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Insula Functional Connectivity in Schizophrenia: Subregions, Gradients, and Symptoms

Ye Tian, Andrew Zalesky, Chad Bousman, Ian Everall, and Christos Pantelis

ABSTRACT

BACKGROUND: The insular cortex is connected to a diverse network of cortical and subcortical areas. This study aimed to investigate whether the diversity in functional connectivity across the insula's topography is altered in individuals with schizophrenia and relates to the clinical symptoms of the disorder.

METHODS: Insula-to-whole-brain functional connectivity was mapped using resting-state functional magnetic resonance imaging at the resolution of voxels in individuals with schizophrenia (n = 49) and healthy comparison individuals (n = 52). Diversity in functional connectivity across the insula's topography was represented as discrete subregions and gradients of continuous variation. Canonical correlation analysis was used to relate interindividual variation in insula connectivity to clinical symptoms.

RESULTS: Insula connectional diversity was parcellated into two subregions: dorsoanterior and ventroposterior. Compared with the healthy comparison group, subjects with schizophrenia were associated with an overall reduction in insula functional connectivity as well as reduced differentiation in connectivity profiles between these subregions. A significant interaction effect between diagnosis and insula subregion indicated that the anterior subregion in schizophrenia was connected with increased strength to the somatosensory, motor, occipital, and parietal cortices, whereas the posterior subregion showed increased connectivity with the thalamus and prefrontal cortex. Insula connectivity with the anterior cingulate and auditory cortices was significantly associated with cognitive impairment, negative symptoms, poor psychosocial functioning, and longer duration of illness (r = .64, p = .03).

CONCLUSIONS: Diversity in functional connectivity across the insula's rostrocaudal axis is reduced in schizophrenia, resulting in reduced differentiation between anterior and posterior insula. Interindividual variation in insula connectivity explains variability in some of the clinical symptoms of schizophrenia.

Keywords: Clustering, Diversity curve, Insular cortex, Parcellation, Resting-state fMRI, Schizophrenia

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Functional dysconnectivity of the insular cortex is consistently observed in psychosis spectrum disorders, including individuals with first-episode psychosis (1–3), individuals with chronic schizophrenia (4,5), and individuals at high risk for the disorder (6–8). Specifically, the insula shows aberrant patterns of connectivity with the cingulate cortex as well as regions comprising the default mode network and central executive network (4–7). Most previous neuroimaging studies investigating the connectivity of the insula in schizophrenia have treated the insula as a single, homogeneous region (3) or have considered only its anterior aspect (4,5,7–9). In particular, neuroimaging data are typically averaged over all voxels comprising the insula to provide a summary characterization, and thus any potential variation in connectivity across the topography (spatial extent) of the insula may be overlooked. We refer to this topographic variation as connectional diversity. A region with greater connectional diversity shows greater variation across its topography with respect to the cortical and subcortical regions to which it connects.

Recently, functional and structural magnetic resonance imaging (MRI) studies have sought to characterize the insula's connectional diversity using one of two models: 1) discrete subregions (clusters) (10) or 2) continua of variation (diversity curves) (11). These studies have generally been limited to healthy individuals. According to the subregions model, the insula can be parcellated into clusters, where each cluster is associated with a distinct connectivity pattern. Many studies employing the subregions model suggest that the insula is best represented with a bipartite subdivision comprising anterior and posterior subregions (10,12–16), whereas other studies provide evidence of a tripartite clustering in which the anterior insula is further subdivided into dorsal and ventral components (4,17,18) as well as parcellations comprising more than three subdivisions (14,19–27). Particular subregions have been linked to specific aspects of cognition, emotion, and somatosensory sensations (18,28,29). More recently, it has been suggested that the insula's connectional diversity can be represented more parsimoniously as a continuum of gradual variation along a rostrocaudal axis, rather than with discrete subregions (30). Interindividual variation in the rate of variation along this axis of the insula has been found to explain significant variation in behavioral traits assessed in healthy adults (11).

