Altered resting-state functional connectivity of the insula in individuals with clinical high-risk and patients with first-episode schizophrenia

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ARTICLE INFO

ABSTRACT

Objectives: Abnormalities in insular functional connectivity have been implicated in many clinical features of schizophrenia. The aim of this study was to determine to what degree such abnormalities occur in individuals with clinical high risk for psychosis (CHR), and whether which is associated with symptom severity.
Methods: Resting-state fMRI data were collected from 47 healthy controls, 24 CHR individuals and 19 patients with first-episode schizophrenia. Using the posterior, dorsal and ventral insular subregions as separate seeds, we examined resting-state functional connectivity differences between different groups and the association between concurrent symptom severity and dysconnectivity.
Results: Compared with healthy controls, both CHR individuals and schizophrenia patients showed hypoconnectivity between posterior insula (PI) and somatosensory areas, and between dorsal anterior insula (dAI) and putamen. Schizophrenia patients also showed dAI and ventral anterior insula(vAI) hyperconnectivity with visual areas relative to controls and CHR individuals. Correlation analysis revealed that dAI functional connectivity with superior temporal gyrus was positively correlated with positive symptoms of CHR, and vAI connectivity with dorsolateral prefrontal cortex was negatively correlated with the severity of the symptoms of first-episode schizophrenia.
Conclusions: Our findings suggest that insular functional dysconnectivity with the sensory cortex may be a system-level neural substrate preceding the onset of psychosis.

1. Introduction

Schizophrenia is a severe mental disorder characterized by positive, negative symptoms and cognitive deficits (van Os et al., 2010). Up to date the etiology of schizophrenia is still unclear, which has been hypothesized as a disorder of distributed brain dysconnectivity (Konrad and Winterer, 2008). Neurodevelopment deficits of schizophrenia can lead to abnormal neural connectivity, which could cause abnormalities in information processing and integration, which are core

deficits of schizophrenia (Simon et al., 2009).

The insular cortex integrates external sensory input with the limbic structure, was the important integration area of the brain, which has hence been ascribed an integrative role, linking information from diverse functional systems (Kurth et al., 2010; Li et al., 2017; Moran et al., 2013). The functions of insula alteration in schizophrenia include the processing of both visual and auditory emotional information, pain, and neuronal representations of the self (Wylie and Tregellas, 2010). The last of these functions plays a crucial role in identifying between self-

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generated and external information, which suggest that insula dysfunction may contribute to positive symptom, a primary feature of schizophrenia (Cascella et al., 2011).

Recently, the rest state functional connectivity (rsFC) of the insula cortex has attracted significant attention in schizophrenia research (Gogolla, 2017; Wylie and Tregellas, 2010). For example, one study showed that significant inverse correlations between the severity of delusions and volumes of the right insula (Cascella et al., 2011). The right anterior insula cortex (AIC) has been shown to modulate activity in the central executive networks (CEN) and default mode networks (DMN) in individuals with schizophrenia (Moran et al., 2013). Another study found an aberrant dependence of DMN/CEN interactions on anterior insular salience network activity (Gogolla, 2017). Furthermore, patients with schizophrenia presented increased rsFC from the ventral AIC and dorsal AIC to the right caudate (Li et al., 2017). The study by Pang et al. showed that reduced right insula-Heschl's rsFC was present in drug naive patients with first episode schizophrenia (FEP), which might be related to the manifestation of clinical symptoms (Pang et al., 2017).

Schizophrenia is a neurodevelopmental disorder associated with brain abnormalities that likely occur before the onset of clinical symptoms (Lord et al., 2011). A study examined the association of thalamic dysconnectivity and conversion to psychosis in youth and young adults in clinical high risk for psychosis (CHR), and they found marked thalamic hyperconnectivity with sensory motor areas in those who converted to full-blown illness (Anticevic et al., 2015). Other studies found that a complex interplay of alterations in right superior temporal lobe (Witthaus et al., 2008), dorsal and ventral corticostriatal systems (Dandash et al., 2014), caudate (Hannan et al., 2010), hippocampus (Witthaus et al., 2009), cerebello-cortical (Bernard et al., 2015), and anterior cingulate cortex (Lord et al., 2011). Whole-brain structural covariance analyses revealed subtle changes of connectivity of the default mode, executive control, salience, motor, and auditory networks in CHR (Heinze et al., 2015). Furthermore, it has been shown that the early patterns of salience network topology were associated with risk of developing clinical psychosis, characterized by loss of network segregation and disruption of network communities (Wang et al., 2018).

There are few studies that focused on the insular connectivity in CHR. A study indicated that CHR subjects exhibited reduced rsFC between the left ventral anterior insula and other salience network regions, and reduced white-matter microstructures along tracts in close proximity to regions of disrupted rsFC (Wang et al., 2016). Furthermore, studies have also found that insular cortex gray matter abnormalities in CHR (Takahashi et al., 2009).

The insular cortex, however, is not a homogeneous structure, and consists of subregions with distinct connectivity patterns (Wylie and Tregellas, 2010). The purpose of the present study was to examine whether different insular networks were differentially affected in CHR and their relationships with the severity of symptoms. Based on previous published insular foci involving in sensorimotor, cognitive and affective processing, rsFC between different insular subregions and the whole brain was compared among three groups: healthy control, CHR subjects and FEP patients. We then examined the relationship between insular rsFC and symptom severity in the CHR and patient groups. We tested the following 2 hypotheses: (1) CHR is associated with distinct rsFC pattern for each insular subregion; (2) specific insular dysconnectivity is associated with severity of psychotic symptoms.

2. Materials and methods

2.1. Subjects

Nineteen FEP patients, 24 CHR individuals, and 47 healthy controls (CN) were included in this study (Table 1). The FEP and CHR subjects were recruited from outpatient clinics and inpatient units at Beijing

Table 1
Demographic characteristics of the participants in this study.

