Brain-Wide Analysis of Functional Connectivity in First-Episode and Chronic Stages of Schizophrenia

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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 (FE) and chronic patients from controls using resting-state functional MRI, and determine changes that are specifically associated with disease onset, a clinical staging model is adopted. We analyze functional-connectivity differences in prodromal, FE (mostly drug naïve), and chronic patients from their matched controls from 6 independent datasets involving a total of 789 participants (343 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 FE 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-sensorimoter 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 FE 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/thalamus

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,[13] the temporal lobe,[14] limbic structures,[15,16] 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 FE 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 disorder.[2] There are additional studies that have focused on the early changes in schizophrenia, including.[6,12,15,29–32]

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 controls,[2] 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 FE 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 FE 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 FE patients were becoming evident in the prodromal group, as this would further implicate these differences in the onset stage of schizophrenia. Here the FE 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,[33,34] for both FE and chronic schizophrenia. Our results for FE 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 (479 patients) in our study, which were from 6 different datasets that have all been reported. 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 Positive and Negative Syndrome Scale (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 FE patients, 146 chronic schizophrenia). These subjects are from Dataset 1[#] (Shanghai Mental Health Centre, prodromal, described in supplementary text S1); Dataset 2[#] (Huaxi hospital,[19] FE); Dataset 3[#] (Central South University,[35] FE); Dataset 4[#] (The Center for Biomedical Research Excellence-COBRE,[36] chronic); Dataset 5[#] (National Taiwan University Hospital,[37] chronic); Dataset 6[#] (First Affiliated Hospital of China Medical University,[38] FE and chronic). Detailed demographics of the patients are provided in table 1. The FE schizophrenia patients were defined as having illness duration ≤ 12 months (with a mean of 0.27 mo in our dataset), while the chronic patients were defined as having illness duration > 12 months (with a mean of 8.2 y, ie, 98.4 mo in our dataset). 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[39] by qualified psychiatrists utilizing all available

Table 1. Demographic and Clinical Characteristics of Patients in Datasets 1# to 6#

Sites	Number	Age (y)	P	Sex (Male/Female)	P	P Scale	N Scale	G Scale	Duration of Illness (y)	Medication
1# Shanghai Mental Health Center	Control 23 (23)	27.8±8.7	.52	14/9	.99					
	Patient 17 (18)	26.1±8.1		10/7					Prodromal	Drug-naïve
2# Huaxi	Control 150 (180)	25.8±8.7	.19	80/70	.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	.24	32/25	.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 (0.3±0.3) FE	25 Medicated; 14 Drug-naïve
4# COBRE	Control 53 (74)	34.8±11.3	.40	42/11	.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	.08	25/37	.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[a] (154)	27.1±10.6	.20	31/32	.06					
	Patient 45 (53)	24.7±9.3		19/26					Matched with FE patients 0.30±0.26 (FE)	22 Drug-naïve
	Control 84[a] (154)	32.8±13.4	.33	40/44	.91				Matched with chronic patients	
	Patient 23 (25)	30±9.2		10/13					4.4±2.8 Chronic	4 Drug-naïve

Note: FE, 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 FE (197 patients) dataset, and 345 subjects in chronic stage dataset (146 patients). Note that dataset 6# does not have Positive and Negative Syndrome Scale (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.

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

clinical information including a diagnostic interview, clinical case notes and clinician's observation. Symptom severity was measured using the PANSS[40] assessment (for high-risk subjects for psychosis, ie, Dataset 1[#] see supplementary text S1). The FE patients were all confirmed as schizophrenic 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 United States). All subjects underwent resting-state functional MRI (fMRI) 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 template[41]). We carefully performed the following procedures to achieve motion correction: (1) Three-dimensional motion correction and (2) Data scrubbing as described by Power et al.[42] 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 methods 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-analysis[43] to integrate the results of patients of the same stage (FE: 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 automatic anatomical labelling [AAL] region-based

network with altogether 4005 edges) was constructed. A 2-sample, 2-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. 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 method[43] which is well-validated in integrating results from individual datasets (eg, MRI[31]) 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.[32]

