

Original citation:

Li, Tao, Wang, Qiang, Zhang, Jie, Rolls, Edmund T., Yang, Wei, Palaniyappan, Lena, Zhang, Lu, Cheng, Wei, Yao, Ye, Liu, Zhaowen et al.. (2016) Brain-wide analysis of functional connectivity in first-episode and chronic stages of schizophrenia. Schizophrenia Bulletin.

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Title

Brain-wide analysis of functional connectivity in first-episode and chronic stages of schizophrenia

Running title: Functional connectivity changes in schizophrenia

Authors

Fei Wang^{1*}, Qiang Wang^{2*}, Jie Zhang^{3,4*#}, Edmund T. Rolls^{5,6*}, Wei Yang^{7*}, Lena Palaniyappan^{8,9}, Lu Zhang⁷, Wei Cheng³, Ye Yao³, Zhaowen Liu^{10,3}, Qiang Luo³, Timothy J. Crow¹¹, Matthew R. Broome^{12,13,14}, Ke Xu¹, Chunbo Li¹⁵, Jijun Wang¹⁵, Zhening Liu¹⁶, Guangming Lu⁴, Tao Li^{2#}, Jianfeng Feng^{3,17,18},

Affiliations, and author addresses

1. Department of Radiology, The First Affiliated Hospital, China Medical University, Shenyang, Liaoning, PR China
2. The Mental Health Center and the Psychiatric Laboratory, West China Hospital, Sichuan University, Chengdu, China
3. Institute of Science and Technology for Brain Inspired Intelligence, Fudan University, Shanghai, PR China
4. Department of Medical Imaging, Jinling Hospital, Nanjing University School of Medicine, Nanjing 210002, PR China.
5. Oxford Centre for Computational Neuroscience, Oxford, UK

-
6. Department of Computer Science, University of Warwick, Coventry
CV4 7AL, UK
 7. Shanghai Center for Mathematical Sciences, Shanghai, 200433, P.R
China
 8. Departments of Psychiatry, Neuroscience and Medical Biophysics
& Robarts Research Institute, Western University, London, Ontario,
Canada.
 9. Lawson Health Research Institute, London, Ontario, Canada.
 10. School of Computer Science and Technology, Xidian University,
Xi'an 710071, Shanxi, PR China
 11. SANE POWIC, University Department of Psychiatry, Warneford
Hospital, Oxford OX3 7JX, UK.
 12. Department of Psychiatry, Medical Science Division, University of
Oxford, Oxford, UK
 13. Oxford Health NHS Foundation Trust, Oxford UK.
 14. Division of Mental Health and Wellbeing, Warwick Medical School,
University of Warwick, Coventry, UK.
 15. Shanghai Mental Health Center, Shanghai, PR China.
 16. Mental Health Center, Xiangya Hospital, Central South University,
Changsha, China
 17. Collaborative Innovation Center for Brain Science, Fudan
University, Shanghai, 200433, PR China

18. Shanghai Center for Mathematical Sciences, Shanghai, 200433, P.R
China

* These authors contributed equally to the work

Corresponding author information:

Professor Jie Zhang

Institute of Science and Technology for Brain Inspired Intelligence, Fudan
University, Shanghai, PR China

Email: jzhang080@gmail.com

Professor Tao Li

The Mental Health Center and the Psychiatric Laboratory, West China
Hospital, Sichuan University, Chengdu, China

Email: xuntao26@hotmail.com

Word Count: Abstract: 250; MS: 3823

3 figures, 2 tables and a supplement document involving 1 figure and
8 tables.

Abstract

Published reports of functional abnormalities in schizophrenia remain divergent due to lack of staging point-of-view and whole-brain analysis. To identify key functional-connectivity differences of first-episode and chronic patients from controls using resting-state fMRI, and determine changes that are specifically associated with disease onset, a clinical staging model is adopted. We analyze functional-connectivity differences in prodromal, first-episode (drug naïve), and chronic patients from their matched controls from 6 independent datasets involving a total of 789 participants (360 patients). Brain-wide functional-connectivity analysis was performed in different datasets and the results from the datasets of the same stage were then integrated by meta-analysis, with Bonferroni correction for multiple comparisons. Prodromal patients differed from controls in their pattern of functional-connectivity involving the inferior frontal gyri (Broca's area). In first-episode patients, 90% of the functional-connectivity changes involved the frontal lobes, mostly the inferior frontal gyrus including Broca's area, and these changes were correlated with delusions/blunted affect. For chronic patients, functional-connectivity differences extended to wider areas of the brain, including reduced thalamo-frontal connectivity, and increased thalamo-temporal and thalamo-sensorimotor connectivity that were correlated with the positive, negative and general symptoms, respectively. Thalamic changes became

prominent at the chronic stage. These results provide evidence for distinct patterns of functional-dysconnectivity across first-episode and chronic stages of schizophrenia. Importantly, abnormalities in the frontal language networks appear early, at the time of disease onset. The identification of stage-specific pathological processes may help to understand the disease course of schizophrenia and identify neurobiological markers crucial for early diagnosis.