Variable	CN	CHR	FEP	p value[a]
Sample size	47	24	19	—
Age (years)	26.0 ± 4.8	24.6 ± 5.3	24.3 ± 7.1	0.392
Sex (male/female)	29/18	15/9	10/9	<0.05
Education (years)	13.8 ± 3.2	13.2 ± 3.3	12.7 ± 3.5	0.431
Head motion	0.113 ± 0.051	0.116 ± 0.065	0.096 ± 0.059	0.459
PANSS	—	86.7 ± 12.6		—
SIPS				
Positive	—	—	8.0 ± 3.3	—
Negative	—	—	9.1 ± 5.3	—
Disorganization	—	—	5.0 ± 2.6	—
General	—	—	4.8 ± 3.3	—

[a] Group differences were tested using ANOVA for continuous variables, and chi-square test for sex. CN, control; CHR, clinical high-risk; FEP, first-episode psychosis.

Anding Hospital, Capital Medical University (Beijing, China), and the CN subjects were recruited from the local community in the same regions by advertisements. All participants were right-handed. None of them had major head trauma, history of alcohol or drug dependence, or history of neurological disorders. Structured interviews by experienced psychiatrists were conducted with each participant. The study protocol was reviewed and approved by the research ethics committee (IRB) of Beijing Anding Hospital and it was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to data collecting and brain scanning.

The inclusion criteria for FEP were defined as individuals who met the diagnosis of schizophrenia according to the DSM-IV criteria and had symptoms for less than 12 months. The symptoms of all FEP patients were assessed with the Positive and Negative Symptom Scale (PANSS). The CHR subjects were assessed using to the Structured Interview for Prodromal Syndromes (SIPS) (Dandash et al., 2014). The CHR subjects meet at least one of the prodromal states of psychosis criteria: attenuated positive syndrome (APS), brief intermittent psychotic syndrome (BIPS), and/or genetic risk with deterioration (GRD). At the time of enrollment, seven FEP patients were receiving antipsychotics, including risperidone ($n = 4$), olanzapine ($n = 1$), haloperidol ($n = 1$), and paliperidone ($n = 1$). Moreover, six CHR subjects in this study were medicated with antipsychotics, including risperidone ($n = 1$), olanzapine ($n = 3$), quetiapine ($n = 1$) and amisulpride ($n = 1$). One CHR subjects was also receiving anti-depressant (fluvoxamine). Average daily dose of antipsychotics was calculated using olanzapine equivalent (Leucht et al., 2016).

2.2. Data acquisition and preprocessing

The subjects underwent resting-state scans on a SIEMENS 3T Trio MRI scanner (Siemens Magnetom Trio, Germany) using a 12-channel head coil. Subjects were instructed simply to keep their eyes closed, to relax, and to remain awake and perform no specific cognitive exercise. After each session, subjects were asked whether they were awake in the previous session and all the subjects confirmed that they were awake. The functional MRI images were collected by using a gradient-echo EPI sequence. The imaging parameters were as follows: 31 axial slices, repetition time = 2000 ms, echo time = 30 ms, flip angle = 90°, slice thickness = 4.8 mm, slice gap = 0 mm, acquisition matrix = 64 × 64, field of view = 200 mm. Each functional resting-state session lasted 8 min, and 240 vol were obtained.

Functional scans were preprocessed using the SPM8 software (Wellcome Department of Imaging Neuroscience, University College London, UK; http://www.fil.ion.ucl.ac.uk/spm). Prior to preprocessing, the first 10 vol of each scan were discarded for magnetic saturation. The remaining 230 vol were corrected for within-scan acquisition time differences between slices, and realigned to the first volume to correct

for inter-scan head motions. All subjects in this study had less than 2 mm translation and 2° of rotation in any of the x, y, and z axes. Then, the volumes were normalized to the standard EPI template in the Montreal Neurological Institute (MNI) space and resliced to $3 \times 3 \times 3 \text{ mm}^3$. The resulting images were spatially smoothed with a Gaussian filter of 6 mm full-width half-maximum kernel.

For functional connectivity analyses, several additional steps were conducted to reduce spurious variance that was unlikely to reflect neuronal activity. These steps included: (1) multiple regression of nuisance variables, (2) temporally filtered with a band-pass filter (0.01–0.1 Hz), and (3) linear detrending to remove any residual drift. The nuisance variables included signals averaged from white matter, cerebrospinal fluid and the whole brain, six parameters obtained by head motion correction, and outlier volumes flagged by the artifact detection tool (ART; http://www.nitrc.org/projects/artifact_detect). A volume was defined as an outlier and scrubbed if the absolute head motion was > 0.5 mm from previous scan, or if the scan-to-scan global signal change was > 3 standard deviations of the global brain signal. Outlier volumes in the global mean signal intensity and motion were scrubbed by including them as nuisance regressors (i.e. one regressor per outlier) during the multiple regression procedure. After scrubbing, the 3 groups did not differ in residual head motion in the scanner (Table 1; control: 0.113 ± 0.051 mm; CHR: 0.116 ± 0.065 mm; patient: 0.096 ± 0.059 mm; one-way ANOVA, $p = 0.459$).

2.3. Functional connectivity analysis

To compute the rsFC of the insula, six spherical seed regions of interest (ROIs) (radius = 6 mm) were defined using previous published foci of insular subdivisions(Manoliu et al., 2014; Uddin, 2015; Uddin et al., 2014), each corresponding to the left and right ventral anterior insula (MNI coordinates: −33, 13, −7 and 32, 10, −6), the left and right dorsal anterior insula (MNI coordinates: −38, 6, 2 and 35, 7, 3), and the left and right posterior insula (MNI coordinates: −38, −6, 5 and 35, −11, 6) (Fig. 1A). The time series of each seed region were calculated by averaging the fMRI time series over all voxels within the region. For each subject and for each seed region, the correlation map was created by calculating Pearson's correlation coefficients between the seed time series and time series of all voxels in the brain. These correlation maps were converted to Z-value maps using Fisher's r-to-z transformation, to improve the normality of the correlation coefficients.