First-Episode. The meta-analytic approach involving FE 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 (figures 1a and 1b and supplementary table S2, the most significant link has $P = 1.2 \times 10^{-12}$), with 35 (43%) involving the opercular (Brodmann area BA 44) and
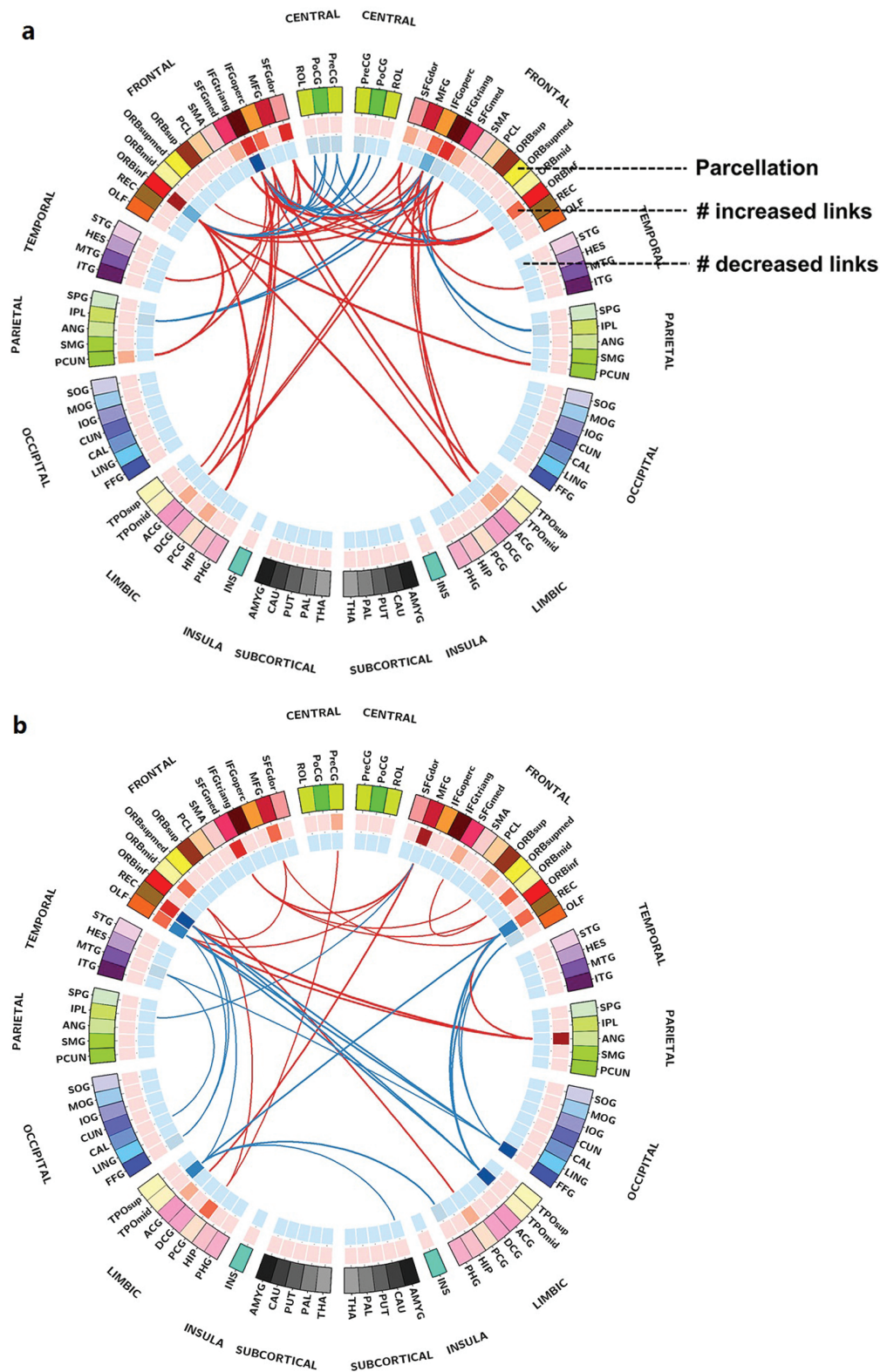
Fig. 1. Significantly altered functional connectivity for first-episode schizophrenia by meta-analysis involving Datasets 2#, 3#, and 6#. The color of the 3 circles (from outside to inside) denotes: the 90 different automatic anatomical labelling (AAL) regions (First circle); the number of increased links (Second circle, deep red means a region has more increased links); the number of decreased links (Third circle, deep blue means a region has more decreased links). The thickness of the links is proportional to –log10 (P 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 (ie, mean (FC_patient) − mean(FC_control) > 0), and blue links indicate the opposite.