Key Words: resting-state fMRI, whole brain functional-connectivity analysis, clinical staging model, Broca's area, meta-analysis

Introduction

The pathophysiology of schizophrenia involves distributed functional dysconnectivity involving a number of brain regions ^{1, 2}, including the frontal lobe ³⁻¹⁰, and its language-related areas in the inferior frontal gyrus ^{11, 12}, sensory-motor areas ¹⁶, the temporal lobe ¹⁵, limbic structures ^{13, 14} and thalamus ¹⁷⁻²⁴. Despite numerous leads, the reported findings are somewhat inconsistent and the core regions associated with the pathogenesis of schizophrenia still remain controversial.

One important factor may be heterogeneity in patients regarding the stage of illness and medication ²⁵. Recently, the clinical staging model provides an approach to this problem ²⁶⁻²⁸ by offering a lifespan perspective of psychosis, ranging from the prodromal (ultra high-risk) stage through the

first-episode (FE) stage to the chronic stage of illness. It avoids an assumption of homogeneity and a grouping together of patients with different levels of illness severity and chronicity, all of which can influence the observed neurobiological landscape of psychiatric disorders. In a review of investigations that did compare first-episode and chronic stages of schizophrenia, it was concluded that schizophrenia is associated with dysconnectivity that is particularly evident in the connections involving the frontal lobe, and that this applies across all stages of the disorder². There are additional studies that have focused on the early changes in schizophrenia, including^{6,12,15, 29, 30,42,43}.

Another important factor in the heterogeneity of the findings may be that many studies have focused on functional connectivity between regions specified a priori, rather than adopting a whole-brain analysis. Consequently the reported findings are influenced by choice of the regions of interest, and may not cover the most significantly different areas that may reflect the core pathological changes in schizophrenia. A third important factor is that many studies have utilized relatively few patients and controls, often in the region of 10-40 patients and controls², and this may somewhat limit the reproducibility of the findings.

Given this background, the investigation described here first aims to provide evidence on functional connectivity differences in a large group of drug-naïve first-episode patients with schizophrenia using brain-wide

resting-state functional connectivity analyses. This is especially important for understanding the changes at the onset of the disorder. We further aim to identify differences in functional connectivity from controls of another large group of chronic schizophrenic patients (who are receiving medication), for patients at this stage are the usual participants in functional connectivity studies, and it is important to know how the connectivity differences in first-episode unmedicated patients differ from what is usually studied. In addition, we measured functional connectivity differences from controls in a small prodromal (ultra-high-risk) group, some of whom are likely to develop schizophrenia, to investigate whether some of the differences in the first-episode patients were becoming evident in the prodromal group, as this would further implicate these differences in the onset stage of schizophrenia. Here the first episode patients were defined as having illness duration less than a year, while chronic patients were defined as having illness duration longer than a year.

To achieve these goals, we adopted a whole-brain analysis using resting-state datasets from multiple neuroimaging centers to provide large numbers of patients and controls, and integrated the results by a meta-analytic approach that we developed ^{31, 32}, for both first-episode and chronic schizophrenia. Our results for first-episode patients (largely antipsychotic-naïve) are crucial to identify the core regions relevant to pathogenesis before the patients are exposed to antipsychotic treatment ²³. Combining

these results with chronic schizophrenia patients, we evaluate whether there are more widespread neurobiological differences, and whether the profile of functional dysconnectivity is different, across stages, to provide evidence on the pathophysiology of schizophrenia.

Methods

Participants

There were altogether 1050 subjects enrolled (497 patients) in our study. As we combine multi-center data with possibly large variation, it is important to set up a protocol to ensure data quality. The exclusion criteria were: 1. Subjects with poor structural scans, or without complete demographic information and PANSS scores, or age<16. 2. Head movement: subjects with >10% displaced frames in a scrubbing procedure or maximal motion between volumes in each direction > 3 mm, and rotation about each axis > 3° were excluded. 3. Patients and controls were screened in each dataset so that age, gender, education, head movements, and the total root mean square displacements did not show significant differences.

