Internetwork dynamic connectivity effectively differentiates schizophrenic patients from healthy controls

Hui Shen\textsuperscript{a}, Zhenfeng Li\textsuperscript{a}, Ling-Li Zeng\textsuperscript{a}, Lin Yuan\textsuperscript{a}, Fanglin Chen\textsuperscript{a}, Zhening Liu\textsuperscript{b} and Dewen Hu\textsuperscript{a}

Increasingly more neuroimaging studies have shown that the complex symptoms of schizophrenia are linked to disrupted neural circuits and disconnectivity of intrinsic connectivity networks. Previous studies have assumed temporal stationarity of resting-state functional connectivity, whereas temporal dynamics have rarely been explored. Here, we utilized resting-state functional MRI with a sliding window approach to measure the amplitude of low-frequency fluctuations (ALFFs) in functional connectivity in 24 patients with schizophrenia and 25 healthy controls. We found that there were significant differences in the ALFFs of specific connections, the majority of which were located between the intrinsic connectivity networks. Importantly, the experimental results of a multivariate pattern analysis of these ALFF measures showed that 81.3% ($P < 0.0009$) of the participants were correctly classified as either schizophrenic patients or healthy controls by leave-one-out cross-validation. Our results show significant abnormality in the dynamics of internetwork functional connectivity in schizophrenia, which contributes toward the characterization and differentiation of schizophrenic patients, and may be used as a potential biomarker. *NeuroReport* 00:000–000 © 2014 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Keywords: intrinsic connectivity network, low-frequency fluctuation, MRI, resting state, schizophrenia

*College of Mechatronics and Automation, National University of Defense Technology and \textsuperscript{b}Mental Health Institute, Second Xiangya Hospital, Central South University, Changsha, Hunan, China

Correspondence to Dewen Hu, College of Mechatronics and Automation, National University of Defense Technology, Changsha, Hunan 410073, China E-mail: dwhu@nudt.edu.cn

Received 2 August 2014 accepted 26 August 2014

**Introduction**

In past decades, numerous neuroimaging studies on schizophrenic patients have been carried out to identify the neurobiological markers of this complex disorder. These studies have shown that abnormal functional integration between specific brain regions is associated with the pathophysiological mechanism of schizophrenia [1]. This disconnectivity hypothesis of schizophrenia has been supported by increasing evidence from functional and structural connectivity studies [1–3]; however, the pathophysiological mechanism of this complex disorder is still unknown.

A typical assumption that has been used in previous functional connectivity studies of schizophrenia is temporal stationarity, and functional connectivity has been measured over the entire course of the functional MRI (fMRI) scan [2,4]. Recent fMRI studies have shown that brains are remarkably active even in the absence of overt behavior, leading to increased concerns regarding the dynamics of functional connectivity in the resting state [5,6]. Changes related to disease states in the dynamic properties of functional connectivity have also been reported [7], further suggesting a neural origin and raising the intriguing possibility that temporal features of functional connectivity could serve as a disease biomarker. The nonstationary correlational structures of fMRI data were believed to provide an opportunity to extract more network information, enhancing our understanding of the properties of brain networks in brain diseases such as schizophrenia.

In the present study, we hypothesize that abnormal temporal changes occur in the low-frequency oscillations (LFOs) of the intrinsic connectivity networks (ICNs) of schizophrenic patients. We first calculated the amplitude of low-frequency fluctuations (ALFFs) from the dynamic functional connectivity matrix extracted using a sliding window approach. Then, considering the ALFF measures as classification features, we used support vector machines (SVMs) to differentiate schizophrenic patients from healthy controls.

