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**SPIE.**

# Similarity analysis of functional connectivity with functional near-infrared spectroscopy

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**Abstract.** One of the remaining challenges in functional connectivity (FC) studies is investigation of the temporal variability of FC networks. Recent studies focusing on the dynamic FC mostly use functional magnetic resonance imaging as an imaging tool to investigate the temporal variability of FC. We attempted to quantify this variability via analyzing the functional near-infrared spectroscopy (fNIRS) signals, which were recorded from the prefrontal cortex (PFC) of 12 healthy subjects during a Stroop test. Mutual information was used as a metric to determine functional connectivity between PFC regions. Two-dimensional correlation based similarity measure was used as a method to analyze within-subject and intersubject consistency of FC maps and how they change in time. We found that within-subject consistency ( $0.61 \pm 0.09$ ) is higher than intersubject consistency ( $0.28 \pm 0.13$ ). Within-subject consistency was not found to be task-specific. Results also revealed that there is a gradual change in FC patterns during a Stroop session for congruent and neutral conditions, where there is no such trend in the presence of an interference effect. In conclusion, we have demonstrated the between-subject, within-subject, and temporal variability of FC and the feasibility of using fNIRS for studying dynamic FC. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: [10.1117/1.JBO.20.8.086012](https://doi.org/10.1117/1.JBO.20.8.086012)]

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## 1 Introduction

Due to large variations in neuroscientific findings, researchers have long avoided investigating moment-to-moment variability of neuronal activity and have chosen to comment on the grand average of ensemble data. The source of this variability is usually attributed to the presence of many forms of exogenous and endogenous noise that increase the complexity of data, specifically the neuroimaging data. The ultimate goal of neuroimaging researchers, hence, is to extract meaningful information from an ensemble of data by proposing methods to minimize the variations.<sup>1</sup> Other than instrumentation noise [ $i(t)$ ], the natural consequence of operation of the brain leads to generation of physiological noise [background noise,  $b(t)$ ]. Buried under these two types of noise is also the true moment-to-moment variability of neuronal activity [ $n(t)$ ], which should be distinguished from instrumentation and background noise.<sup>2</sup> Hence, a generic neuroimaging data model  $d(t)$  can be written as the sum of these independent or weakly dependent (as in the case of the background physiological noise with the neural activity) data:  $d(t) = n(t) + b(t) + i(t)$ . One easy way to eliminate the uncorrelated noise signals is to perform a task  $N$  times, where the expectation of the neuronal activity [ $\bar{n}(t) = \sum_k n_k(t)$ ,  $k = 1 \dots N$ ] converges to a hypothetical true neuronal activity [ $n_H(t)$ ], while the noise terms approach zero [ $\bar{i}(t) \approx 0$  and  $\bar{b}(t) \approx 0$ ]. Here the main assumption is that  $n_k(t)$  is stationary, meaning each time the task is repeated the neuronal signal is very similar to the previous ones.

Functional connectivity (FC) refers to the statistical relations of activations of distinct neuronal populations without any reference to causal or anatomic connections.<sup>3</sup> Conventionally, FC is computed by correlating a time series signal from one area [functional magnetic resonance imaging (fMRI) voxel or electroencephalography (EEG) electrode] with a signal from another area. The result of this analysis provides an FC matrix, which can then be further analyzed to provide a map or a network topology. Usually the whole time series are used to compute these correlation matrices, which inherently come with the assumption that the initial condition of the brain remains constant throughout the task; hence, neuroimaging data are stationary [i.e.,  $n_H(t) \approx \bar{n}(t)$ ]. This assumption surely poses a limitation to investigate the dynamical properties of the FC matrices. Even when several task blocks are used, an average of signals corresponding to these task blocks are computed [i.e.,  $\bar{n}^T(t)$ ,  $T$  is the task type] in order to increase the strength of the statistics.<sup>4</sup> Only recently have scientists started investigating the dynamical properties of FC networks and how FC maps obtained from the beginning of a task resembled the maps obtained toward the end of a session (which is termed the “consistency of FC maps”).<sup>5,6</sup> Several studies have shown that FC vary due to differences in mental tasks and changes in FC are observed even in the same imaging session.<sup>7,8</sup> Consequently, dynamic FC, investigation of temporal properties of FC, is becoming an emerging topic.

Although dynamic properties of FC have been studied recently, most of these studies use fMRI as an imaging tool.

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There has been no study in dynamic FC with functional near-infrared spectroscopy (fNIRS) as an imaging tool, to our knowledge. fNIRS is a promising functional imaging tool, especially when temporal resolution is considered: it is very difficult to have higher temporal resolution with fMRI, whereas with fNIRS, much higher temporal resolution might be possible. Therefore, fNIRS might be an important tool for investigation of dynamic FC. Previous studies have shown that it is feasible to apply FC methods to fNIRS signals.<sup>9-13</sup> In this study, we explored within-subject and intersubject consistency and temporal variability of FC networks using fNIRS in the prefrontal cortex (PFC) of the brain. We questioned whether it is feasible to study the temporal changes in FC computed from fNIRS signals that represent the neuronal dynamics during a cognitive task [i.e.,  $n_k^T(t)$ ].