Group analyses were performed for the correlation maps of each seed region. For between-group comparisons, all individual-subject maps were entered into one-way ANOVA with three between-group levels (FEP, CHR, and CN). Then, we investigated the associations between clinical measures and insular functional connectivity strength in the CHR and FEP subjects by multiple linear regression analysis. Each analysis was adjusted using nuisance covariates for age, sex, education level and residual head motion. Olanzapine equivalent was also used as covariates for the regression analysis with clinical measures. The statistical map was thresholder at $p < 0.005$ at the voxel level, then corrected for multiple comparisons to $p < 0.05$ by using a cluster threshold. The *3dClustSim* method in AFNI was used to compute the cluster size threshold based on 1000 Monte Carlo simulations. Intrinsic smoothness of the data was estimated in AFNI *3dFWHMx*. The simulations were performed on a group GM mask (defined by voxels with a value greater than 0.1 in the averaged GM images across all the subjects) and yielded a minimum cluster size of 84 voxels.

3. Results

3.1. Demographic and clinical characteristics analysis

The original study sample included 19 FEP, 24 CHR, and 47 healthy control subjects. Group differences were tested using ANOVA for continuous variables, and chi-square test for sex. Table 1 shows demographic and clinical characteristics of the study sample. There were no significant differences between the patient group, CHR group and control group in age, education and head motion. A significant difference in sex was found among three groups ($p < 0.05$), therefore sex was used as a covariate for between-group comparisons.

3.2. Seed regions of interest

The resting-state functional connectivity patterns of different insula subregions are shown in Fig 1B-D, respectively. The PI was mainly connected with sensorimotor cortices; the dAI connected with the dorsal anterior cingulate cortex and dorsolateral prefrontal cortex (which is thought to mediate cognitive processes), and the vAI primarily connected with the pregenual anterior cingulate and limbic cortices (which mediate affective processes). The 3 insular subregions were then used as seed regions of interest to study altered rsFC of the insula in FEP and CHR.
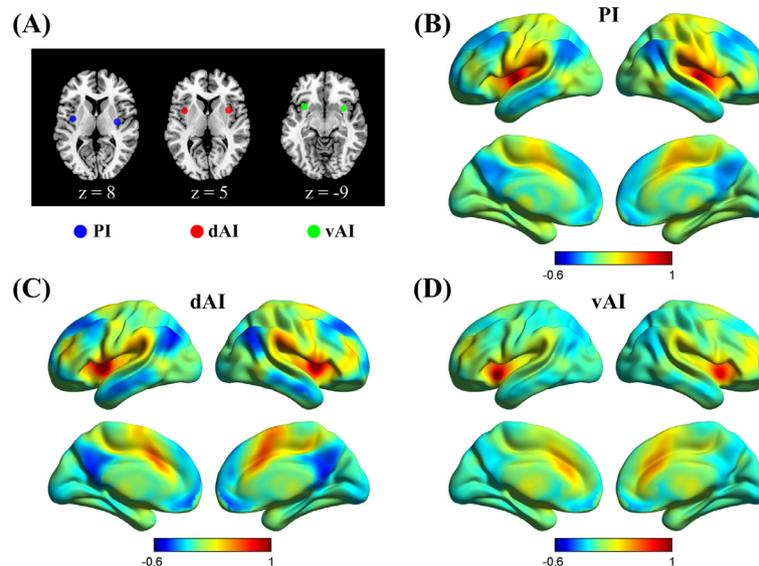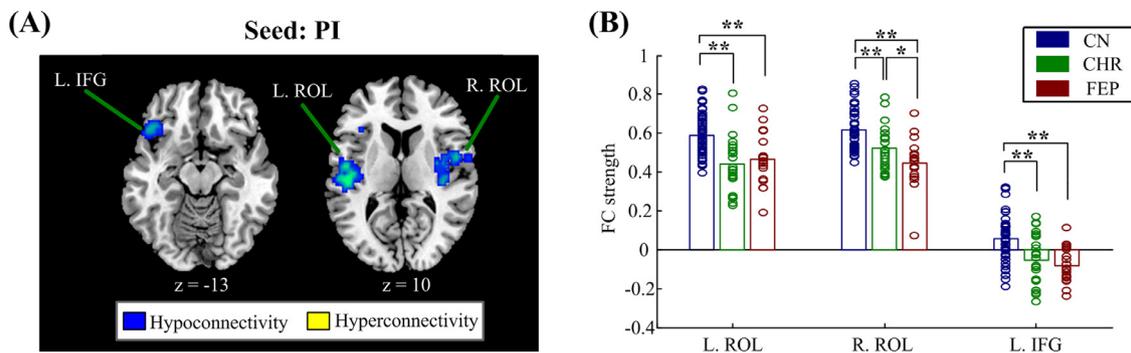

Fig. 1. Seed regions of interest. (A) The spatial distribution of the insula subregions used as seeds for rsFC analyses. (B), (C) and (D) are the resting-state functional connectivity patterns of different insula subregions. PI, posterior insula; dAI, dorsal anterior insula; vAI, ventral anterior insula.

Fig. 2. Between-group comparisons of the functional connectivity with the posterior insula. (A) Representative slices showing regions of altered functional connectivity with the posterior insula. (B) Scatter plots of functional connectivity strength of the regions in panel (A). *: $p < 0.05$, **: $p < 0.001$. ROL, rolandic operculum; IFG, inferior frontal gyrus.