**Materials and methods**

**Participants**

A total of 49 participants were enrolled in this study, including 24 patients with schizophrenia and 25 healthy controls. The patients were recruited from the Department of Psychiatry, Second Xiangya Hospital of the Central South University, Changsha, China. All of the patients fulfilled the criteria for schizophrenia according to the DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders*, 4th ed.). The positive and negative syndrome scales were used to assess the symptom severity of the patients [8]. None of the patients had a history of neurological disorders, substance...
abuse, or electroconvulsive therapy. At the time of image acquisition, 18 of the patients with schizophrenia were receiving atypical psychotropic drugs [risperidone ($n=10$, 2–6 mg/day), clozapine ($n=4$, 200–350 mg/day), quetiapine ($n=4$, 400–600 mg/day), and sulpiride ($n=1$, 200 mg/day)], whereas the remaining six were medication free. All of the controls were recruited from Changsha city, China, and none of them was a relative of the schizophrenic patients. The participants were demographically similar with respect to age, sex, and education levels (Table 1). All participants were right-handed Chinese speakers and provided written informed consent. This study was approved by the ethics committee of the Second Xiangya Hospital of the Central South University.

Table 1  Demographic and clinical profiles of the participants in this study (mean ± SD)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Schizophrenia patients ($n=24$)</th>
<th>Healthy control ($n=25$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (males/females)</td>
<td>12/12</td>
<td>13/12</td>
</tr>
<tr>
<td>Education (years)</td>
<td>12.28 ± 2.5</td>
<td>13.52 ± 2.85</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.36 ± 6.32</td>
<td>25.48 ± 5.45</td>
</tr>
<tr>
<td>PANSS total</td>
<td>87.24 ± 12.2</td>
<td></td>
</tr>
<tr>
<td>PANSS positive</td>
<td>21.92 ± 4.74</td>
<td></td>
</tr>
<tr>
<td>PANSS negative</td>
<td>23.38 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>PANSS general</td>
<td>41.96 ± 6.4</td>
<td></td>
</tr>
</tbody>
</table>

PANSS, Positive and Negative Syndrome Scale.

Resting experiment and data acquisition

MRIs were acquired using a 1.5-T GE Signa scanner (GE Medical Systems). All fMRI images were collected using a gradient-echo planar imaging sequence. The imaging parameters were as follows: TR = 2000 ms, TE = 40 ms, FOV = 24 cm, FA = 90°, matrix = 64 × 64, slice thickness = 5 mm, gap = 1 mm, and slices = 20. During image acquisition, the participants were instructed to relax, keep their eyes closed, remain awake, and perform no specific cognitive exercise. To minimize head motion, the participants’ heads were fixed using foam pads and earplugs were used to reduce scanner noise. For each participant, the fMRI scan lasted 6 min, resulting in 180 volumes.

Data preprocessing

The flowchart of the entire analysis is shown in Fig. 1. First, resting-state fMRI images were preprocessed using SPM8 software (Welcome Department of Cognitive Neurology, Institute of Neurology, London, UK, http://www.fil.ion.ucl.ac.uk/spm). Before image preprocessing, the first five volumes of each functional time series were discarded for magnetic saturation effects. The remaining 175 volumes were corrected for head motion by registering and reslicing. Next, the volumes were normalized to the standard echo planar imaging template in the Montreal Neurological Institute (MNI) space (3-mm isotropic voxels). The resulting images were spatially smoothed with a Gaussian filter with an 8-mm full width at half maximum kernel and then temporally filtered with a Chebyshev band-pass filter (0.01–0.08 Hz).

We further regressed out the global, white matter, and cerebrospinal fluid average signals, as well as the effects of head movement [9,10]. Regional mean time series were calculated for each individual by averaging the fMRI time series over all voxels within each of the 160 regions of interest (ROIs) (radius = 8 mm) used in the previous study [11]. Therefore, for each participant, we generated a 160 × 175 data matrix. Then, we used a sliding-window approach to calculate the dynamic functional connectivity matrices.

Construction of dynamic networks

Empirically, window sizes of ~30–60 s can produce robust results in conventional acquisitions [6,12]. In this study, a time window of fixed length (width = 20, TRs = 40 s) was selected, and the data points within the window were used to calculate the functional connectivity matrix [5,13]. Then, for each participant, the window was shifted in time by a single data point, resulting in 156 windows, each containing a 160 × 20 data matrix. In each window, we evaluated functional connectivity between each pair of ROIs using Pearson correlation coefficients. Fisher’s z-transform was then applied to the correlation values to ensure normality. Thus, for each participant, we obtained a 156 × 12 720 matrix in which each column is the time series of one correlation coefficient.