Hence, we decided to focus our study on a pure cognitive task and used the well-known Stroop test to obtain the FC maps. In short, we questioned whether the brain dynamics during a specific instant ( $t$ ), a specific stimulus type ( $T$ ), for a given person ( $k$ ) [hence  $n_k^T(t)$ ] remain unchanged as the subject performs the task over and over during the course of a study. Hence, the main question is how similar (or different) the individual brain responses are for a given task [i.e.,  $n_k^{T(i)}(t) \stackrel{?}{=} n_k^{T(j)}(t)$ ], where  $T(i)$  and  $T(j)$  are the  $i$ 'th and  $j$ 'th blocks of the same  $T$  stimulus type (i.e., congruent blocks).

## 2 Methods

### 2.1 Data Collection

#### 2.1.1 Subjects

Twelve healthy subjects recruited from college graduate students volunteered for this study (seven males, ages  $24 \pm 2.3$ ). The study was approved by the Ethics Board of Bogazici University and informed consent forms were signed and collected from the participants.

#### 2.1.2 Protocol

A modified version of the color-word matching Stroop task was used in the study.<sup>14</sup> This task consists of three different stimulus conditions: neutral (N), congruent (C), and incongruent (IC) (Fig. 1). The subjects were asked to detect if the word below defines the color of the word above correctly or not. In the neutral case, a nonverbal stimulus was introduced in the upper

Color-word matching stroop task		
Neutral	Congruent	Incongruent
XXXX BLUE	RED BLUE	GREEN BLUE
XXXX BLUE	BLUE BLUE	GREEN BLUE

**Fig. 1** Three different stimulus conditions in the Stroop task: neutral, congruent, and incongruent. The words in the upper row are the cases where the word below wrongly defines the color of the word above. The other three questions given at the bottom row represent the opposite case; the word below correctly defines the color of the word above.

word, as a series of X's. Subjects made a left mouse click with their right index finger to indicate a match case and a right mouse click with their middle finger for nonmatching cases.

Five blocks were applied for each condition (Fig. 2). Each block consisted of six trials with an interstimulus interval of 4 s. Word pairs were presented on the screen until the response was given with a maximum time of 2.5 s. Between each task block, there was a short resting period of 20 s. The order of task blocks were randomized for each subject. The randomization algorithm prevented tasks of the same type to be presented successively.

### 2.1.3 Functional near-infrared spectroscopy device

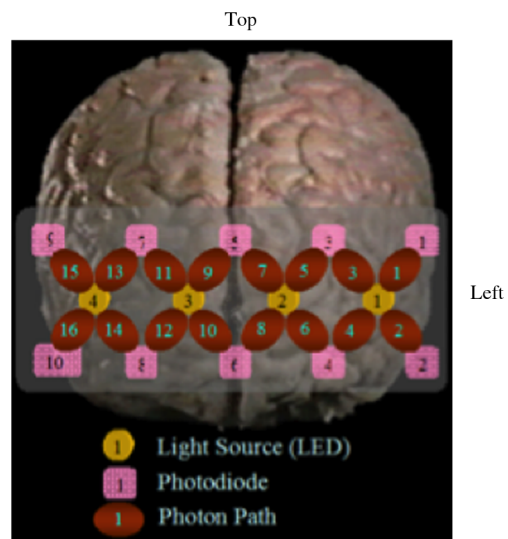
In this study, a 16-channel continuous wave-fNIRS device (NIROXCOPE 301) was used, which was developed in the Neuro-Optical Imaging Laboratory of Boğaziçi University.<sup>15-17</sup> This device has four sources of light, surrounded by 10 optical sensors. The probe geometry is a rectangular formation, each source surrounded by four detectors with a source-detector spacing set at 2.5 cm. At a given time, only one of these light sources and the surrounding four detectors are active. Therefore, 16 time-series data were collected from each subject corresponding to 16 regions in the PFC region, given in Fig. 3. Signals were acquired every 0.57 s, which gives the temporal resolution of the time-series signals collected. The data were previously recorded and published in another study.<sup>17</sup>

### 2.2 Data Preprocessing

During test sessions, time-series signals from 16 regions of PFC defined in Fig. 3 were recorded. The raw data collected were



**Fig. 2** A sequence for the three different stimuli in the Stroop task: neutral, congruent, and incongruent. Black lines indicate 20 s of rest. An initial 60 s of rest precedes the task.



**Fig. 3** Approximate anatomical sampling of the ARGES Cerebro™ functional near-infrared spectroscopy (fNIRS) system from the prefrontal cortex.

first used to generate the time-series of changes in oxyhemoglobin ([HbO<sub>2</sub>]) and deoxyhemoglobin ([Hb]), by using a modified version of the Beer-Lambert law. A previous study suggested that [HbO<sub>2</sub>] has the strongest correlation with the BOLD signal measured by fMRI.<sup>18</sup> Therefore, [HbO<sub>2</sub>] concentrations were computed for the 16 channels over the PFC. Then these 16 time-series signals were filtered digitally by a fourth-order Butterworth bandpass filter at the range of 0.03 to 0.25 Hz to eliminate slow drifts and physiological noise by using the `butter(4, [0.03, 0.25] / (Fs/2))` code in MATLAB<sup>®</sup>.<sup>16,17,19</sup>