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The aim of the present study was to characterize the diversity in functional connectivity across the topography of the insular cortex in adults with schizophrenia. To this end, we acquired resting-state functional MRI (fMRI) data in individuals with schizophrenia and healthy comparison individuals and used these data to map the functional connectivity profile of each insula voxel with respect to all other cortical and subcortical voxels. We clustered insula voxels into distinct subregions based on similarity in their connectivity profiles as well as modeled variation in connectivity among insula voxels continuously using the recently proposed concept of a diversity curve. An individual's diversity curve quantifies how rapidly the insula's connectivity with the rest of the cortex changes per unit length along the insula's rostrocaudal axis (11). We then performed statistical analyses to compare the functional connectivity profiles of distinct insula subregions between the schizophrenia and healthy comparison groups. Finally, we assessed whether patterns of insula functional connectivity in the schizophrenia group were associated with interindividual variation in the severity of psychosis symptoms, cognition, and psychosocial functioning. Given the widespread reductions in functional connectivity previously reported in this cohort (31) and the known association between insula connectional diversity and cognitive-affective function (11), we hypothesized that schizophrenia would be associated with reduced insula connectional diversity and reduced segregation (differentiation) between the connectivity profiles of putative insula subregions. Our hypothesis is supported by evidence of reduced segregation on the global scale of whole-brain functional networks in the disorder (32) as well as loss of functional specialization within specific areas such as the prefrontal cortex (33). This is the first study to comprehensively characterize connectional pathology across the topography of the insula in individuals with schizophrenia.

METHODS AND MATERIALS

Participants, Image Acquisition, and Image Preprocessing

Participants. This study was approved by the Melbourne Health Human Research Ethics Committee (ID No. 2012.069). All participants gave written consent before participation. Individuals with established schizophrenia (n = 49, mean age 41.02 ± 9.93 years, 15 female subjects) and healthy comparison individuals matched for average age, sex, and handedness (n = 52, mean age 39.85 ± 10.46 years, 17 female subjects) were included in this study. The majority of individuals with schizophrenia (n = 44) were considered resistant to treatment (34) and currently prescribed and taking clozapine. The diagnosis of schizophrenia was further confirmed by the Mini International Neuropsychiatric Interview (35). Healthy comparison individuals were recruited from the local community. Individuals satisfying at least one of the following exclusion criteria were not considered: current or history of psychiatric diagnoses, substance dependence, mental retardation (IQ <70), first-degree relatives with psychiatric illness, neurological disorders, impaired thyroid function, diabetes, and other major medical conditions.

The severity of clinical symptoms was assessed using the Positive and Negative Syndrome Scale (PANSS) (36), Scale for the Assessment of Positive Symptoms (37), and Scale for Assessment of Negative Symptoms (SANS) (38) on the day of screening. For all individuals, social, occupational, and psychological functioning were measured with the Global Assessment of Functioning (GAF) (39), Social and Occupational Functioning Assessment Scale (SOFAS) (40), and Wechsler Adult Intelligence Scale for cognition (41) (Table 1). Antipsychotic dosage was converted to chlorpromazine equivalent dose using an established conversion methodology (42).

Table 1. Demographic and Clinical Characteristics

	Schizophrenia Group (n = 49)	Comparison Group (n = 52)	Statistic	p Value
Age, Years	41.02 (±9.93)	39.85 (±10.46)	t = 0.58	.56
Sex, Male/Female	34/15	35/17	χ^2 = 0.05	.82
Handedness, Right/Left	45/4	50/2	χ^2 = 0.84	.36
Current IQ	91.34 (±18.70)	111.51 (±14.04)	t = 5.91	< .001
GAF Score	47.85 (±13.50)	79.27 (±11.34)	t = 12.52	< .001
SOFAS Score	48.38 (±14.81)	79.44 (±11.63)	t = 11.66	< .001
Age at Illness Onset, Years	22.71 (±6.44)	—	—	—
Duration of Illness, Years	17.95 (±9.16)	—	—	—
PANSS-POS	16.08 (±5.72)	—	—	—
PANSS-NEG	18.5 (±7.90)	—	—	—
PANSS-GEN	22.25 (±9.72)	—	—	—
PANSS-T	51.32 (±24)	—	—	—
SAPS Total Score	20.37 (±16.31)	—	—	—
SANS Total Score	40.60 (±19.28)	—	—	—
Clozapine Dosage, mg/day	383.70 (±134.20)	—	—	—
CPZ Equivalent, mg/day	498.80 (±281.10)	—	—	—

Values are presented as n or mean (±SD).