For the time series of each correlation coefficient, we calculated the ALFF value from the typical frequency band (0.01–0.08 Hz). Consequently, the dynamic functional connectivity matrix of an individual participant was transformed into a 12 720 dimensional feature vector, representing the ALFF map of the dynamic functional connectivity for that participant.

Feature selection and support vector classification

We used two-tailed two-sample t-tests to extract the connections with significant ALFF differences between the patients and the controls ($P<0.0009$, uncorrected), which spanned the feature space for classification. SVMs with a polynomial kernel function were used to solve the classification problem [14]. Because of our limited number of samples, we used a leave-one-out cross-validation (LOOCV) strategy to estimate the generalization ability of our classifier. Permutation tests (10 000 times) were used to estimate the statistical significance of the observed classification accuracy [15,16].

On every fold of LOOCV, the features with lower $P$-values than the threshold were selected. The selected features differed slightly from fold to fold because of a slightly different subset for training on each fold. However, 11 features were included on every fold of LOOCV, which constitute the ‘consensus’ features for
the schizophrenia classification. For each ROI, we also computed the ‘region weight’, which was computed by summing the feature occurrence number in LOOCV.

Results

The nonlinear SVM classifier with the polynomial kernel achieved an accuracy of 81.3% (83.3% for patients and 80.0% for healthy controls, \( P < 0.0001 \), area under the curve = 0.835). The classification results with different kernel functions (linear and Gaussian kernel functions) are shown in Table 2.

After performing two-tailed two-sample \( t \)-tests in LOOCV, 11 ‘consensus’ connections were identified by intersecting the feature sets of all folds with \( P \)-values lower than 0.0009 (Fig. 2). By performing modularity optimization on the average adult functional connectivity matrix, the 160 ROIs could be divided into six resting-
state functional networks: cingulo-opercular (CON), frontoparietal, default mode (DMN), sensorimotor, occipital, and cerebellar (see [11] for detail). On the basis of the network affiliations of each ROI, the functional connections whose temporal variability was decreased in schizophrenia were mainly distributed between the default network and the CON and between the frontoparietal network and occipital cortex, whereas those with increased variability were located between the default network and the cerebellum and between the DMN and the sensorimotor network.

To assess the relative contributions of the different networks toward discrimination of schizophrenic patients from healthy controls, we summed the feature occurrence number in a given network (Fig. 2d). Functional connections were separated on the basis of whether they connected ROIs belonging to the same or different networks. Figure 2d shows that the vast majority of abnormal dynamic connections were located across functional networks.

In addition, Fig. 2 shows the relative contributions of different ROIs and individual features in schizophrenia discrimination by computing their ROI and connectivity weights. In particular, the DMN showed the greatest sum total of feature weights, meaning that it is relatively more important in accounting for the abnormal temporal variability of resting-state functional connectivity in schizophrenia.

**Discussion**

In the current study, we observed significant changes in the ALFF in the spontaneous oscillation of resting-state functional connectivity in schizophrenia. The majority of these abnormal connections were located across the ICNs, suggesting remarkable abnormality of internetwork functional interaction in schizophrenia.
Furthermore, on the basis of a multivariate pattern analysis of these abnormal connections, 83.3% of the 24 schizophrenic patients and 80.0% of the 25 healthy control participants were classified correctly. Thus, we believe that symptoms of schizophrenia can be effectively decoded by the amplitude of LFO in internetwork functional connectivity, which could provide potential dynamic connectivity signatures for the characterization and differentiation of schizophrenic patients.