### 2.3 Functional Connectivity Matrices

Functional connectivity between brain regions can be evaluated using various different measures, including correlation,<sup>20</sup> partial correlation,<sup>21</sup> coherence,<sup>22</sup> mutual information,<sup>23</sup> and autoregressive models.<sup>24</sup> Mutual information was used in this study to compute the functional connectivity. Mutual information has an advantage compared to correlation since it can detect nonlinear dependencies of neural signals,<sup>25,26</sup> and it has been used in the literature as a metric to estimate FC in EEG and fMRI studies.<sup>23,27,28</sup>

Zhou et al. proposed a method for computing mutual information of two signals based on their coherence in the frequency domain<sup>25</sup> which was used in this study. The mutual information of the *i*'th and *j*'th time-series in frequency domain is given as

$$\phi(i, j) = [1 - \exp(-2\delta_{ij})]^{\frac{1}{2}}, \quad (1)$$

where

$$\delta_{ij} = \frac{1}{2\pi} \int_{\lambda_1}^{\lambda_2} \log[1 - \text{coh}_{ij}(\lambda)] d\lambda, \quad (2)$$

and the cross-coherence function  $\text{coh}_{ij}(\lambda)$  is given as

$$\text{coh}_{ij}(\lambda) = |R_{ij}(\lambda)|^2 = \frac{|f_{ij}(\lambda)|^2}{f_{ii}(\lambda)f_{jj}(\lambda)}, \quad (3)$$

where  $f_{ij}(\lambda)$  is the cross spectral density between the *i*'th and *j*'th time-series; and  $f_{ii}(\lambda)$  and  $f_{jj}(\lambda)$  are the spectral densities of the *i*'th and *j*'th time-series, respectively.<sup>25</sup>

In Eq. (2), mutual information is computed as a sum of coherence values in a frequency band. In Eq. (1), this value is normalized into the range of (0,1).

Let  $\phi(i, j)$  denote the mutual information of signals *i* and *j*. All pair-wise mutual information (MI) of 16 signals obtained by an fNIRS constructs a 16 × 16 MI matrix  $A = [a_{ij}]$ , where  $a_{ij} = \phi(i, j)$ . We use the properties of mutual information to simplify this matrix. For signals *i* and *j*, (1) MI of a signal with itself is 1, i.e.,  $\phi(i, i) = 1$  for all *i* and (2) MI is symmetric, i.e.,  $\phi(i, j) = \phi(j, i)$ . Therefore, the upper triangular part of the 16 × 16 MI matrix carries all the information (Fig. 4). Redefine  $A = a_{i,j}$  as

$$a_{ij} = \begin{cases} \phi(i, j), & j > i, \\ \bar{A}, & \text{otherwise} \end{cases}$$

where  $\bar{A}$  is the mean value, that is,

$$\bar{A} = \sum_{i=1}^C \sum_{j=i+1}^C \phi(i, j).$$

This revised matrix  $\bar{A}$  is called the FC matrix.

FC matrices were computed from signals corresponding to the time range of each block. Since there were 15 stimulus blocks in total (five neutral, five congruent, five incongruent) with rest periods in between, 15 FC matrices were generated per subject corresponding to 15 block periods (rest periods are ignored). This is described in Fig. 5. Given the 0.57 s of temporal resolution and 24 s period for each task block, each task block included ~42 time-points.

The following conventions are used in the equations in the following sections:

$N = 12$ , the number of subjects.

$M = 15$ , the total number of task blocks ( $5N + 5C + 5IC$ ).

$T = 5$ , the number of blocks of each test type.

$C = 16$ , the number of fNIRS channels.

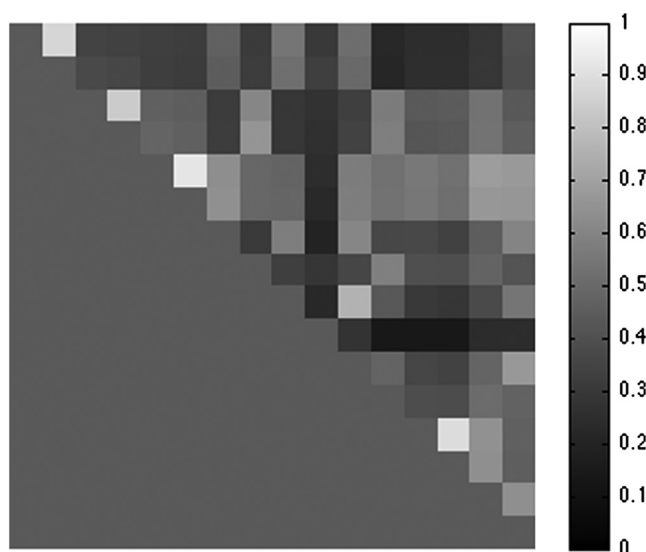
For each subject, there are 16 × 16 FC matrices for each person. For a person *k*, we group these 15 matrices such that the first five are the neutral FC matrices denoted by  $A_k^1, \dots, A_k^5$ . The second five are the congruent ones,  $A_k^6, \dots, A_k^{10}$ . Finally, the last five are the incongruent FC matrices, namely,  $A_k^{11}, \dots, A_k^{15}$ .