After data quality control, 789 subjects were left, including 343 patients (17 high-risk subjects, 197 first-episode patients, 146 chronic schizophrenia). These subjects are from Dataset 1[#] (Shanghai Mental Health Centre, prodromal, described in supplement text S1); Dataset 2[#]

(Huaxi hospital, FE, ¹⁹); Dataset 3[#] (Central South University³³, FE); Dataset 4[#] (The Center for Biomedical Research Excellence-COBRE, chronic); Dataset 5[#] (National Taiwan University Hospital³⁴, chronic); Dataset 6[#] (First Affiliated Hospital of China Medical University, FE and chronic). Detailed demographics of the patients are provided in Table 1. The first-episode group had illness duration ≤ 12 months while the chronic patients has an illness duration > 12 months with a mean of 8.8 years. A small prodromal group consisted of participants who are putatively prodromal for psychosis due to being at clinical high risk as defined by the SIPS/SOPS.

All patients were diagnosed by the DSM-IV diagnostic criteria ³⁵ by qualified psychiatrists utilizing all available clinical information including a diagnostic interview, clinical case notes and clinician's observation. Symptom severity was measured using the Positive and Negative Syndrome Scale (PANSS) ³⁶ assessment (for high-risk subjects for psychosis, i.e., Dataset 1[#] see supplementary text S1). **The first-episode patients were all confirmed as schizophrenia by at least a 6-month follow-up.** All healthy controls were assessed by clinicians in accordance with DSM-IV criteria as being free of schizophrenia and other Axis I disorders, and none had neurological diseases, head trauma or substance dependence. Written informed consent was obtained from all individual participants, and ethical guidelines were approved by the Institutional Review Boards

(IRB) of the respective hospitals (Taiwan, mainland China and USA). All subjects underwent resting-state functional MRI scanning for 5-7 minutes, and were asked to relax and think of nothing in particular and not to fall asleep. The imaging acquisition protocols for 6 datasets are provided in the Supplementary Methods S2.

Image Preprocessing

All fMRI data were preprocessed using SPM8: the data were realigned and normalized to a standard template (Montreal Neurological Institute) and resampled to $3 \times 3 \times 3$ mm voxels. All fMRI time-series underwent band-pass temporal filtering (0.01-0.08 Hz), nuisance signal removal from ventricles, deep white matter, global mean signal removal, and 6-parameter rigid-body motion correction. 90 regional time series were extracted by averaging voxel time series within each anatomically defined region (using the Automated-Anatomical-Labeling template³⁷). We carefully performed the following procedures to achieve motion correction. 1. Three-dimensional motion correction. 2. Data scrubbing as described by Power et al³⁸. The frames whose frame-wise displacement > 0.5 mm were all deleted together with 1 preceding and 2 succeeding frames (see Supplementary Methods S1 for data scrubbing details). A discussion of global signal removal can be found in the Supplementary Method S1.

Statistical analysis

Clinical staging model. To identify stage-specific functional-connectivity changes, we first took a staging model perspective and performed whole brain functional connectivity analysis for each dataset by comparing patients with matched controls. We then used meta-analysis³⁹ to integrate the results of patients of the same stage (First-episode: involving Datasets 2[#], 3[#] and 6[#]; Chronic: involving Datasets 4[#], 5[#] and 6[#]).

Whole-Brain Functional-Connectivity Analysis in each dataset. The Pearson cross-correlations between all pairs of regional BOLD signals were calculated, and the whole-brain functional connectivity network (90*90 AAL region-based network with altogether 4005 edges) was constructed. A two-sample, two-tailed t-test was performed to obtain the p value for every functional connectivity link in each dataset, with age, sex, root-mean-square displacements of head movement, and dosage (if the dataset contains both medicated and drug-naïve patients) being regressed out. ~~We~~—If the dataset contained patients at different stages, we performed the analysis separately for each stage.

Meta-analysis to integrate results for the same stage from different imaging centers. We used a Liptak-Stouffer z-score method³⁹ which is well-validated in integrating results from individual datasets (e.g., MRI⁴⁰) as follows. For a specific stage of schizophrenia, the p-value of each

functional connectivity in the relevant dataset i was converted to the corresponding z score: $z_i = \Phi^{-1}(1 - p_i)$, where Φ is the standard normal cumulative distribution function. Then a combined z -score for a functional connectivity was obtained using the Liptak-Stouffer formula:

$$Z = \frac{\sum_{i=1}^k w_i z_i}{\sqrt{\sum_{i=1}^k w_i^2}}$$

where w_i is the inverse of the variance of z_i . Z follows a standard normal distribution under the null hypothesis and is transformed into its corresponding p-value, with Bonferroni correction used to correct for multiple comparisons.

Finally, we perform Pearson correlation analysis between each identified functional connectivity (through meta-analysis) with the PANSS scores of schizophrenia, with Bonferroni correction used to correct for multiple comparisons.