CPZ, chlorpromazine; GAF, Global Assessment of Functioning; PANSS-GEN, Positive and Negative Syndrome Scale–General Psychopathology subtotal score; PANSS-NEG, Positive and Negative Syndrome Scale–Negative subtotal score; PANSS-POS, Positive and Negative Syndrome Scale–Positive subtotal score; PANSS-T, Positive and Negative Syndrome Scale total score; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; SOFAS, Social and Occupational Functioning Assessment Scale.

Image Acquisition and Image Preprocessing. Image acquisition and resting-state fMRI data preprocessing are described in the Supplement.

Insula Discrete Subregions Model

Cluster Analysis. For each individual, the left and right insula were separately parcellated into distinct subregions using cluster analysis. In brief, functional connectivity was mapped between each insula voxel and all other gray matter voxels, and Ward's linkage was then used to cluster insula voxels according to similarity in connectional profiles. The morphology of the resulting clusters (i.e., volume and length measurements) was then tested for differences between the schizophrenia and healthy comparison group using the Wilcoxon rank sum test. Functional connectivity mapping and cluster analysis is described in detail in the Supplement. Modularity analysis (Newman's spectral community detection algorithm) was used to combine each individual's insula parcellation to generate a group-level consensus parcellation of the insula for each of the two groups. The modularity Q value was then used to test whether the degree of differentiation in functional connectivity profiles between the anterior and posterior insula subregions differed between the two groups (Supplement).

Whole-Brain Functional Connectivity Profile of Insula Subregions. Using the cluster analysis described above, we found that the insula was most parsimoniously partitioned into two clusters for the majority of individuals with schizophrenia (88%) and healthy comparison (87%) individuals. While substantial variation in the shape and location of clusters across individuals was evident (Supplemental Figure S1), the group-level consensus parcellation comprised two modules that mapped to anterior and posterior insula subregions. We sought to test whether whole-brain functional connectivity patterns differed between anterior and posterior insula or between the schizophrenia and healthy comparison groups. To this end, the intersection between the separate anterior and posterior insula masks for each group was computed to yield a single anterior and posterior insula mask that was common to both groups (Figure 1C and Supplemental Table S1). For each individual, the fMRI signal was averaged across all voxels comprising the anterior and posterior mask, and this averaged signal was then correlated (Pearson's correlation) with all other gray matter voxels, yielding a whole-brain anterior and posterior insula functional connectivity map for each person. For each gray matter voxel comprising this map, within-subjects analysis of variance was used to test the main effects of diagnosis (schizophrenia/control), subregion (anterior/posterior), and laterality (left/right) on insula connectivity as well as the interaction between diagnosis and the latter two main effects. Nonparametric cluster-based inference, as implemented in Randomise (43), was used to control the familywise error across the set of all voxels and to identify voxel clusters for which the main or interaction effects were significant (permutations: n = 10,000; cluster forming threshold: t = 2.5, $p < .05$).

Insula Continuum Model and Diversity Curves

It has recently been suggested that connectional diversity across the insula's topography is more parsimoniously represented as continua of gradual variation, rather than with discrete subregions (11). Given this consideration, we employed the recently proposed concept of a diversity curve (11) to represent the insula's connectional diversity for each individual as a continuum of variation. In brief, Laplacian eigenmaps (44) were mapped for each individual's insula using established methods (45), and the eigenmaps were projected onto a rostrocaudal curvilinear trajectory through the insula representing the trajectory of maximal change in the eigenmap (11). This yielded a separate diversity curve for each individual. Further details are provided in the Supplement. We compared the diversity curves between the schizophrenia and healthy comparison groups to test the hypothesis that schizophrenia would be associated with a reduction in insula connectional diversity. To this end, a two-sample t test was performed to test for a difference in diversity between the schizophrenia and healthy comparison individuals at each point along the diversity curves. The false discovery rate was used to control for multiple comparisons across the set of all points.