### 2.4 Similarity and Consistency Analysis

Two-dimensional (2-D) correlation is a method commonly used to compute the similarity of two images or patterns, especially for registration purposes.<sup>29</sup> The similarity of two FC networks can be computed by 2-D correlation.<sup>5</sup> In this study, the similarity was computed as

$$s(A, B) = \frac{\sum_i \sum_j [(a_{ij} - \bar{A})(b_{ij} - \bar{B})]}{\sqrt{[\sum_i \sum_j (a_{ij} - \bar{A})^2][\sum_i \sum_j (b_{ij} - \bar{B})^2]}}. \quad (4)$$

Here, *A* and *B* are two different FC matrices and  $\bar{A}$  and  $\bar{B}$  are the means of them, respectively. Note that (1) the similarity of a matrix with itself is 1, i.e.,  $s(A, A) = 1$  for all *A*, (2) the value of



**Fig. 4** Functional connectivity (FC) matrix computed for a subject based on fNIRS signals recorded during the Stroop task. Diagonal and lower triangle entries are removed since the matrix is symmetric and diagonal entries are equal to 1.

the similarity is within  $(-1,1)$  range, and (3) the similarity is also symmetric, i.e.,  $s(A,B) = s(B,A)$ . Since lower triangle and diagonal entries hold no additional information, these entries were replaced by the mean of the upper triangle values.<sup>5</sup>

The similarity of the matrices can be used to investigate the consistency of FC maps for subjects. Now, define  $C_{k,l}$ , the consistency of subjects  $(k, l)$ , as

$$C_{k,l} = \frac{1}{\binom{M}{2}} \sum_{i=1}^M \sum_{j=i+1}^M s(A_k^i, A_l^j).$$

$C_{k,k}$  is called the within-subject consistency. Intersubject consistency can be computed by the average of every pair of subjects as

$$C_{IS} = \frac{1}{\binom{N}{2}} \sum_{k=1}^N \sum_{l=k+1}^N C_{k,l}. \tag{5}$$

Finally, we computed the within-task and intertask consistency of FC matrices for each subject. If the within-task consistency is significantly higher than the intertask consistency, this means that FC patterns differ for different cognitive tasks. To measure within-task consistency, we computed the average similarity of FC matrices of the same stimulus condition (neutral, congruent, or incongruent) for each subject.

Let  $b$  be 0,5,10, representing the index of the  $A_k^b$  FC matrices for the neutral, congruent, and incongruent task blocks, respectively. Then the average within-task (of the same stimulus condition) similarity  $S^T$  for any condition is given as

$$S^b = \frac{1}{N \binom{5}{2}} \sum_{k=1}^N \sum_{i=1}^T \sum_{j=i+1}^T s(A_k^{b+i}, A_k^{b+j}). \tag{6}$$

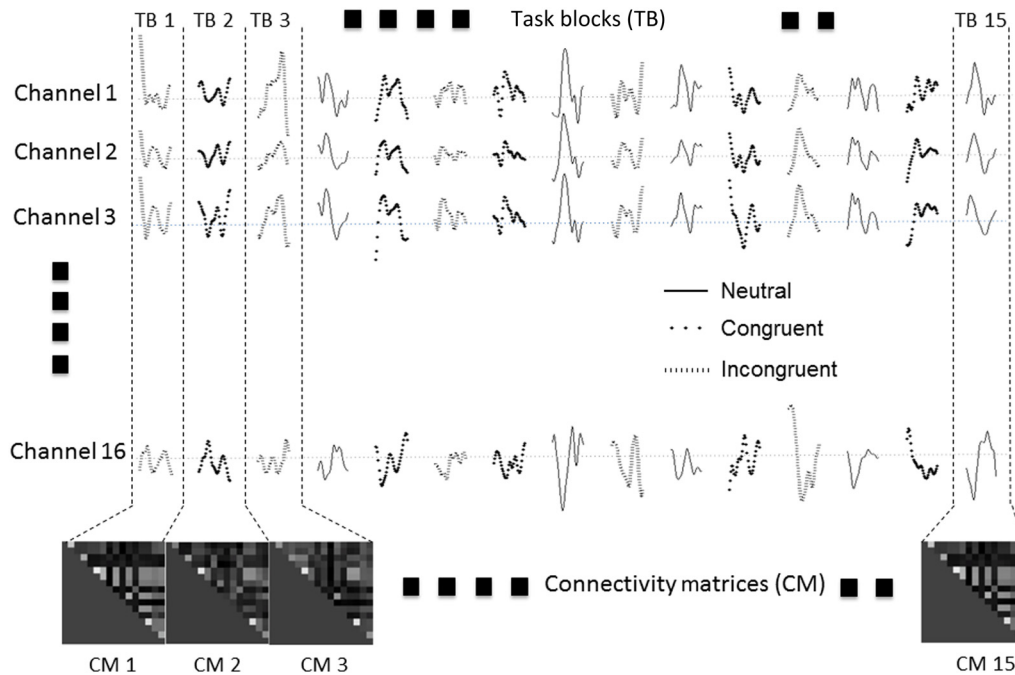
The within-task consistencies for congruent and incongruent stimulus conditions are computed in the same way.

To measure the intertask consistency, we computed the average similarity of FC matrices corresponding to different tasks for each subject. We computed the average neutral-congruent FC map similarity and repeated it for neutral-incongruent and congruent-incongruent.