Results

Stage-specific functional-connectivity alterations

Prodromal stage (ultra high-risk subjects)

In patients, functional connectivity differed from controls in the frontal regions, in particular between the orbital part of the inferior frontal gyrus and the angular gyrus; the inferior frontal gyrus triangular part and the precuneus; and the medial part of the superior frontal gyrus and the inferior

temporal gyrus, as shown in Supplementary Table S1. These inferior frontal gyrus regions include parts of Broca's area and are implicated in language ⁴¹.

First-episode

The meta-analytic approach involving first-episode patients from multiple centers (2[#], 3[#] and 6[#]) revealed 82 functional connections that significantly differed from controls (Bonferroni correction). 77 (94%) of these involved the frontal lobe (Fig. 1a,b and Supplement Table S2, the most significant link has $p=5.8 \times 10^{-7}$), with 35 (43%) involving the opercular ([Brodmann area BA 44](#)) and triangular ([BA 45](#)) parts of the inferior frontal gyrus (Broca's area) and 18 (22%) involving the orbital part of the inferior frontal gyrus which includes part of Broca's area ⁴¹ ([and corresponds in part with BA 47](#)). Striking changes involved Broca's area (IFGoperc and IFGtriang) which had increased functional connectivity with the superior frontal gyrus, medial frontal gyrus, anterior cingulate cortex, precuneus, middle temporal gyrus and temporal pole; and the orbital part of the inferior frontal gyrus (Frontal_Inf_Orb) which had increased connectivity with the superior frontal gyrus, the precuneus and the posterior cingulate cortex; and decreased connectivity with the pre- and postcentral cortex (motor and somatosensory) cortex, inferior parietal cortex and Rolandic operculum (Fig. 1a and Supplement Table S2). The other connectivity differences included for the gyrus rectus (part of the ventral medial prefrontal cortex)

increased connectivity with the angular gyrus and decreased connectivity with the fusiform gyrus and temporal pole; and for the middle frontal gyrus (Frontal_Mid) and an anterolateral part of the orbital cortex (Frontal_Mid_Orb) that may include part of Brodmann area 10 some increased functional connectivity links (Fig. 1b and Supplement Table S2).

Chronic stage

For the chronic stage, the meta-analytic approach involving chronic stage datasets from multiple centers (4[#], 5[#] and 6[#]) showed that functional connectivity differences from controls became much more widespread (with 162 altered links, Bonferroni correction), most prominently in the thalamus bilaterally (58 links, the most significant link has $p=1.2 \times 10^{-19}$), and the cingulate cortex (49 links, mainly to occipital and subcortical regions, most significant link has $p=7.6 \times 10^{-12}$), see Fig. 2a-c, Fig. 3 and Supplement Table S3a. The increased functional connectivity between the thalamus and the pre- and post-central gyrus, middle temporal gyrus and nearby temporal cortex areas, and the fusiform and lingual gyri was striking (Fig. 2a and Supplement Table S3). In contrast, there was decreased functional connectivity between the thalamus and some frontal cortical areas (Fig. 2a and Supplement Table S3a). The cingulate cortex had increased functional connectivity with occipital areas, and decreased functional connectivity with subcortical structures including the thalamus and basal ganglia (Fig. 2b and Supplement Table S3b). Finally, the basal

ganglia had increased functional connectivity with the middle temporal gyrus, and decreased functional connectivity with the supramarginal and inferior parietal gyri~~us~~ (Fig. 2c and Supplement Table S3c).

Correlations with the symptom scores and illness duration

For the first-episode patients (Supplement Table S4a), functional links involving Broca's area (Frontal_Inf_operc and Frontal_Inf_triag) were correlated with the PANSS positive and negative sum scores. Functional connectivity of Frontal_Inf_Orb (which is part of or related to Broca's area and the lateral orbitofrontal cortex) ~~was~~^{ere} correlated with Lack of judgement and insight. Functional connectivity links of Frontal_Mid_Orb (an anterolateral part of the orbital cortex that includes part of area 10) were correlated with Difficulty in c thinking and Uncooperativeness. Functional connectivity links of Frontal_Sup_Medial (medial prefrontal cortex area 10) were correlated with the negative and positive sum scores, with Difficulty in abstract thinking, Uncooperativeness, and Lack of judgement and insight (Supplement Table S4a). Functional connectivity links of the middle frontal gyrus (Frontal_Mid) were correlated with the positive sum score, and with Uncooperativeness (and for the superior frontal gyrus with Lack of Judgement and Insight).