The DMN, considered a hub for distant connections and a core functional network, plays a vital role in fundamental functions such as self-relevant internal information processing [17] and monitoring of the external environment [18]. The dysfunction of the DMN and its disconnection with other regions have been linked to positive and negative symptoms in patients with schizophrenia [19]. Garrity et al. [4] found that altered temporal frequency and spatial location of the DMN is associated with schizophrenia and suggested that these altered temporal fluctuations in patients might result from a change in the connectivity of some key regions with other brain networks. In the current study, the DMN showed the highest occurrence rate in the consensus connections, which is consistent with its role as a hub for integrating distributed information. In the patient group, the DMN–cerebellar and DMN–sensorimotor connectivity increases showed increased temporal fluctuations, whereas the DMN–thalamus connection presented decreased fluctuations over time. The two types of opposite patterns in the changed amplitude of LFO associated with the DMN may suggest abnormal information communication among these regions or disease-related compensation effects in terms of functional integration in schizophrenia.

We found that the cerebellum of the patients showed stronger LFO in dynamic connections with the anterior prefrontal cortex, precuneus, and the angular gyrus of the DMN. Previous studies examining structural and functional abnormalities of the cerebellum have found that the cerebellar regions involved in feedback and feedforward loops with cortical regions are affected in schizophrenia [20,21]. Our previous report also showed high discriminative power of the frontal–cerebellar circuit in schizophrenia discrimination [2]. Furthermore, this observation is consistent with the latest report, in which schizophrenic patients present stronger fluctuations over time in connections between the cerebellum and the left frontoparietal cortex [22]. This may confirm cerebellar dysfunction of specific regions in schizophrenia, which leads to weak frontal–cerebellar functional connections [20] and disease-related modulations in schizophrenia [22].

As a newly defined control network, the CON is responsible for various cognitive processes that have consistently been found to be impaired in schizophrenia [23]. Here, abnormalities were found in the dynamic functional connections related to the CON, including the basal ganglia–fusiform, thalamus–angular, thalamus–occipital, and middle insula–occipital connections, with decreased LFO amplitudes. Structural and functional damage within these regions have been reported in schizophrenia. For example, the volume of the basal ganglia was linked to treatment response to antipsychotics in schizophrenia [24]. In the typical frequency band of 0.01–0.08 Hz, the middle occipital gyrus, precuneus, posterior insula, and other regions show significantly lower ALFF values in schizophrenic patients than in controls [25]. This implies that the abnormal dynamic interaction among these regions may partially result from the dysfunction of individual regions. In particular, the weak LFO in the dynamic connections of the CON with other networks in schizophrenia may suggest a possible abnormality in the processing and transfer of cognitive information across these networks, resulting in cognitive damage in schizophrenic patients.

In the consensus connections, we can see that the internetwork functional connections are more highly represented than the intranetwork connections (Fig. 2d). In previous studies, researchers have used stationary functional connectivity analyses to examine the differences in intranetworks between patients with schizophrenia and healthy controls [1]. According to these investigations, the abnormality in the functional connectivity had been identified within the DMN [4], the CON [23], the visual cortical areas, and the cerebellum network [26]. The current results extend these findings, suggesting that there is significant abnormality of internetwork dynamic interactions in the brains of schizophrenic patients, in addition to their intranetwork connection alterations. These observations, together with our recent findings on between-network connectivity abnormality in schizophrenia [27], further highlight the importance of exploring the functional interaction across the ICNs of schizophrenic patients to better understand the pathophysiology of this complex disorder.

Limitations
Some limitations in this study are worth noting. Because of the limited sample size, our findings need to be confirmed with a larger sample size in the future. In addition, some of the patients with schizophrenia in this study were medicated. Previous studies have suggested that antipsychotic treatments tend to alter aberrant connectivity [28].

Conclusion
This study shows that multivariate pattern analysis on the basis of the ALFF of dynamic functional connectivity can effectively discriminate schizophrenic patients from healthy controls. The majority of the connections with the most significant differences were located across the
ICNs, indicating a remarkable abnormality of internetwork dynamic functional interaction in schizophrenic patients. The results provide new insight into the pathophysiology of this complex disorder and contribute toward its characterization and discrimination.

Acknowledgements
The authors thank the volunteers and patients for their participation in the study. This research was supported by the National Basic Research Program of China (2011CB707802) and the National Science Foundation of China (61375111).

Conflicts of interest
None declared.

References