### 2.5 Time-Varying Changes in FC Networks

In many studies of functional imaging, connectivity values are computed over the whole time-series to find the FC map, which is based on the assumption that FC map characteristics do not change during the recording session. In order to challenge this assumption, we investigated the resemblance of the consecutive FC matrices for a subject. We tested whether the similarity between two FC matrices of the same subject remained the same or not when the question blocks were presented closer to each other. In the case of a progressive change in the patterns of FC maps of subjects, test block pairs that are distant in time should show less similarity in their FC maps, compared to the test block pairs that are closer in time.

The time-differences of pairs of Stroop blocks versus their similarity in FC matrices were plotted. Since there are 15 task blocks in our Stroop test protocol with the same duration, the time-difference between each arbitrarily selected task block can have, at most, 14 different values for a subject. And considering that the randomization algorithm prevents successive



**Fig. 5** This figure describes how time series data from 16 fNIRS channels was segmented and how mutual information based connectivity matrices were computed. The fNIRS signals of a single subject are seen in the figure. There are 15 consecutive task blocks (randomly distributed 5 N, 5 C, 5 IC task blocks), and there are resting periods between task blocks. The connectivity matrix corresponding to each task block is computed by taking the signals from the beginning instant of the task block to the end of the same task block. In the end, we have 15 connectivity matrices for each subject.

presentation of two tasks of the same type, there are 13 possible time-difference values for a given task type. Since the order of congruent, neutral, and incongruent questions were arranged randomly, there is a variable number of block pairs that have certain differences in their times of presentation. We averaged FC similarity values of all task block pairs that have the same time distance in all subjects. We computed these values for neutral, congruent, and incongruent task types independently and observed the correlation between the time-difference and FC map similarity variables.

## 2.6 Behavioral Differences in Task Types and Time-Varying Changes in Behavioral Responses

We explored the behavioral differences between task types and temporal changes in the behavioral response of the subjects. We investigated behavioral differences of task types by comparing the reaction time and accuracy of responses of the subjects, by using nonpaired *t* tests between each pair of task types.

Temporal changes in the behavioral response were investigated in terms of changes in the response times in the Stroop task. We averaged reaction times of each task block for each subject, and we compared the first and last task blocks of each task type in terms of their reaction times by using paired *t* tests. We also plotted the average reaction times of task blocks ordered in time for each task type. In order to be able to observe the trends in reaction time without the effects of outliers, we applied outlier elimination, which removed the reaction time values outside of the 30 percent window of the mean reaction time of the subject on a specific task type. These outlier values were replaced by mean values.

## 3 Results

### 3.1 Behavioral Results

#### 3.1.1 Behavioral differences between cognitive tasks

The comparison of reaction times for three different stimulus conditions neutral (N), congruent (C), and incongruent (I) are

given in Fig. 6, and the mean values are 0.98 s, 1.05 s, and 1.17 s [ $F(2,33) = 3.15$ ,  $p = 0.056$ ], respectively. Reaction times were significantly longer in the incongruent condition compared to the neutral ( $p < 0.0001$ ) and congruent ( $p = 0.0003$ ) conditions, while differences between reaction times for congruent and neutral conditions were marginally significant ( $p = 0.056$ ).

A comparison of correct answers among neutral, congruent, and incongruent conditions is given in Fig. 7. The average number of correct answers was 29.5 (98.3%) for neutral condition, 29.2 (97.2%) for the congruent condition, and 27.8 (92.8%) for the incongruent condition out of 30 questions [ $F(2,33) = 3.49$ ,  $p = 0.046$ ]. A *t* test showed that the numbers of correct answers that subjects gave to the congruent and neutral questions are not significantly different. On the other hand, it is seen that number of correct answers decreases significantly for incongruent questions.

#### 3.1.2 Temporal changes in behavior during the Stroop test

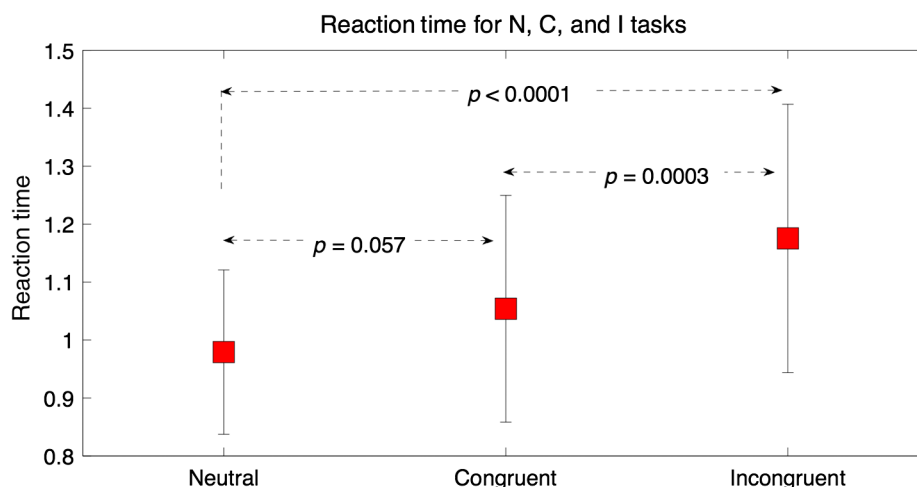
Table 1 shows the reaction times for the first and last task blocks for each task type. Results of the paired *t* tests are also given. First task blocks have a higher reaction time compared to last task blocks for all task types.