For the chronic patients (Supplement Table S4b), the positive symptoms (delusions, hallucinations and suspiciousness) were correlated with the functional connectivities of the thalamus with the pre/postcentral

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gyrus, superior medial and middle frontal gyrus. The negative symptoms (blunted affect and social withdrawal) were positively correlated with functional connectivity of the posterior cingulate with the lingual and fusiform gyrus, and superior occipital gyrus. Posterior cingulate-fusiform connectivity was positively correlated with the general subscores of motor retardation, disturbance of volition, and preoccupation (Supplement Table S4b). The connectivity associated with precuneus and subcortical structures correlated significantly with illness duration, see Supplement Table S5.

Stage-specific functional alteration across stages

For the first-episode patients (190 patients and 270 controls), 46 brain regions (Fig. 3a) showed functional connectivity changes (involving 82 links), compared with 67 regions (Fig. 3b, involving 162 links) for the chronic stage (146 patients and 199 controls). These more widespread functional connectivity changes in the chronic stage imply an apparent spreading of functional alterations to more regions after disease onset. We also found stage-specific changes: alterations in the first episode patients primarily involved the frontal lobe, including Broca's area (IFGoperc and IFGtriang); while for the chronic stage, posterior cingulate, subcortical areas (especially the thalamus), the lingual gyrus, cuneus and occipital areas showed the most prominent changes (pink regions in Fig 3). The data shown in Fig. 3 use Bonferroni correction. To examine to what extent the

frontal regions with altered functional connectivity were still evident in chronic schizophrenia, we show similar data to those in Fig. 3, but now with FDR statistical correction in Supplement Fig. S1. This shows that some frontal functional connectivity differences are still evident in chronic patients, but that other areas are now more involved. The differences in these frontal vs other patterns of involvement in first-episode and chronic patients were still statistically significant with the FDR-corrected data (Chi-square=553.21, df=44, $p < 10^{-50}$).

Discussion

We report a functional neuroimaging analysis examining the functional dysconnectivity at different stages of schizophrenia using 6 independent datasets. To our knowledge, this is the first imaging study to directly examine the effect of functional connectivity changes at first-episode and chronic stages in schizophrenia with large multi-center datasets, with large numbers of patients, and with the almost unique data from drug-naïve first-episode patients with schizophrenia. The first-episode schizophrenia patients demonstrated most prominently localized changes in the frontal lobes, especially Broca's area and the orbital part of the inferior frontal gyrus, indicating that the frontal differences from controls have an early-onset nature. For the chronic stage of schizophrenia, much wider functional-connectivity changes were found, mostly prominently involving

the thalamus.

Both thalamic and frontal abnormalities have been widely reported in schizophrenia. However, the results for the regions responsible for the core pathogenesis remain inconsistent, partly due to a number of factors such as the lack of a staging point of view, small sample sizes, and confounding factors such as the effects of medication. The thalamus is connected to all cortical areas and conveys information to the neocortex, including information from the basal ganglia. Though a number of other studies have identified thalamic connectivity changes in the chronic stage of schizophrenia^{17, 19, 22-24}, such studies provide little information about early illness pathophysiology. In contrast, studies of first-episode schizophrenia have the advantage to specify brain changes at illness onset, thus providing crucial information about the pathogenesis of schizophrenia. Our functional-connectivity analysis of 197 first-episode schizophrenia patients (76% being drug-naïve, and the results for the drug-naïve subset shown in Supplement Table S7) shows localized changes in the frontal lobes, suggesting these to be core regions for schizophrenia pathogenesis. Further, we found that subjects at high risk of psychosis (the prodromal stage) also had functional connectivity changes primarily associated with the frontal lobes (especially the inferior frontal gyrus which includes Broca's area). These results in combination suggest a key neuropathological role of the frontal lobe in the onset of schizophrenia. The

data presented in this investigation provide an important contribution to this evidence, for we analyzed data from hundreds of first-episode patients who were drug-naïve (i.e. who had never received treatment), and therefore provide key information about the early changes in the brain in schizophrenia.

Functional abnormalities of the frontal lobe have been one of the most consistent findings in schizophrenia, although they have not generally been localized to the inferior frontal gyrus^{4-10, 42, 43}. Part of the importance of the present investigation is that it shows that frontal functional connectivity changes involving the inferior frontal gyrus are present in first-episode, antipsychotic-naïve patients. A recent review² of functional connectivity changes in schizophrenia involved typically much smaller sample sizes than those reported here, and reported typically altered functional connectivity in patients sometimes involving the frontal lobe, but without much emphasis on the thalamus, and with no marked stage-specific differences in functional connectivity. In contrast, with the larger sample size and brain-wide approach described here, we found that Broca's area in the inferior frontal gyrus had increased functional connectivity, which is the most significant changes in first-episode schizophrenia, and that increased thalamic connectivity including with somatosensory and motor areas in the post- and precentral gyrus was prominent in chronic schizophrenia. We further note the smaller prominence of frontal

connectivity changes in for example the inferior frontal gyrus in the chronic stage, though differences from controls were still evident with FDR correction as shown in Supplement Table S6.