Clinical Associations

We hypothesized that interindividual variation in the extent of insula connectivity reductions with the anterior cingulate cortex (ACC) and superior temporal gyrus (STG) would be associated with clinical severity in the schizophrenia group. This hypothesis is supported by suggestions that 1) the ACC and anterior insula are key regions of the salience network (28), which is known to show aberrant network switching in schizophrenia (5,46), and 2) the STG is crucial for language (Wernicke's area) and auditory (Heschl's gyrus and planum temporale) processing, and dysconnectivity of this region has been associated with auditory hallucinations in schizophrenia (47–49). To test this hypothesis, for each individual with schizophrenia, fMRI data were averaged across all voxels comprising the ACC and STG clusters identified with Randomise as well as the masks delineating the posterior and anterior insula subregions. Note that the ACC cluster identified with Randomise included a portion of the supplementary motor area (SMA), and thus we refer to this cluster as ACC/SMA. The resulting regionally averaged time series were then correlated (Pearson's correlation) to yield functional connectivity estimates between 1) anterior insula and ACC and 2) posterior insula and STG. Finally, canonical correlation analysis (50) was used to test for a multivariate association between these two functional connectivity estimates and several measures of symptom severity: PANSS, Scale for the Assessment of Positive Symptoms, SANS, GAF, SOFAS, Wechsler Adult Intelligence Scale, and duration of illness (Supplement).

Numeric computation was performed in MATLAB R2017b (The MathWorks, Inc., Natick, MA). Images were visualized using BrainNetViewer1.6 (51) and FSLView software.

RESULTS

Demographic and clinical characteristics are shown in Table 1. The schizophrenia and healthy comparison groups were matched in terms of mean age, sex, and handedness, and thus these factors were not controlled when performing group comparisons. However, IQ and measures of social and general

