict processing in the prefrontal cortex.

**Results**

**Behavioral Results**

Mean reaction times (RTs) of correct answers were calculated for each participant in each condition. A paired t-test was performed with the congruent, incongruent and neutral conditions as a dependent variables in order to reveal significant statistical differences in RT between three Stroop trials. The mean RT for the neutral (1018.43±142.34ms) and congruent conditions (1120.26±163.65ms) were shorter than the incongruent condition (1223.43±131.21ms) and the average errors were very low (congruent = ~ 3% and non-congruent = ~ 4.5%).

**fNIRS Results**

The analysis of statistical parametric Mapping (SPM) depicting concentration changes in hemodynamic parameters (oxy-Hb, deoxy-Hb and total Hb) of brain regions were extracted during the Stroop paradigms. The analysis of GLM random effects revealed significant differences between the congruent, incongruent and neutral conditions in the left dorsolateral prefrontal cortex (LDLPC) [(-46, 36, 12) mm in MNI coordinates; p<0.05], right dorsolateral prefrontal cortex (RDLPFC) [(46, 36, 12) mm in MNI coordinates; p<0.05] and in the dorsomedial prefrontal cortex (DMPFC) [(3,
Spatially, in congruent condition, approximately similar activations were observed on the both sides of the cerebral hemisphere, including LDLPFC and RDLPFC regions (see Figure 4a, panels A, B and C). Whereas, during incongruent condition, an increased activity was recorded in the LDLPFC (see Figure 4b, panels A, B and C) and under neutral condition, the increase in activity in LDLPFC was even more pronounced compared to the other two Stroop conditions (see Figure 4c, panels A, B and C). Then, the voxels with significant activation within LDLPFC, RDLPFC and DMPFC were selected as the source positions for DCM analysis.

DCM Analysis

Stroop task-independent connectivity determines the neuronal connectivity patterns between activated brain regions, which are constant in all experimental conditions. The event related alterations of neural interactions were investigated based on optical density signals derived from fNIRS data. Initially, models were specified to compare the unique influence of regional stimulation on effective connectivity parameters involved in the Stroop interference effect. Then, the locations of the three regions of interest (ROIs) contributed toward the Stroop tasks were identified in each individual (voxel-level corrected for pFWE < 0.05) (Table 2 and Figure 5a). A repeated measure analysis of variance (ANOVA) was conducted for each measurement area to determine differences between the different conditions (congruent vs. incongruent vs. neutral Stroop tasks).

The Stroop interference effect was evaluated according to the task-dependent changes in connectivity between the LDLPFC, RDLPFC, DMPFC regions under incongruent condition (see Figure 5b). The results indicated an increase in connectivity strength from RDLPFC to DMPFC (RDLPFC→DMPFC), LDLPFC to DMPFC (LDLPFC→DMPFC) and LDLPFC to RPFC (LDLPFC→RPFC) while other communications do not change. Strength of similar increased connectivity was observed from DMPFC to LDLPFC (DMPFC→LDLPFC) and RDLPFC to LPFC (RDLPFC→LPFC), also from DMPC to RDLPFC (DMPC→RDLPFC) under congruent and neutral conditions, respectively (Table 3). In addition, among different Stroop conditions, the effect of task was more dominant in neutral trial on LDPFC and DMPFC.

After statistical analysis, the connectivity of

![Figure 4: SPM t-statistic map of regional concentration changes of (A) oxy-Hb (B) deoxy-Hb and (C) total Hb during (a) the congruent condition (b) the incongruent condition (c) the neutral condition (GLM random effect analysis for all contrasts were displayed at pFWE < 0.05).]

<table>
<thead>
<tr>
<th>Cortex Regions</th>
<th>Nodes Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LDLPFC</td>
</tr>
<tr>
<td>2</td>
<td>RDLPFC</td>
</tr>
<tr>
<td>3</td>
<td>DMPFC</td>
</tr>
</tbody>
</table>

Table 2: Coordinates of nodes for DCM model.
ROIs for all three conditions (e.g. congruent, incongruent and neutral) were evaluated using ANOVA test. The connection indexes have been shown in Table 3 and the strongest connections were presented in bold.

**Discussion**

Cognitive responses require a proper understanding of the various stimuli in the environment. It is also possible that they are due to the semantic processing of the different characteristics of a given stimulus in different cortical regions of the human brain. The semantic processing, which is the basis of cognitive responses, is so extensive that they cannot be achieved by a single region in the cortex and without connecting to other areas [28, 29]. On the other hand, it has been demonstrated that Stroop interference effect in normal subjects changes neural activity of brain regions involved in the cognitive conflict processing, but the effective connectivity among these regions has not been investigated yet [30, 31]. In the present study, the fNIRS measurements of

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**Table 3:** Estimation of Stroop task dependent on connectivity parameters: Mean and Standard Deviation values of Stroop DCM model.