3.3. rsFC results

Fig. 2 shows the results of the seed-based analysis for between-group comparisons of the PI rsFC. Compared with the CN subjects, the CHR and FEP patients showed decreased rsFC in the primary and secondary somatosensory cortices (i.e. the rolandic operculum and related areas), and the left inferior frontal gyrus. There was also significantly difference between FEP and CHR in the right rolandic operculum ($p < 0.05$). To further characterize the effects, we extracted the mean PI rsFC from the identified regions of interest. We found marked rsFC differences between the FEP and CN groups in all regions of interest (effect sizes > 0.9). Across areas, the CHR group was associated with similar but attenuated functional dysconnectivity comparing with the individuals in the FEP and CN groups, confirmed via formal effect sizes (CHR vs. FEP:0.20–0.61; CN vs. CHR: 0.82–1.06) (Table 2).

Fig. 3 shows the results of the seed-based analysis for between-group comparisons of the dAI rsFC. Compared with CN subjects, the CHR and FEP patients showed decreased rsFC in the bilateral putamen, whereas increased rsFC was found in the middle occipital gyrus. FEP patients also showed significantly increased rsFC in the fusiform relative to CN and CHR subjects. Effect size analysis also indicated marked rsFC differences between the FEP and CN groups in all regions of interest (effect sizes > 0.9). Except for the fusiform, the CHR group showed similar functional dysconnectivity with the FEP group, but exhibited more subtle abnormalities (CHR vs. FEP: < 0.4; CN vs. CHR: > 0.7) (Table 2).

Using the vAI as seed region, we observed that FEP patients exhibited functional hyperconnectivity in the left fusiform and right cuneus relative to healthy controls ($p < 0.001$; effect sizes > 0.9). However, there was no significantly difference between CN and CHR in these regions ($p > 0.05$; effect sizes < 0.3) (Fig. 4 and Table 2).
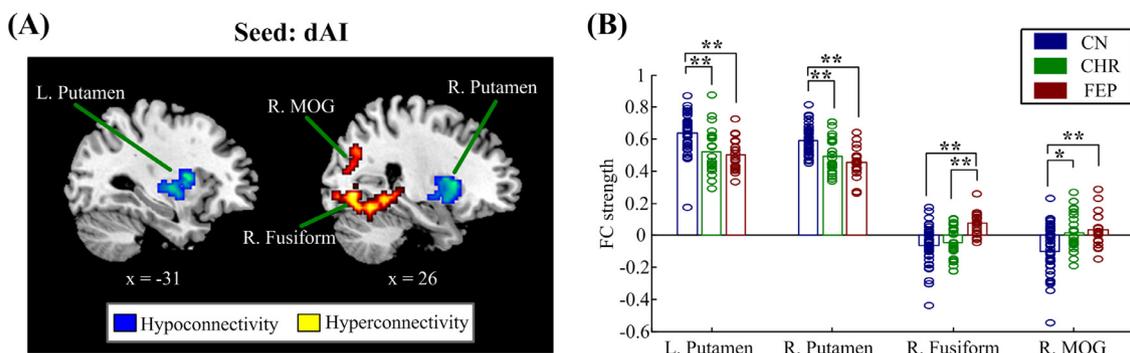
3.3. Association between symptom severity and insular connectivity

We first performed region-of-interest analysis in clusters with group differences, but did not detect any significant associations (absolute r values < 0.41, $p > 0.07$). Exploratory analyses were also performed in voxel-wise whole brain level, to give a full description of possible symptom-related insular functional connectivity. We found that concurrent positive symptoms on the Scale of Prodromal Symptoms (SOPS) was positively correlated with the rsFC between dAI and right superior temporal gyrus across the CHR subjects ($r = 0.732$, $p < 0.001$) (Fig. 5A). Moreover, we found that the rsFC between vAI and left dorsolateral prefrontal cortex (DLPFC) was negatively correlated with total scores of PANSS across the FEP patients ($r = -0.724$, $p < 0.001$) (Fig. 5B).

4. Discussion

In this study, we found that the CHR was associated with different abnormal rsFC patterns in subregions of insular cortex. Compared to controls, the rsFC alterations in CHR and FEP were similar between the PI and sensory cortex, and between dAI and putamen. FEP patients also showed dAI and vAI hyperconnectivity with several visual areas, but such abnormalities were not presented in CHR. Furthermore, rsFC between dAI and superior temporal gyrus was positively correlated with positive symptoms in CHR. All these findings suggested that insular functional dysconnectivity maybe a possible biomarker for the episode of schizophrenia.

The insular cortex is thought to integrate perceptions, emotions, thoughts, and plans into one subjective image of "our world" (Kurth et al., 2010). Previous studies found that mid-posterior regions are densely connected to primary and secondary sensory and motor

Table 2

Brain areas showing functional connectivity differences with insular subdivisions in between-group comparisons.

Anatomical area	Side	Num. of voxels	MNI coordinates	Effect size CN vs. FEP	CHR vs. FEP	CN vs. CHR
Seed: PI						
Rolandic operculum	L	193	(−51, −9, 12)	**0.96**	0.20	**1.06**
Rolandic operculum	R	150	(60, −3, 15)	**1.26**	0.61	**0.82**
Inferior frontal gyrus	L	97	(−36, 30, −9)	**1.07**	0.25	**0.82**
Seed: dAI						
Putamen	L	96	(−30, −9, 3)	**1.07**	0.15	**0.87**
Putamen	R	181	(27, 12, 6)	**1.25**	0.37	**0.95**
Fusiform	R	398	(27, −75, −3)	**1.13**	**1.10**	0.18
Middle occipital gyrus	R	96	(33, −69, 24)	**0.91**	0.16	0.79
Seed: vAI						
Fusiform	L	116	(−27, −72, −6)	**0.93**	**1.27**	0.28
Cuneus	R	192	(15, −75, 21)	**1.00**	**1.02**	0.03

Effect sizes with values greater than 0.8 are labeled in bold.