Figure 8 illustrates how reaction times change during the Stroop test for neutral, congruent, and incongruent tasks. The values are the average of all 12 subjects. Therefore, initially there were 360 reaction time values for each type of task (12 people  $\times$  5 blocks per task  $\times$  6 stimuli per block). Four of these 360 values were detected as outliers of the corresponding subjects for the corresponding tasks and, therefore, were replaced by corresponding mean values. It is observed that reaction time tends to decrease during the Stroop test for the congruent and incongruent task types. For neutral type questions, reaction times did not decrease further after an initial drop.

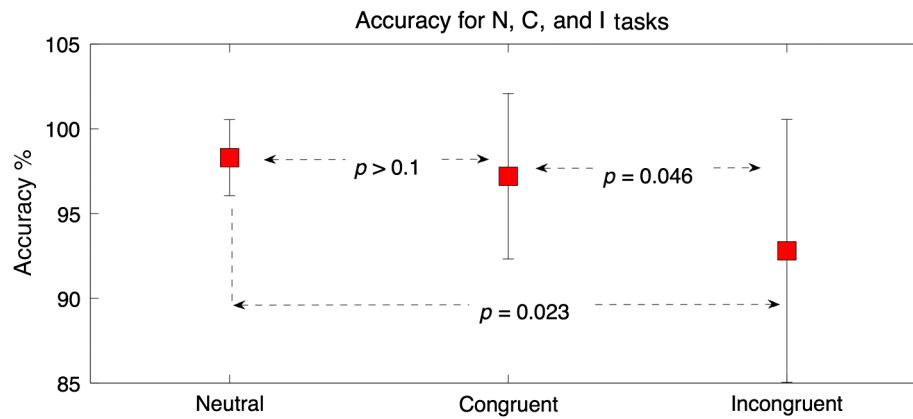
### 3.2 Functional Connectivity Results

#### 3.2.1 Consistency of functional connectivity networks

The average within-subject consistency of the FC matrices was computed as  $0.61 \pm 0.09$  for 12 healthy control subjects. The



**Fig. 6** The comparison results for reaction times in three different stimulus conditions: neutral (N), congruent (C), and incongruent (I) conditions.



**Fig. 7** The comparison results for accuracy in three different stimulus conditions: neutral (N), congruent (C), and incongruent (I) conditions.

**Table 1** Average reaction time values for first and last task blocks for each task type.

	Neutral	Congruent	Incongruent
First task block	1.077 s (0.181)	1.132 s (0.296)	1.259 s (0.311)
Last task block	0.948 s (0.158)	0.973 s (0.174)	1.086 s (0.2)
<i>p</i> values	0.024	0.009	0.003

result of intersubject consistency was found as  $0.28 \pm 0.13$ , which is significantly lower than the within-subject consistency ( $p < 0.001$ ). The comparison of within-subject consistency and intersubject consistency is given in Fig. 9.

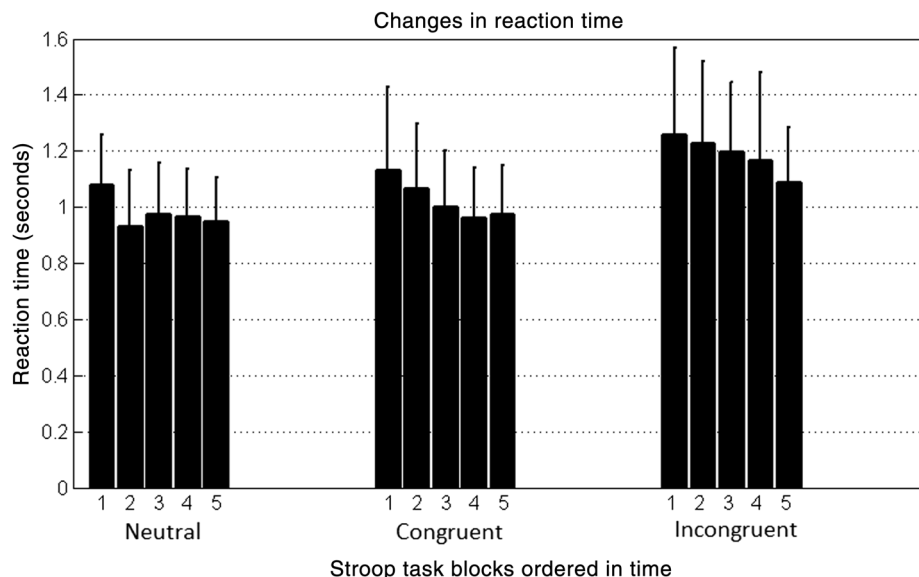
It was found that within-subject consistency does not change with task type (stimulus condition). Both within-task and inter-task consistency values are in the range between 0.605 and 0.615, and *t* test confirmed that there is no significant difference between them.