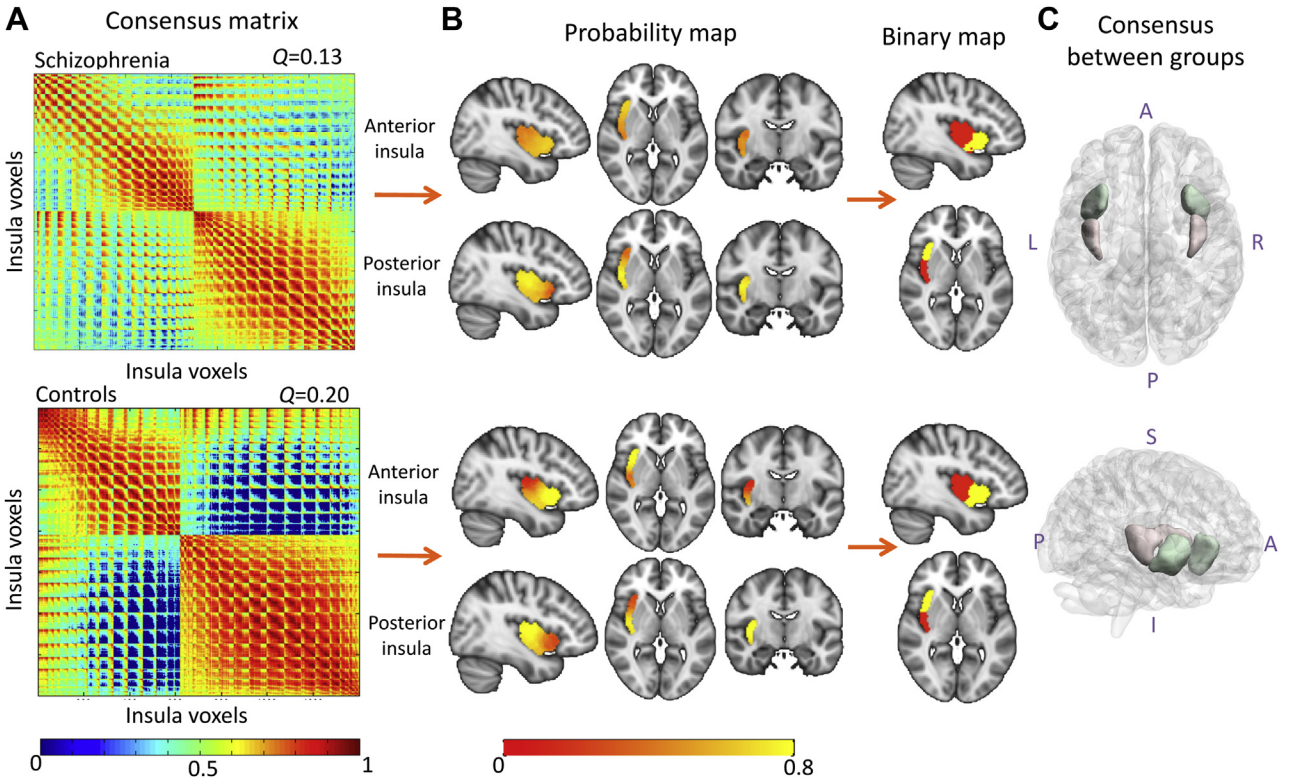

Figure 1. Bipartite parcellation of the right insular cortex based on resting-state functional connectivity in individuals with schizophrenia and healthy comparison individuals. **(A)** Group consensus matrices (insula voxels × insula voxels) for the schizophrenia (upper matrix) and healthy comparison groups (lower). Each cell stores the proportion of individuals for which a given pair of insula voxels comprised the same cluster. Warm tones (red) indicate pairs of voxels consistently comprising the same subregions, whereas cool tones (blue) indicate pairs of voxels consistently comprising distinct subregions. While two modules were identified in both groups, modular separation was significantly reduced in the schizophrenia group ($Q = 0.13$, $p = .0038$) compared with the healthy comparison group ($Q = 0.20$). The rows and columns of the consensus matrices are ordered such that all insula voxels comprising the anterior insula module are listed first, followed by all voxels in the posterior insula. **(B)** The consensus matrices are mapped to the insula to yield probabilistic maps of the anterior and posterior insula subregions. The color of each insula voxel is modulated by the proportion of individuals for which the voxel comprised the relevant subregion (module). Yellow tones indicate voxels that consistently comprise the relevant subregion across individuals, whereas red tones indicate voxels that rarely comprise the subregion. Probability maps were converted to binary (hard) segmentations to delineate discrete anterior (yellow) and posterior (red) insula subregions for both groups. **(C)** The intersection of the anterior insula subregion delineated for each of the two groups was determined to define a consensus anterior insula subregion for both groups (green) and similarly for the posterior insula subregion (pink). Slice coordinates (Montreal Neurological Institute, mm): x = 40, y = −10, z = 2. A, anterior; I, inferior; L, left; P, posterior; R, right; S, superior.

functioning were significantly reduced in the schizophrenia group.

Reduced Differentiation Between Anterior and Posterior Insula Subregions

Bipartite parcellation of the insula into anterior and posterior subregions yielded the most parsimonious representation in the majority of individuals with schizophrenia (88%) and healthy comparison individuals (87%). For the remaining individuals, insula voxels were partitioned into two clusters according to the individual's best-fitting bipartite solution. To facilitate between-group comparison, only bipartite insula parcellations were analyzed here. The volume (cluster size) of each insula subregion was estimated by enumerating the number of voxels assigned to the cluster of voxels delineating each subregion and multiplying this number by the volume of each individual voxel (8×10^{-3} cm^3). The anterior insula subregion was significantly reduced in the schizophrenia group (right: $z = 3.81$, schizophrenia: 4.59 ± 2.2 cm^3, comparison group: 6.41 ± 2.2 cm^3, $p < .001$; left: $z = 4.47$, $p < .001$), whereas the posterior insula was significantly increased (right: $z = 3.44$, schizophrenia: 5.60 ± 2.3 cm^3, comparison group: 4.40 ± 2.2 cm^3, $p < .001$; left: $z = 3.92$, $p < .001$) and extended further anteriorly (right: $z = 3.89$, $p < .001$; left: $z = 3.13$, $p = .0017$) and laterally (right: $z = 3.89$, $p < .001$; left: $z = 3.87$, $p < .001$) relative to the healthy comparison group (Supplemental Figure S2). We found that the differentiation between these two putative insula subregions was significantly reduced in the schizophrenia group (Q value right insula, schizophrenia: 0.13, comparison group: 0.20, $p = .0038$; Q value left insula, schizophrenia: 0.12, comparison group: 0.21, $p = .002$) (Figure 1A, B and Supplemental Figure S3).

Altered Insula Functional Connectivity

Having parcellated the insula into two distinct subregions (anterior and posterior), we next sought to investigate whether

the functional connectivity between these two subregions and the rest of the cortex was altered in schizophrenia. We investigated three main effects (subregion, diagnosis, laterality) and two interaction effects (diagnosis × subregion, diagnosis × laterality). We identified regional clusters associated with each of these effects.