<table>
<thead>
<tr>
<th>Connectivity Type</th>
<th>Mean±SD connection strength of congruent</th>
<th>Mean±SD connection strength of incongruent</th>
<th>Mean±SD connection strength of Neutral</th>
<th>p-value (ANOVA test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMPFC ► RDLPC</td>
<td>0.178±0.06</td>
<td>0.189±0.023</td>
<td>0.196±0.05</td>
<td>0.035</td>
</tr>
<tr>
<td>RDLPC ► DMPFC</td>
<td>0.226±0.014</td>
<td>0.301±0.116</td>
<td>0.150±0.011</td>
<td>0.041</td>
</tr>
<tr>
<td>LDDLPC ► DMPFC</td>
<td>0.154±0.030</td>
<td>0.252±0.036</td>
<td>0.210±0.019</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMPFC ► LDDLPC</td>
<td>-0.018±0.019</td>
<td>-0.013±0.014</td>
<td>-0.011±0.017</td>
<td>0.026</td>
</tr>
<tr>
<td>LDDLPC ► RDLPC</td>
<td>0.003±0.017</td>
<td>0.009±0.065</td>
<td>0.007±0.06</td>
<td>0.028</td>
</tr>
<tr>
<td>RDLPC ► LDDLPC</td>
<td>0.100±0.019</td>
<td>0.052±0.036</td>
<td>0.026±0.004</td>
<td>0.011</td>
</tr>
<tr>
<td>TASK ► DMPFC</td>
<td>0.011±0.005</td>
<td>0.001±0.025</td>
<td>0.012±0.009</td>
<td>0.013</td>
</tr>
<tr>
<td>TASK ► LDDLPC</td>
<td>0.003±0.017</td>
<td>0.005±0.065</td>
<td>0.008±0.012</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Figure 5:** (a) the location of the selected nodes for DCM model in Stroop task. Blue, red and green nodes show LDDLPC, DMPFC, and RDLPC, respectively. (b) The structure of DCM for Stroop interference effect. The model consists of three regions, including, left DLPFC, DMPFC and right DLPFC and input according to the Stroop paradigm.
healthy participants were adapted to monitor as follows: (1) brain activation patterns based on GLM and (2) inter-regional effective connectivity based on DCM, during the processing of a standard color and word Stroop task.

According to our behavioral data, RTs were slower for incongruent compared to neutral and congruent conditions, exposing the expected Stroop effect. This suggests that prolonged activation for incongruent stimuli in comparison to congruent stimuli created conflict in response selection. The spatial maps of hemodynamic parameters for different Stroop tasks demonstrated activation in the lateral and dorsal prefrontal cortex, which is in accordance with previous functional imaging studies [32-34].

During the incongruent condition, the study showed increased activity in LDLPFC while only a minor difference was observed for bilaterally activation pattern during the congruent condition. In addition, the increase in DLPFC activation was more pronounced for neutral task, as revealed by the fNIRS analysis. In the Stroop task, first, the person pays particular attention to the images; thus, he/she can distinguish differences between the word color and color name, then select appropriate answer. Since, the Stroop test requires proper attention and cognition as well as the design and execution of appropriate responses, its results can determine the neural communications between different areas of the prefrontal cortex. In this regard, brain networks that respond to Stroop interference, especially the prefrontal cortex region, were considered for the determination of ROIs in subsequent DCM analysis.

The DCM results indicated that the best matching of the connectivity model with the fNIRS data is obtained among areas of LDLPFC, RDLPFC and DMPFC where ascending or descending regional couplings are modulated by Stroop conflict processing. According to the effective connectivity results, Stroop effect was associated with an increase in the RDLPFC→DMPFC, LDLPFC→DMPFC and LDLPFC→RDLPFC coupling.

These findings suggest that the brain prefrontal cortex, especially DLPFC and DMPFC are key regions in response to conflict effect, known to be active during Stroop tasks. The DMPFC region receives inputs from different sensory areas by information processing and identifies the characteristics of the stimulus such as its shape, color, and nature. The prefrontal cortex that extends in both hemispheres of the brain plays an important role in identifying errors, making appropriate decisions and controlling actions. So that the disturbances in this region leads a lack of concentration, and one will not be able to organize programs effectively [35, 36]. This DMPFC also has outputs to the DLPFC components, which are responsible for performing various tasks, such as identifying differences in selection and making decision, designing and governing actions [37].

With regard to the results, it seems that under incongruent condition, the input from all sensory pathways increases to the left DLPFC region, indicating the LDLPFC contribution in understanding the difference between colors and names, as well as controlling the appropriate response. In addition, under congruent condition, output from the LDLPFC expands to other areas (middle and right) of prefrontal cortex, without any more differences. In addition, the middle and right regions play a role in controlling the cognition related proper response. In neutral conditions, there is also sensory input to the DMPFC area, but a significant increase in activity is observed on both sides of the DLPFC, as color and name matching recognition are not required.

In this study, the efficacy of DCM in fNIRS was demonstrated to examine the effective connectivity among brain-activated regions during the Stroop tasks. In addition, this research can provide some useful information about the response of the human to conflict stimuli, as shown in Stroop interference effect.
It seems that for better understanding of neural mechanisms involved in the main cognitive functions, identification of the brain activity patterns, directionality of neural interactions and their changes should be considered.

Conclusion
The results of the present study indicate that fNIRS is an effective neuroimaging tool to study the neural correlates of response inhibition, selective attention and interference during the execution of the cognitive functions. In this report, DCM model was used to quantify conflict processing associated with the Stroop task and provide complementary information about the connectivity patterns among brain activated regions. These findings could be used in future neurological research and psychotherapy application.

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Conflict of Interest
None

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