Fig. 3. Between-group comparisons of the functional connectivity with the dorsal anterior insula. (A) Representative slices showing regions of altered functional connectivity with the dorsal anterior insula. (B) Scatter plots of functional connectivity strength of the regions in panel (A). *: $p < 0.05$, **: $p < 0.001$. MOG, middle occipital gyrus.

areas (Friedman et al., 1986). In the current study, the CHR and FEP patients showed hypoconnectivity in the primary and secondary somatosensory cortices. This finding is consistent with a previous study (Kurth et al., 2010), which found that sensorimotor tasks activated the mid-posterior insular cortex. Another study also found that the posterior region was functionally connected with primary and secondary somatomotor cortices (Deen et al., 2011). In addition, the CHR and FEP patients showed hypoconnectivity in the inferior frontal gyrus, the weaker connectivity, the more severe the symptoms. The symptom may be caused by the reduced control of higher-level central nervous system to the insular cortex, such as the inferior frontal gyrus (Li et al., 2017).

As the insular is thought to integrate perceptions, emotions, thoughts, and plans into one subjective image, it is often been ascribed an integrative role, linking information from diverse functional systems (Cauda et al., 2011; Kurth et al., 2010). A study by Kurth et al. points that the dAI acts as a multimodal integration region (Kurth et al., 2010). In the current study, compared with the CN subjects, the CHR and FEP patients showed hypoconnectivity in the bilateral putamen, whereas hyperconnectivity in the fusiform and several visual areas. Our findings are consistent with the study by Kurth et al. (2010), which conducted a conjunction analysis across these domains found that putamen, fusiform and visual areas overlapped on the dAI (Kurth et al., 2010). This overlap might constitute a correlate for a functional integration between different functional systems and reflect a link between them that is necessary to integrate different qualities into a coherent experience of the world (Dupont et al., 2003; Kurth et al., 2010).

In general, across areas, the CHR group was associated with an intermediate level of dysconnectivity between the individuals in the FEP and CN groups, confirmed via formal through calculating the effect sizes of the rsFC. However, there were no significant differences between FEP and CHR in most of the regions, which demonstrated that neurocognitive deficits were similar in both FEP and CHR (Iwashiro et al., 2016; Park et al., 2015), thus the dysconnectivity of the

insular cortex in CHR may be considered as a nearly biological sign of schizophrenia (Park et al., 2015).

On the other hand, using the vAI as seed regions, we also observed that FEP patients exhibited functional hyperconnectivity in several visual areas relative to healthy controls and CHR, which is in line with previous studies that have reported abnormalities of visual brain networks in schizophrenia patients (Chen et al., 2015; Robson et al., 2016). The absent of rsFC abnormalities in visual areas in CHR individuals suggests that these changes are not prominent in the psychosis-risk syndrome, and may be secondary to factors associated with chronicity of the illness or antipsychotic drug treatment.

In this manuscript, we found that composite positive symptoms on the SOPS in the CHR group was positively correlated with rsFC between dorsal anterior insula and the right superior temporal gyrus, and furthermore PANSS total score was negatively correlated with the rsFC between ventral anterior insula and dorsolateral prefrontal cortex (DLPFC) in the FEP group. In general, the brain regions associated with symptoms concentrated in sensory cortex, such as superior temporal gyrus in CHR group, the stronger connectivity, the more severe the symptoms. The functions of insula alteration in schizophrenia include the processing of sensory information (Wylie and Tregellas, 2010), superior temporal gyrus is auditory cortex, activation of the superior temporal gyrus and insular may cause hallucinations, which are the most common symptoms in CHR (Cascella et al., 2011). A previous study examined the association of thalamic dysconnectivity and conversion to psychosis in youth and young adults at elevated clinical risk and they found that there was marked thalamic hyperconnectivity with sensory motor areas in those who converted to full-blown illness (Anticevic et al., 2015) . However, in patients, symptoms may be associated with rsFC between insula and higher-level central nervous system, such as DLPFC, the weaker connectivity, the more severe the symptoms, and the reduced control of higher-level central nervous system may lead to symptoms. The previous study showed anterior
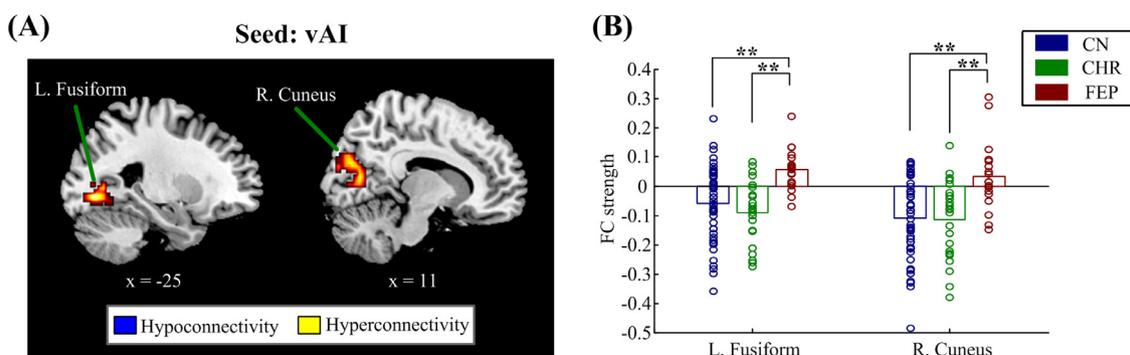
Fig. 4. Between-group comparisons of the functional connectivity with the ventral anterior insula. (A) Representative slices showing regions of altered functional connectivity with the ventral anterior insula. (B) Scatter plots of functional connectivity strength of the regions in panel (A). *: $p < 0.05$, **: $p < 0.001$.