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[32] (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 precentral and postcentral cortex (motor and somatosensory) cortex, inferior parietal cortex and Rolandic operculum (figure 1a and supplementary 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 (figure 1b and supplementary 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 figures 2a–c, figure 3 and supplementary table S3a. The increased functional connectivity between the thalamus and the precentral and postcentral gyrus, middle temporal gyrus and nearby temporal cortex areas, and the fusiform and lingual gyri was striking (figure 2a and supplementary table S3). In contrast, there was decreased functional connectivity between the thalamus and some frontal cortical areas (figure 2a and supplementary 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 (figure 2b and supplementary 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 (figure 2c and supplementary table S3c).

Correlations With the Symptom Scores and Illness Duration

For the FE patients (supplementary table S4a), functional links involving Broca's area (Frontal_Inf_operc and Frontal_Inf_triang) 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 correlated with Lack of judgment 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 abstract 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 judgment and insight (supplementary 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 Judgment and Insight).

For the chronic patients (supplementary table S4b), the positive symptoms (delusions, hallucinations and suspiciousness) were correlated with the functional connectivities of the thalamus with the pre/postcentral 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 (supplementary table S4b). The connectivity associated with precuneus and subcortical structures correlated significantly with illness duration, see supplementary table S5.

Stage-Specific Functional Alteration Across Stages

For the FE patients (197 patients and 270 controls), 46 brain regions (figure 3a) showed functional connectivity changes (involving 82 links), compared with 67 regions (figure 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 FE 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 figure 3). The data shown in figure 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 figure 3, but now with FDR statistical correction in supplementary figure 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 FE and chronic patients were still statistically significant with the FDR-corrected data ($\chi^2 = 553.21$, $df = 44$, $P < 10^{-50}$).