The inferior frontal gyrus, which includes Broca's area, is critically involved in speech production and language processing⁴⁴. It is functionally connected with the temporal lobe to form a language network. Our findings of increased functional connectivity of the inferior frontal gyrus including Broca's area with temporal cortex and cingulate areas, and the correlation of increased functional connectivity with the positive/negative symptoms, implicate language-related frontal areas as key regions related to the onset of schizophrenia. As Broca's area is important for syntactic functions⁴⁵, we suggest that this increased connectivity may be related to the thought disorders in schizophrenia, as syntax is important in multi-step thinking⁴⁶. It is interesting to note that the predisposition to schizophrenia may be a component of a *Homo sapiens*-specific variation associated with the capacity for language⁴⁷. Our results are consistent with this hypothesis.

We note that thalamic changes have been reported in first-episode schizophrenia^{21, 48} and high-risk subjects⁴⁹. In our dataset, however, we did not find prominent thalamic functional connectivity differences from controls in first episode schizophrenic patients or high-risk subjects. This seeming discrepancy may be due to the different methodology we adopted. In our work we used ~~the~~ whole-brain functional-connectivity

analysis with strict Bonferroni correction. Therefore only the most significant changes of the whole brain (herein the functional connectivity alterations of the inferior frontal gyrus) are identified. In contrast, the work^{21, 49} used a seed-based approach and focussed exclusively on the thalamus, and ~~that~~ the changes ~~in~~ other parts of the brain ~~have~~were not ~~been~~ systematically evaluated. Consequently our results do not contradict ~~with~~ previous works, but revealed brain-wide changes rather than changes associated with pre-defined regions of interest. It is important to note that we do not exclude the possible neuropathological role of the thalamus in first-episode schizophrenia or the prodromal stage. In fact with FDR correction ($q=0.05$) adopted, thalamic changes are also present in first-episode patients in our data, as shown in Supplement Fig. S1 and Table S6. Our results suggest that thalamic alterations cannot be ruled out in first-episode schizophrenia, but are less evident than at the chronic stage.

Several strengths and limitations should be noted. A strength is that we used the largest multisite dataset reported with a meta-analytic approach to address inter-site variations. Meta-analysis is more suitable than traditional validation approaches that test if the same observation survives a type-I error, as it considers the effect-size distribution to be more important than if observations survive arbitrarily set thresholds. We note that separate analysis in each dataset (with Bonferroni correction) yields similar results, see Table 2. For first-episode patients, both dataset 2[#] and 3[#] demonstrate

increased connectivity between the inferior frontal gyrus (orbital part) and superior frontal gyrus. For the chronic stage, both datasets 4[#] and 5[#] demonstrate decreased connectivity between the thalamus and middle frontal gyrus, and decreased connectivity between the thalamus and postcentral gyrus. Another strength is the involvement of hundreds of first-episode patients the great majority of whom were not receiving medication, and the changes in functional connectivity that we found in these patients provide important data for understanding the pathogenesis of schizophrenia.

A limitation is that most of the chronic patients were receiving medication, so it is difficult to be sure about whether the differences between the first-episode and chronic patients are related to disease progress or the effects of medication on the functional connectivity. Nevertheless, the data presented here are important, for they provide insight into how the functional connectivity of untreated first episode patients is different from that of most patients seen clinically, who will be receiving medication. To provide some evidence on the possible role of medication, for first episode datasets 3[#] and 6[#] (containing both medicated and drug-naïve patients), we examined whether medicated and drug-naïve patients showed significant differences in functional connectivity, as shown in Supplement Table S8a. Only a few links had $p < 0.05$ (uncorrected). We furthermore used only drug-naïve first-episode patients in the meta-analysis shown in Supplement

Table S7, and found 62 significantly changed connectivities, 50 of which overlap with those obtained by using all first-episode patients (Supplement Table S2). For the chronic dataset 4[#] (all patients are medicated), we performed a correlation analysis between the significantly different functional connectivities and the medication dosage, as shown in Supplement Table S8b. Only 3 links (out of 162) show correlation with the dose of medication ($p < 0.05$, uncorrected). Furthermore, though antipsychotic medication may vary across sites, separate analysis of chronic Datasets 4[#] and 5[#] revealed the same thalamus-post/precentral dysconnectivity (Table 2) that correlated with tension, suggesting that thalamic changes may not depend critically on the type of medication. Another limitation is that we used the automatic anatomical labelling (AAL) atlas to define the different brain areas to be analyzed³⁷. It would thus be interesting to use functional atlases⁵⁰ or extend the investigation to voxel-level. **Finally, there is no follow up data in Dataset #1 (high risk subjects) to indicate the transition, and the sample size is not large. We also note that the chronic patients in this study had illness durations that were typically of many years (see Table 1).**

Conclusions

With large multicenter resting-state fMRI datasets and whole-brain analysis, we demonstrate the utility of a staging model of schizophrenia.