(A) Seed: dAI

(B) Seed: vAI
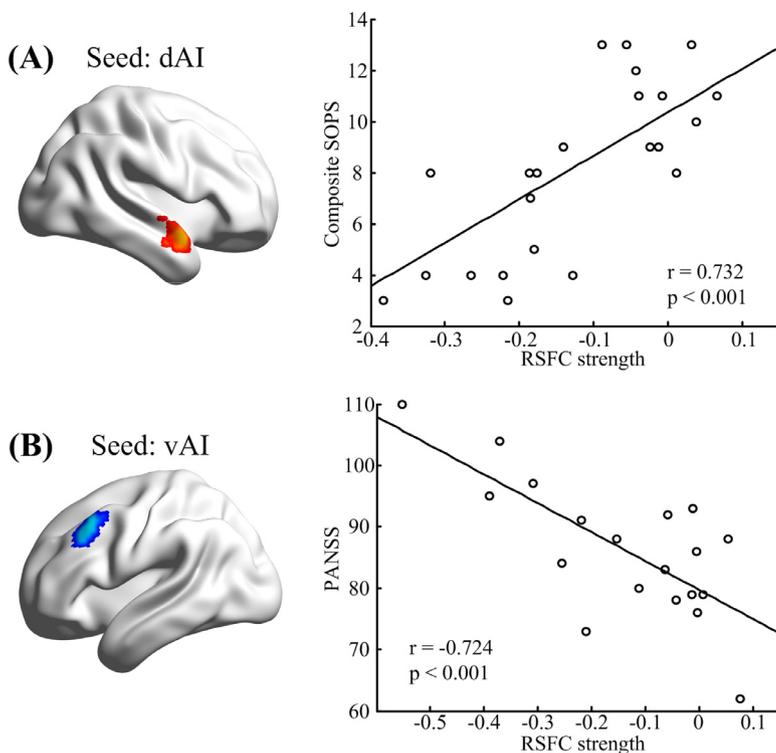
Fig. 5. Significant associations between insular functional connectivity and clinical symptom severity. (A) Using the dorsal anterior insula as seed region, connectivity in the right superior temporal gyrus was positively correlated with composite positive symptoms on the SOPS in the CHR group. (B) Using the ventral anterior insula as seed region, connectivity in the left dorsolateral prefrontal cortex was negatively correlated with PANSS total score in the FEP group.

insular cortex modulate activity in the executive networks in individuals with schizophrenia, which might be an emergent pathophysiological gateway in schizophrenia (Moran et al., 2013). It should be noted that the brain regions showing associations with symptom did not exhibit significant differences in group comparisons. A possible reason is that abnormalities in these regions may be normalized by antipsychotic treatment, as suggested by other studies (Guo et al., 2017; Sarpal et al., 2015). Although olanzapine equivalent was used as nuisance covariate in our study, medication effects on functional connectivity may not be dose-dependent. Studies exploring insular dysconnectivity in nonmedicated psychosis are required to exclude such effects.

Due to nature of our design, our findings only showed associations and we could not address upstream causal mechanisms. To map such upstream cellular mechanisms, translational research from animal experiments (Schobel et al., 2013), experimental pharmacologic neuroimaging studies (Anticevic et al., 2012), and computational models may generate mechanistic and testable predictions (Coyle, 2006; Murray et al., 2012). Schizophrenia likely involves alterations in glutamatergic, dopaminergic, and inhibitory gamma-aminobutyric acid (GABA) neurotransmission (Gonzalez-Burgos et al., 2010; Gonzalez-Burgos and Lewis, 2012; Howes and Kapur, 2009; Kegeles et al., 2012). Theoretical models of the illness repeatedly implicate these neurotransmitter systems inthalamo-striatal-cortical circuits, in our current study we found altered rsFC between insular cortex and putamen, sensorimotor cortex, which was consistent to the models. However, it currently remains unknown which upstream mechanisms and at which locus (subcortical or cortical) produces such widespread insular cortex dysconnectivity.

Several limitations of this study should be noted. First, the sample size of the study was relatively small, thus limiting the generalization of our findings. Second, as in most studies involving connectivity, head movements during imaging data acquisition is a concern. To limit the effects of possible head movements, we used careful movement censoring methods for all data and used movement (percentage of frames scrubbed) as a covariate in the analysis, which did not change our findings. Finally, we started our analysis from the 3 subregions first,

while this may be a well-justified first pass approach, it is possible that our analysis failed to capture other potentially important discrepancies across insular cortex.

In conclusion, this study focused on the comparison of rsFC of different insular subregions across CN, CHR and FEP. The results suggest that CHR individuals have insular functional dysconnectivity with the sensory cortex and putamen as that seen in schizophrenia. Besides, specific insular connectivity was associated with severity of psychotic symptoms. Our findings suggest that insular functional dysconnectivity may be a system-level neural substrate preceding the onset of psychosis.

Role of funding source

Declaration of Competing Interest

The authors have declared that no competing interests exist.

References

Anticevic, A., Gancsos, M., Murray, J.D., Repovs, G., Driesen, N.R., Ennis, D.J., Niciu, M.J., Morgan, P.T., Surti, T.S., Bloch, M.H., 2012. NMDA receptor function in large-scale anticorrelated neural systems with implications for cognition and schizophrenia. Proc. Natl. Acad. Sci. 109 (41), 16720–16725.
Anticevic, A., Haut, K., Murray, J.D., Repovs, G., Yang, G.J., Diehl, C., McEwen, S.C., Bearden, C.E., Addington, J., Goodyear, B., Cadenhead, K.S., Mirzakhanian, H., Cornblatt, B.A., Olvet, D., Mathalon, D.H., McGlashan, T.H., Perkins, D.O., Belger, A., Seidman, L.J., Tsuang, M.T., van Erp, T.G., Walker, E.F., Hamann, S., Woods, S.W., Qiu, M., Cannon, T.D., 2015. Association of thalamic dysconnectivity and conversion to psychosis in youth and young adults at elevated clinical risk. JAMA Psychiatry 72 (9), 882–891.