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 FE and chronic stages in schizophrenia with large multi-center datasets, with large numbers of patients, and with the almost unique data from drug-naive FE patients with schizophrenia. The FE schizophrenia patients demonstrated most prominently localized changes in the frontal lobes, especially Broca's
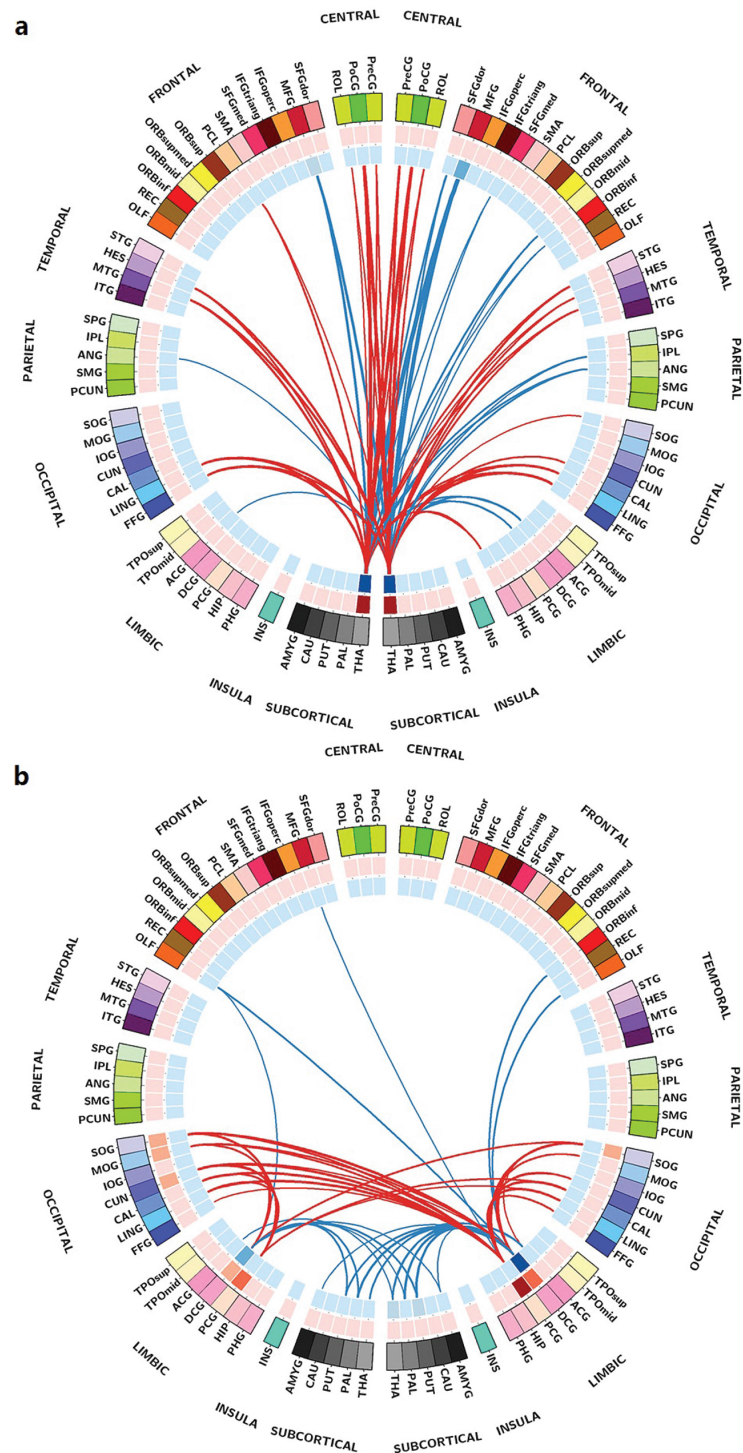
Fig. 2. Significantly altered functional connectivity for chronic stage schizophrenia by meta-analysis involving Datasets 4#, 5#, and 6#. (**a**) illustrates altered links involving the thalamus (58 links), and (**b**) is for links involving the cingulate cortex (49 links). (**c**) is for the remaining significantly different links.
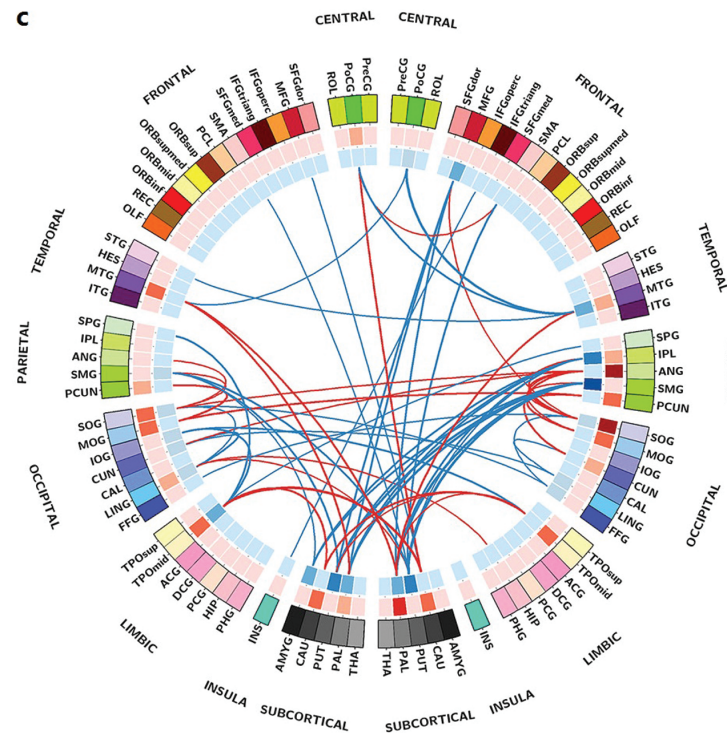
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Fig. 2. *Continued*