Main Effect of Subregion (Anterior vs. Posterior). Across all individuals (individuals with schizophrenia and healthy comparison individuals combined), we found that the anterior insula was preferentially connected to the frontolimbic and paralimbic areas (peak $t = 24.2$, $p < .001$) and the posterior cerebellar lobes (crus I and crus II) (peak $t = 8.17$, $p = .006$), whereas the posterior insula was characterized by extensive parietotemporo-occipital and anterior cerebellar connectivity (peak $t = 22.4$, $p < .001$) (Figure 2A).

Main Effect of Diagnosis (Schizophrenia vs. Healthy Comparison Group). Functional connectivity was significantly reduced in the schizophrenia group between the insula and multiple cortical and subcortical areas. Specifically, schizophrenia was associated with significant reductions in connectivity between the anterior insula and regions located at the bilateral ACC, SMA, paracingulate cortex, and superior frontal gyrus (peak $t = 5.51$, $p = .0118$) as well as regions in the frontal and central operculum; orbitofrontal cortex; inferior frontal gyrus; temporal pole; ventral medial prefrontal cortex; and subcortical areas such as the amygdala, putamen, parahippocampal gyrus, and pallidum (peak $t = 5.7$, $p = .0035$) (Figure 2B, upper panel). In contrast, the posterior insula showed significantly reduced connectivity with the bilateral STG, planum temporale, Heschl's gyrus, middle temporal gyrus, and parietal and central operculum (right cluster: peak $t = 5.31$, $p = .0018$; left cluster: peak $t = 4.59$, $p = .0091$). Furthermore, functional connectivity between the posterior insula and the bilateral precentral and postcentral gyrus, posterior and anterior cingulate cortex, precuneus, and SMA was significantly reduced in the schizophrenia group (peak $t = 5.4$, $p = .0018$) (Figure 2B, lower panel). Significant increases in connectivity were not found in the schizophrenia group ($p > .05$).

Main Effect of Laterality (Left vs. Right). Whereas the insula's cerebral connections showed an ipsilateral preference (anterior: peak $t = 16.8$, $p < .001$; posterior: peak $t = 17.9$, $p < .001$), the insula was more extensively connected to the cerebellum in the contralateral hemisphere (peak $t = 4.68$, $p = .025$) (Supplemental Figure S5).

Interaction Effect (Diagnosis × Subregion). A significant interaction was found between diagnosis and subregion. More specifically, the anterior insula in the schizophrenia group