Bernard, J.A., Orr, J.M., Mittal, V.A., 2015. Abnormal hippocampal-thalamic white matter tract development and positive symptom course in individuals at ultra-high risk for psychosis. NPJ Schizophr, 15009.

Cascella, N.G., Gerner, G.J., Fieldstone, S.C., Sawa, A., Schretlen, D.J., 2011. The insula-claustrum region and delusions in schizophrenia. Schizophr. Res. 133 (1–3), 77–81.

Cauda, F., D'Agata, F., Sacco, K., Duca, S., Geminiani, G., Vercelli, A., 2011. Functional connectivity of the insula in the resting brain. Neuroimage 55 (1), 8–23.

Chen, X., Duan, M., Xie, Q., Lai, Y., Dong, L., Cao, W., Yao, D., Luo, C., 2015. Functional disconnection between the visual cortex and the sensorimotor cortex suggests a potential mechanism for self-disorder in schizophrenia. Schizophr. Res. 166 (1–3), 151–157.

Coyle, J.T., 2006. Glutamate and schizophrenia: beyond the dopamine hypothesis. Cell. Mol. Neurobiol. 26 (4–6), 363–382.

Dandash, O., Fornito, A., Lee, J., Keefe, R.S., Chee, M.W., Adcock, R.A., Pantelis, C., Wood, S.J., Harrison, B.J., 2014. Altered striatal functional connectivity in subjects with an at-risk mental state for psychosis. Schizophr. Bull. 40 (4), 904–913.

Deen, B., Pitskel, N.B., Pelphrey, K.A., 2011. Three systems of insular functional connectivity identified with cluster analysis. Cereb. Cortex 21 (7), 1498–1506.

Dupont, S., Bouilleret, V., Hasboun, D., Semah, F., Baulac, M., 2003. Functional anatomy of the insula: new insights from imaging. Surg. Radiol. Anat. 25 (2), 113–119.

Friedman, D.P., Murray, E.A., O'Neill, J.B., Mishkin, M., 1986. Cortical connections of the somatosensory fields of the lateral sulcus of macaques: evidence for a corticolimbic pathway for touch. J. Comp. Neurol. 252 (3), 323–347.

Gogolla, N., 2017. The insular cortex. Curr. Biol. 27 (12), R580–R586.

Gonzalez-Burgos, G., Hashimoto, T., Lewis, D.A., 2010. Alterations of cortical GABA neurons and network oscillations in schizophrenia. Curr. Psychiatry Rep. 12 (4), 335–344.

Gonzalez-Burgos, G., Lewis, D.A., 2012. NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. Schizophr. Bull. 38 (5), 950–957.

Guo, W., Liu, F., Chen, J., Wu, R., Li, L., Zhang, Z., Zhao, J., 2017. Olanzapine modulation of long- and short-range functional connectivity in the resting brain in a sample of patients with schizophrenia. Eur. Neuropsychopharmacol. 27 (1), 48–58.

Hannan, K.L., Wood, S.J., Yung, A.R., Velakoulis, D., Phillips, L.J., Soulsby, B., Berger, G., McGorry, P.D., Pantelis, C., 2010. Caudate nucleus volume in individuals at ultra-high risk of psychosis: a cross-sectional magnetic resonance imaging study. Psychiatry Res. 182 (3), 223–230.

Heinze, K., Reniers, R.L., Nelson, B., Yung, A.R., Lin, A., Harrison, B.J., Pantelis, C., Velakoulis, D., McGorry, P.D., Wood, S.J., 2015. Discrete alterations of brain network structural covariance in individuals at ultra-high risk for psychosis. Biol. Psychiatry 77 (11), 989–996.

Howes, O.D., Kapur, S., 2009. The dopamine hypothesis of schizophrenia: version III–the final common pathway. Schizophr. Bull. 35 (3), 549–562.

Iwashiro, N., Koike, S., Satomura, Y., Suga, M., Nagai, T., Natsubori, T., Tada, M., Gonoi, W., Takizawa, R., Kunimatsu, A., Yamasue, H., Kasai, K., 2016. Association between impaired brain activity and volume at the sub-region of broca's area in ultra-high risk and first-episode schizophrenia: a multi-modal neuroimaging study. Schizophr. Res. 172 (1–3), 9–15.

Kegeles, L.S., Mao, X., Stanford, A.D., et al., 2012. . elevated prefrontal cortex γ-aminobutyric acid and glutamate-glutamine levels in schizophrenia measured in vivo with proton magnetic resonance spectroscopy. Arch. Gen. Psychiatry 69 (5), 449–459.

Konrad, A., Winterer, G., 2008. Disturbed structural connectivity in schizophrenia primary factor in pathology or epiphenomenon? Schizophr. Bull. 34 (1), 72–92.

Kurth, F., Zilles, K., Fox, P.T., Laird, A.R., Eickhoff, S.B., 2010. A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. Brain Struct. Funct. 214 (5–6), 519–534.

Leucht, S., Samara, M., Heres, S., Davis, J.M., 2016. Dose equivalents for antipsychotic drugs: the ddd method. Schizophr. Bull. 42 (Suppl 1), S90–S94.

Li, J., Tang, Y., Womer, F., Fan, G., Zhou, Q., Sun, W., Xu, K., Wang, F., 2017. Two patterns of anterior insular cortex functional connectivity in bipolar disorder and schizophrenia. World J. Biol. Psychiatry 1–9.

Lord, L.D., Allen, P., Expert, P., Howes, O., Lambiotte, R., McGuire, P., Bose, S.K., Hyde, S., Turkheimer, F.E., 2011. Characterization of the anterior cingulate's role in the at-risk mental state using graph theory. Neuroimage 56 (3), 1531–1539.