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 FE 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 FE schizophrenia patients (76% being drug-naïve, and the results for the drug-naïve subset shown in supplementary 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 FE patients who were largely drug-naïve (ie, 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.[4–10,44,45] 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 FE, antipsychotic-naïve patients. A recent review[2] 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 FE schizophrenia, and that increased thalamic connectivity including with somatosensory and motor areas in the postcentral and precentral gyrus was prominent in chronic schizophrenia. We further note
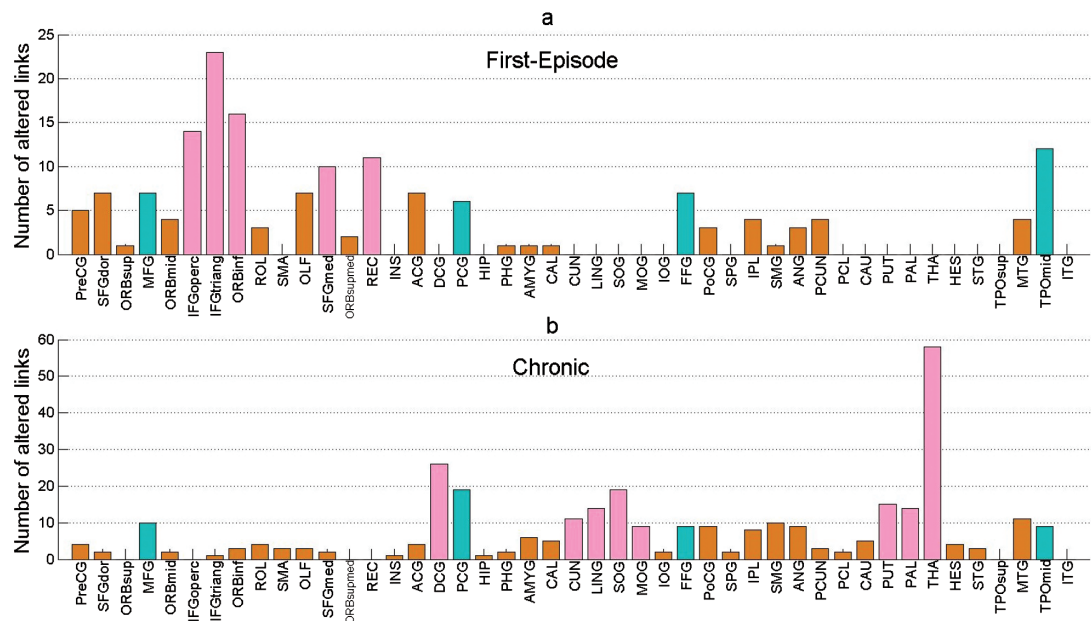
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Fig. 3. The number of significantly different functional connectivity links of each brain region for (**a**) first-episode (FE) and (**b**) chronic stage schizophrenia patients. (a) and (b) were obtained by counting the number of different links in supplementary tables S2 and S3 for each pair of symmetric brain regions (ie, we added the number of links of the left and right corresponding regions). For FE patients, 46 regions (out of 90 automatic anatomical labelling [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 FE 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 FE schizophrenia, and cingulate, subcortical (especially the thalamus), and occipital changes were found in the chronic stage.

the smaller prominence of frontal connectivity changes in, eg, the inferior frontal gyrus in the chronic stage, though differences from controls were still evident with FDR correction as shown in supplementary table S6.

The inferior frontal gyrus, which includes Broca's area, is critically involved in speech production and language processing.[46] 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 functions,[47] we suggest that this increased connectivity may be related to the thought disorders in schizophrenia, as syntax is important in multi-step thinking.[48] 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 language.[49] Our results are consistent with this hypothesis.

We note that thalamic changes have been reported in FE schizophrenia[21,50] and high-risk subjects.[51] In our dataset, however, we did not find prominent thalamic functional connectivity differences from controls in FE schizophrenic patients or high-risk subjects. This seeming discrepancy may be due to the different methodology we adopted. In our work we used 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[21,51] used a seed-based approach and focused exclusively on the thalamus, and the changes in other parts of the brain were not systematically evaluated. Consequently our results do not contradict 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 FE schizophrenia or the prodromal stage. In fact with FDR correction ($q = 0.05$) adopted, thalamic changes are also present in FE patients in our data, as shown in supplementary figure S1 and table S6. Our results suggest that thalamic alterations cannot be ruled out in FE 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 FE patients, both dataset 2# and 3# demonstrate increased connectivity between the inferior frontal gyrus

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)

First-Episode

Brain Region	Brain Region	Control (Mean)		Patient (Mean)		P	
		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	
		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	−0.20 ± 0.29	−0.33 ± 0.27	0.06 ± 0.26	−0.001 ± 0.32	2.2E-06	1.9E-07
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

Note: For FE 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.

(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 FE 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 FE 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 FE 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 FE datasets 3# and 6# (containing both medicated and drug-naive patients), we examined whether medicated and drug-naive patients showed significant differences in functional connectivity, as shown in supplementary table S8a. Only a few links had $P < .05$ (uncorrected). We furthermore used only drug-naïve FE patients in the meta-analysis shown in supplementary table S7, and found 62 significantly changed connectivities, 50 of which overlap with those obtained by using all FE patients (supplementary 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 supplementary table S8b. Only 3 links (out of 162) show correlation with the dose of medication ($P < .05$, 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 AAL atlas to define the different brain areas to be analyzed.[41] It would thus be interesting to use functional atlases[52] 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 (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 FE 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.

Supplementary Material

Supplementary material is available at http://schizophreniabulletin.oxfordjournals.org.

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