We show that for first-episode drug-naïve patients, changes are most evident in language regions such as Broca's area, which may underlie the thought disorders central to schizophrenia symptoms. This is consistent with the hypothesis that schizophrenia is the price that Homo sapiens pays for language made by Crow. For the chronic stage, more widespread changes were found, most prominently in thalamo-cortical connections. These results are of importance for understanding the pathogenesis of schizophrenia, and for providing sensitive neurobiological markers crucial for early diagnosis and treatment.

Acknowledgements

J.Zhang is supported by National Science Foundation of China (NSFC 61104143 and 61573107), and special Funds for Major State Basic Research Projects of China (2015CB856003). J.Feng is a Royal Society Wolfson Research Merit Award holder. J.Feng is also partially supported by the National High Technology Research and Development Program of China (No. 2015AA020507) and the key project of Shanghai Science & Technology Innovation Plan (No. 15JC1400101). The research was partially supported by the National Centre for Mathematics and Interdisciplinary Sciences (NCMIS) of the Chinese Academy of Sciences and Key Program of National Natural Science Foundation of China (No. 91230201). L.Palaniyappan was partly supported by the Wellcome Trust (Research Training Fellowship) during the course of this work. QL is supported by grants from the National Natural Sciences Foundation of China (No. 11101429, No.11471081). Z.Liu was supported by the National Natural Science Foundation of China (81271485, 81471362). J.Wang acknowledges support from the Shanghai Key Laboratory of Psychotic Disorders (13dz2260500). C.Li acknowledges support from the Science and Technology Commission of Shanghai Municipality (13z2260500).

Table 1 Demographic and clinical characteristics of patients in Datasets 1[#] to 6[#]. FE for first-episode. The number in brackets of the column “Number” indicates the number of subjects before quality control. There are 40 subjects in prodromal dataset (17 patients), 467 subjects in first-episode (197 patients) dataset, and 345 subjects in chronic stage dataset (146 patients). Note that dataset 6[#] does not have PANSS scores but BPRS scores, therefore is involved only in the whole-brain analysis but not in the correlation analysis with symptom scores. Patients are matched with corresponding controls in each dataset for age, sex, handedness and head movements.

Sites	Number	Age (year)	p	Sex (male/female)	p	P scale	N scale	G scale	Duration of illness (year)	Medication
1[#] Shanghai Mental Health Center	Control 23 (23)	27.8±8.7	0.52	14/9	0.99					
	Patient 17 (18)	26.1±8.1		10/7						Prodromal Drug-naïve
2[#] Huaxi	Control 150 (180)	25.8± 8.7	0.19	80 / 70	0.36					
	Patient 113 (178)	24.4± 7.8		53 / 60		25.4± 5.6	19.1± 7.0	47.8± 9.0	0.01-1 (0.24±0.27) FE	Drug-naïve

3# Xiangya	Control 57 (60)	26.3± 5.5	0.24	32 / 25	0.51					
	Patient 39 (83)	24.6± 6.6		26 / 14		19.8± 6.3	21.6± 7.7	39.2± 11.8	0.08-1 (0.33.±0.3 4) FE	25 Medicated; 14 Drug- naïve
4# COBRE	Control 53 (74)	34.8± 11.3	0.40	42 / 11	0.13					
	Patient 67 (71)	36.8± 13.7		46 / 21		14.9± 4.6	14.7± 5.2	29.7± 8.2	2-24 (8.9±6.9) Chronic	Medicated
5# Taiwan	Control 62 (62)	31.6± 8.1	0.08	25 / 37	0.21					
	Patient 56 (69)	33.3 ± 9.3		30 / 26		10±5	12.7± 7.5	23.8± 11.8	1-31 (8.8±6.2)	Medicated
6# First Affiliated Hospital of China Medical Univ.	Control 63* (154)	27.1±10.6	0.20	31/32	0.06				Matched with FE patients	
	Patient 45 (53)	24.7±9.3		19/26					FE	22 Drug- naïve
	Control 84* (154)	32.8±13.4	0.33	40/44	0.91				Matched with chronic patients	
	Patient 23 (25)	30±9.2		10/13					Chronic	4 Drug- naïve

*The 84 controls (matched with chronic patients) included all 63 controls that matched with the FE patients in dataset 6#.