Manoliu, A., Riedl, V., Zherdin, A., Muhlau, M., Schwerthoffer, D., Scherr, M., Peters, H., Zimmer, C., Forstl, H., Bauml, J., Wohlschlager, A.M., Sorg, C., 2014. Aberrant dependence of default mode/central executive network interactions on anterior insular salience network activity in schizophrenia. Schizophr. Bull. 40 (2), 428–437.

Moran, L.V., Tagamets, M.A., Sampath, H., O'Donnell, A., Stein, E.A., Kochunov, P., Hong, L.E., 2013. Disruption of anterior insula modulation of large-scale brain networks in schizophrenia. Biol. Psychiatry 74 (6), 467–474.

Murray, J.D., Anticevic, A., Gancsos, M., Ichinose, M., Corlett, P.R., Krystal, J.H., Wang, X.-.J., 2012. Linking microcircuit dysfunction to cognitive impairment: effects of disinhibition associated with schizophrenia in a cortical working memory model. Cerebral Cortex 24 (4), 859–872.

Pang, L., Kennedy, D., Wei, Q., Lv, L., Gao, J., Li, H., Quan, M., Li, X., Yang, Y., Fan, X., Song, X., 2017. Decreased functional connectivity of insular cortex in drug naive first episode schizophrenia: in relation to symptom severity. PLoS ONE 12 (1), e0167242.

Park, S., Yoon, Y.B., Yun, J.-.Y., Jung, W.H., Cho, K.I.K., Kim, S.N., Lee, T.Y., Park, H.Y., Kwon, J.S., 2015. Altered fronto-temporal functional connectivity in individuals at ultra-high-risk of developing psychosis. PLoS ONE 10 (8), e0135347.

Robson, S.E., Brookes, M.J., Hall, E.L., Palaniyappan, L., Kumar, J., Skelton, M., Christodoulou, N.G., Qureshi, A., Jan, F., Katshu, M.Z., Liddle, E.B., Liddle, P.F., Morris, P.G., 2016. Abnormal visuomotor processing in schizophrenia. Neuroimage. Clin. 12, 869–878.

Sarpal, D.K., Robinson, D.G., Lencz, T., Argyelan, M., Ikuta, T., Karlsgodt, K., Gallego, J.A., Kane, J.M., Szeszko, P.R., Malhotra, A.K., 2015. Antipsychotic treatment and functional connectivity of the striatum in first-episode schizophrenia. JAMA Psychiatry 72 (1), 5–13.

Schobel, Scott A., Chaudhury, Nashid H., Khan, Usman A., Paniagua, B., Styner, Martin A., Asllani, I., Inbar, Benjamin P., Corcoran, heryl M., Lieberman, Jeffrey A., Moore, H., Small, Scott A., 2013. Imaging patients with psychosis and a mouse model establishes a spreading pattern of hippocampal dysfunction and implicates glutamate as a driver. Neuron 78 (1), 81–93.

Simon, V., De Hert, M., Wampers, M., Peuskens, J., van Winkel, R., 2009. The relation between neurocognitive dysfunction and impaired insight in patients with schizophrenia. Eur. Psychiatry 24 (4), 239–243.

Takahashi, T., Wood, S.J., Yung, A.R., Phillips, L.J., Soulsby, B., McGorry, P.D., Tanino, R., Zhou, S.Y., Suzuki, M., Velakoulis, D., Pantelis, C., 2009. Insular cortex gray matter changes in individuals at ultra-high-risk of developing psychosis. Schizophr. Res. 111 (1–3), 94–102.

Uddin, L.Q., 2015. Salience processing and insular cortical function and dysfunction. Nat. Rev. Neurosci. 16 (1), 55–61.

Uddin, L.Q., Kinnison, J., Pessoa, L., Anderson, M.L., 2014. Beyond the tripartite cognition-emotion-interoception model of the human insular cortex. J. Cogn. Neurosci. 26 (1), 16–27.

van Os, J., Kenis, G., Rutten, B.P., 2010. The environment and schizophrenia. Nature 468 (7321), 203–212.

Wang, C., Ji, F., Hong, Z., Poh, J.S., Krishnan, R., Lee, J., Rekhi, G., Keefe, R.S.E., Adcock, R.A., Wood, S.J., Fornito, A., Pasternak, O., Chee, M.W.L., Zhou, J., 2016. Disrupted salience network functional connectivity and white-matter microstructure in persons at risk for psychosis: findings from the lyriks study. Psychol. Med. 46 (13), 2771–2783.

Wang, C., Lee, J., Ho, N.F., Lim, J.K.W., Poh, J.S., Rekhi, G., Krishnan, R., Keefe, R.S.E., Adcock, R.A., Wood, S.J., Fornito, A., Chee, M.W.L., Zhou, J., 2018. Large-Scale network topology reveals heterogeneity in individuals with at risk mental state for psychosis: findings from the longitudinal youth-at-risk study. Cereb. Cortex 28 (12), 4234–4243.

Witthaus, H., Brune, M., Kaufmann, C., Bohner, G., Ozgurdal, S., Gudlowski, Y., Heinz, A., Klingebiel, R., Juckel, G., 2008. White matter abnormalities in subjects at ultra high-risk for schizophrenia and first-episode schizophrenic patients. Schizophr. Res. 102 (1–3), 141–149.

Witthaus, H., Kaufmann, C., Bohner, G., Ozgurdal, S., Gudlowski, Y., Gallinat, J., Ruhrmann, S., Brune, M., Heinz, A., Klingebiel, R., Juckel, G., 2009. Gray matter abnormalities in subjects at ultra-high risk for schizophrenia and first-episode schizophrenic patients compared to healthy controls. Psychiatry Res. 173 (3), 163–169.

Wylie, K.P., Tregellas, J.R., 2010. The role of the insula in schizophrenia. Schizophr. Res. 123 (2–3), 93–104.