### 3.2.2 Time-varying changes in FC matrices

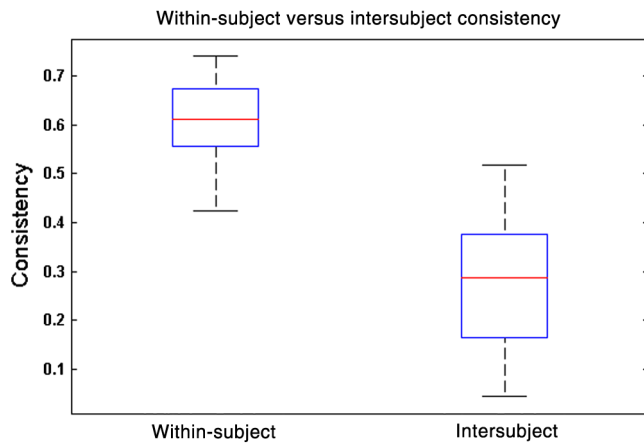
The relation between similarities of FC matrixes and their distance in time is given in Fig. 10. The correlation between time-difference and FC matrix similarity is  $-0.64$  ( $p = 0.019$ ) for the neutral condition and  $-0.79$  ( $p = 0.001$ ) for the congruent condition. It is seen that FC matrixes closer in their presentation times are more similar to each other and the similarity drops when they have a larger time-difference between them. This trend is not statistically significant for incongruent questions ( $p = 0.38$ ).

## 4 Discussion

In this study, we investigated the consistency and temporal variability of FC measured by fNIRS. We questioned whether it is feasible to study dynamic properties of FC with fNIRS. We used a Stroop test instead of measuring resting state brain activity, in order to evoke a measurable PFC activity. Another reason for this approach is that it is not clear what the resting state is, and it has been shown that during a so-called resting state, there might be significant cognitive activity.<sup>30-32</sup> Moreover,



**Fig. 8** Changes in reaction times for each task block during the Stroop test.



**Fig. 9** Within-subject versus intersubject consistency.

previous studies suggest that the Stroop interference effect is related to the activity of PFC,<sup>33–37</sup> from which we measure the fNIRS signals. Functional connectivity during the Stroop task has also been studied in the literature. A study by Kadosh et al.<sup>38</sup> revealed functional networks that are active during the Stroop task and found that these networks include PFC region. A study by Harrison et al. revealed increased connectivity (measured with canonical variants) in the interference condition. Several previous studies have shown that it is possible to study functional connectivity with fNIRS.<sup>11,12,16,39–54</sup> Aydore et al. studied functional connectivity with fNIRS using the Stroop test, and their study revealed increased information transfer in the interference condition.<sup>16</sup> However, most of these studies have ignored the temporal and/or interindividual variability of functional connectivity. Consistency and temporal variability of FC matrices were investigated in this study. Although temporal variability of FC has emerged as an important topic recently, the studies have used fMRI as an imaging tool so far. Our study questions whether it is feasible to study dynamic FC with optical signals, fNIRS. Although the temporal resolution used in this study is only slightly higher than common temporal resolutions used in fMRI, it is possible to achieve a much higher temporal resolution with fNIRS, which might have a key importance in dynamic FC studies in the future.

When we look at the behavioral results in our tests, it can be seen that the incongruent type questions of the Stroop test resulted in significantly higher reaction times compared to the congruent and neutral type questions. This was an expected result from the Stroop test, since there is a well known interference effect in the incongruent type questions.<sup>14</sup> When the verbal and color inputs conflict with each other, which is the case in incongruent type questions, subjects need to process them both and suppress one of them to decide. This is called the interference effect, which results in higher reaction times, as our results also confirm. When reaction times for congruent and neutral conditions are compared, it can be seen that tasks with the congruent condition resulted in higher reaction times, where the difference was marginally significant. This means that the facilitation effect was not observed, which is an effect when two different stimulus modalities are congruent and they facilitate each other, resulting in lower reaction times. But the facilitation effect is not considered as significant as the interference effect and it is not always observed in Stroop tests.<sup>4,12,55</sup> It was suggested that tasks with a congruent condition can result in

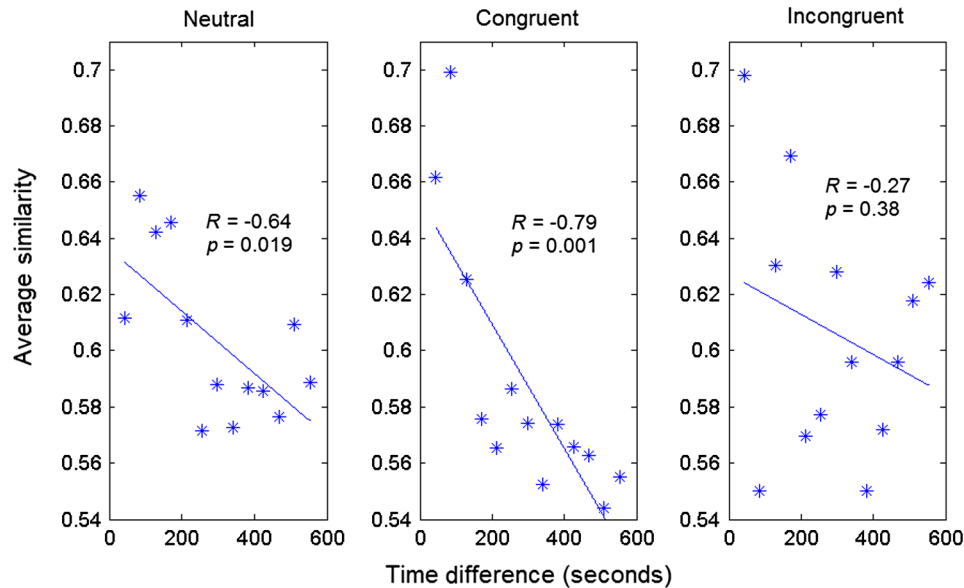
even higher reaction times, due to the conflict that arises in deciding which dimension (color or word) should be attended for responding.<sup>56</sup>