Table 2 Overlapped functional-connectivity (using Bonferroni correction)

in separate analyses of each dataset for FE (#2, #3, and #6) and chronic schizophrenia (#4 and #5). For first-episode patients, both #2 and #3 show significant change in the functional connectivity between the inferior frontal gyrus (orbital) and superior frontal gyrus. #6 also shows changes in the inferior frontal gyrus (triangular and opercular parts). For the chronic stage, both #4 and #5 show significant changes between the thalamus and postcentral gyrus; and between the thalamus and the middle frontal gyrus. Age and sex are regressed out in the whole brain functional-connectivity analysis of each dataset. The mean and standard deviation of the functional connectivity (Pearson correlation) for patients and controls are shown.

First-episode							
Brain region	Brain region	Control (mean)		Patient (mean)		P val	
		Dataset 2 [#]	Dataset 3 [#]	Dataset 2 [#]	Dataset 3 [#]	Dataset 2 [#]	Dataset 3 [#]
Frontal_Inf_Orb_R	Frontal_Sup_L	-0.07±0.22	-0.19±0.21	0.08±0.24	0.03±0.21	1.3E-06	2.4 E-06
		Dataset 6 [#]		Dataset 6 [#]			
Fron_Inf_Tri_R	Fron_Med_Orb_L	-0.30±0.19		-0.11±0.23		4.9 E-06	
Fron_Inf_Oper_L	Precentral_R	0.19±0.20		-0.01±0.24		6.7 E-06	
Chronic							
Brain region	Brain region	Control (mean)		Patient (mean)		P val	
		Dataset 4 [#]	Dataset 5 [#]	Dataset 4 [#]	Dataset 5 [#]	Dataset 4 [#]	Dataset 5 [#]
Thalamus_L	Fron_Mid_R	0.04±0.24	0.20±0.24	-0.21±0.23	-0.15±0.28	2.2E-07	5.0E-10
Thalamus_R	Postcentral_L	-0.23±0.28	-0.32±0.27	0.03±0.25	0.02±0.29	4.8E-07	2.4E-08
Thalamus_R	Fron_Mid_R	0.10±0.25	0.26±0.23	-0.14±0.24	-0.08±0.29	6.4E-07	1.2E-09
Thalamus_R	Postcentral_R	-	-	0.06±0.26	-	2.2E-06	1.9E-07

		0.20±0.29	0.33±0.27		0.001±0.32		
Thalamus_L	Postcentral_R	-0.21±0.29	-0.35±0.26	0.04±0.26	-0.01±0.32	4.9E-06	8.3E-08

Figure legends

Figure 1. Significantly altered functional connectivity for first-episode schizophrenia by meta-analysis involving Datasets 2[#], 3[#] and 6[#]. The color of the 3 circles (from outside to inside) denotes: the 90 different AAL regions (1st circle); the number of increased links (2nd circle, deep red means a region has more increased links); the number of decreased links (3rd circle, deep blue means a region has more decreased links). The thickness of the links is proportional to $-\log_{10}(p \text{ value})$. The right of the brain is on the right of each circular diagram. **a** illustrates altered links of the inferior frontal lobe (51 links, including the opercular, triangular and orbital part), and **b** is for the remaining links. Red links indicate that patients have a higher mean functional connectivity than controls (i.e., $\text{mean}(\text{FC}_{\text{patient}}) - \text{mean}(\text{FC}_{\text{control}}) > 0$), and blue links indicate the opposite.

Figure 2. Significantly altered functional connectivity for chronic stage schizophrenia by meta-analysis involving Datasets 4[#], 5[#] and 6[#]. **a** illustrates altered links involving the thalamus (58 links), and **b** is for links involving the cingulate cortex (49 links). **c** is for the remaining significantly different links.

Figure 3. The number of significantly different functional connectivity links of each brain region for (a) first-episode and (b) chronic stage schizophrenia patients. (a) and (b) were obtained by counting the number of different links in Supplementary Tables S2 and S3 for each pair of symmetric brain regions (i.e. we added the number of links of the left and right corresponding regions). For first-episode patients, 46 regions (out of 90 AAL regions) showed differences from controls, and for chronic stage patients, 67 regions demonstrated differences from controls. Regions marked green are those demonstrating common changes in both first-episode and chronic stages (these regions have more than 2.5% altered-links in both stages), including the middle frontal gyrus, posterior cingulate cortex, the fusiform gyrus, and the temporal pole. Regions marked pink are those demonstrating stage-specific changes. Inferior and medial frontal gyrus changes were found in first-episode schizophrenia, and cingulate, subcortical (especially the thalamus), and occipital changes were found in the chronic stage.

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