The consistency in patterns of FC networks measured during the Stroop task was found to be 0.61, which was measured by correlation of the weighted FC networks. But no difference between the intertask and within-task consistency could be detected. On the other hand, intersubject consistency (0.28) was found to be lower than the within-subject consistency (Fig. 9). Similar results were found in a previous study done with EEG, where within-subject consistency in the same recording session was found to be 0.84 and intersubject consistency was found to be 0.42.<sup>5</sup> In a previous study with fMRI, researchers investigated the consistency of FC networks within different recording sessions, and they found that intersubject consistency was lower than within-subject consistency.<sup>6</sup> Results found in the present study are consistent with these previous studies in the literature.

The low intersubject similarity among the maps might be merely due to different wiring patterns among subjects or systemic error since there is a lack of coregistration algorithm for the probe placement on each subject. Population analysis of FC maps of subjects based on fNIRS signals is being studied in the literature, where FC maps of different subjects are averaged or constructed with a common procedure.<sup>36,57</sup> The low intersubject consistency found in this study implies that making a population analysis of FC matrices of different subjects might be risky, due to differences in patterns of FC matrices of individuals. Although there are some studies for developing registration protocols for fNIRS,<sup>57</sup> there is no standard method in the literature yet. The results of this study show the necessity of such methods before performing a population analysis on the functional connectivity maps computed from fNIRS signals. A proper registration method is required to investigate the differences in FC characteristics between subjects.

The within-subject consistency found for FC matrices implies that a consistent FC pattern exists in PFC during the Stroop task along with a variability of the FC matrices of the same subject. Some part of this variation could be related to noise, but not necessarily noise of the measurement system. Noise is also present in the brain and it is considered to be a natural consequence of the operation of the brain.<sup>9</sup> In several studies, such noise has been termed as task-related spontaneous fluctuations, physiological in origin.<sup>11</sup> In order to understand whether the variability in FC networks is completely caused by spontaneous fluctuations (noise) in the brain, or if there is also a contribution of a temporal change throughout the mental task applied to the subjects, we investigated the time lag–FC network similarity relationship.

The time lag–similarity relationship of FC matrices showed that, for congruent and neutral type questions, FC map patterns of pairs of Stroop question blocks are more similar if they are closer in time (Fig. 10). We interpret this result as a consequence of a progressive change in FC patterns during the Stroop task for neutral and congruent stimulus conditions. When we analyze the temporal dynamics of the behavioral responses, we see that reaction times of the subjects decrease throughout the test. Although this is not the case for the neutral task blocks after the second task block, it can be argued that the reaction time for these task blocks are already very short and it is not possible to decrease further. We interpret these results as the subjects learning and getting used to the questions during the Stroop test.



**Fig. 10** Correlation of FC matrix pairs versus their corresponding distance in time. Y axis in the plot shows the similarity values between connectivity matrices, where the values in the X axis show their distance in time. Each point in the plots is the average of similarities of FC matrix pairs that have a certain difference in time, across all subjects, for a specific task type.

Therefore, the progressive change detected in FC maps of congruent and neutral tasks can be interpreted as a consequence of this habituation effect. It is interesting that no such trend was detected for the incongruent condition. The reason might be that the task is already very difficult to be learned and adapted to in such a short amount of time.

The interpretation of the progressive changes in FC patterns as habituation is consistent with the previous studies that have shown the relation between the activity in PFC and attention.<sup>33–35,38</sup> The most significant result of this study is that we have demonstrated the feasibility of studying the temporal variability of functional connectivity by using fNIRS.

## 5 Conclusion

In this study, we investigated how similar the FC networks are in a single imaging session throughout a cognitive task, and how consistent they are between-subjects based on fNIRS signals obtained from PFC region. The ability of the brain to adapt and develop strategies during a repetitive cognitive task is known to lead to a faster and more accurate completion of such tasks. This adaptation can be clearly seen in the decrease of the reaction times each time the task is repeated. The hypothesis in terms of how this adaptation is best represented in brain imaging is that the connectivity pattern during these repetitive tasks should somehow differ. So we tested both within-subject and intersubject consistency and the temporal variability of FC networks. Our results show that there is a consistency in the FC networks obtained from a single subject during the Stroop task, while there is also a temporal change in these networks during the task. So the strategy development actually leads to a convergence of the FC network while there is an inherent network structure that is preserved throughout the task as observed by the approximately 0.6 within-subject similarity (i.e., 60% similarity). Our results show that fNIRS can be used as an imaging tool for investigating the dynamic properties of FC.

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