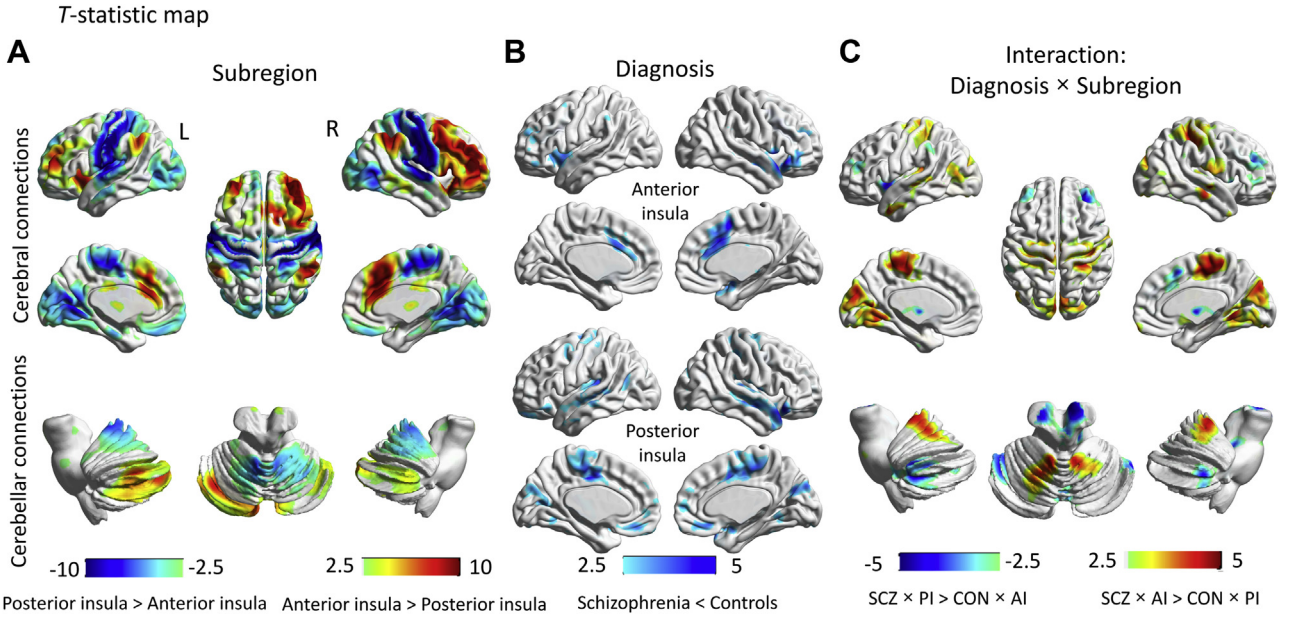

Figure 2. Altered resting-state functional connectivity of the anterior and posterior insular cortex in schizophrenia. Functional connectivity was mapped between the anterior and posterior insula subregions and all other gray matter voxels. Analysis of variance was then used to test the main effect of subregion (anterior vs. posterior) and diagnosis (schizophrenia vs. healthy comparison group) on insula connectivity at each gray matter voxel as well as the interaction between these two main effects. **(A)** Main effect of subregion. The anterior and posterior insula subregions showed unique connectivity patterns: the anterior insula was preferentially connected to frontolimbic areas and the posterior cerebellar lobe, whereas the posterior insula was characterized by increased connectivity with the parietotemporo-occipital cortices and the anterior cerebellar lobe. **(B)** Main effect of diagnosis. Schizophrenia was associated with significant reductions in connectivity between the insula and multiple cortical and subcortical areas, relative to the healthy comparison group. Connectivity increases were not evident in the schizophrenia group. **(C)** Interaction effect. A significant interaction between subregion and diagnosis was found: the anterior insula subregion showed increased connectivity with the somatosensory/motor and occipital cortex in the schizophrenia group, whereas connectivity between the posterior insula and prefrontal cortex and thalamus was increased in schizophrenia. AI, anterior insula; PI, posterior insula; SCZ, schizophrenia group; CON, healthy comparison (control) group. Clusters are colored according to *t* statistic. All images pertain to right insula.

showed increased connectivity with the precentral and postcentral gyrus (i.e., primary motor and somatosensory cortex; peak $t = 5.3$, $p = .001$), the occipital cortex and anterior lobe of the cerebellum (peak $t = 5.34$, $p < .001$), and a cluster located in the primary and secondary auditory cortices (peak $t = 4.99$, $p = .023$). In contrast, the posterior insula in the schizophrenia group showed increased connectivity to the prefrontal cortex (peak $t = 4.37$, $p = .04$), bilateral thalamus (peak $t = 5.27$, $p = .036$), and pons and posterior cerebellar lobes (peak $t = 5.75$, $p = .017$). Comparable findings were evident for the left insula (Supplemental Figure S4).

Interaction Effect (Diagnosis × Laterality). No significant interaction was found between diagnosis and laterality, suggesting that the disorder does not preferentially affect the left or right insula.

Diversity Curves

Diversity curves based on Fielder eigenvectors were mapped for each individual to continuously characterize the rate at which the insula's connectional profile to the rest of the cortex changed along the insula's rostrocaudal axis. Figure 3A shows group-averaged diversity curves for the schizophrenia (green curve) and healthy comparison groups (blue curve). Consistent with previous work (11), the diversity curves show a relatively constant slope, suggesting that the insula's connectivity profile varies gradually and continuously across its topography, from dorsoposterior to ventroanterior. The connectional diversity for the schizophrenia group was reduced in the anterior portion of the insula, indicated by a reduction in the diversity curve's slope (dashed box in Figure 3A); however, this between-group difference did not survive correction for multiple comparisons ($p < .05$) (Figure 3A). Group-consensus eigenmaps were projected to insula voxels in Montreal Neurological Institute space to enable anatomical visualization (Figure 3B).

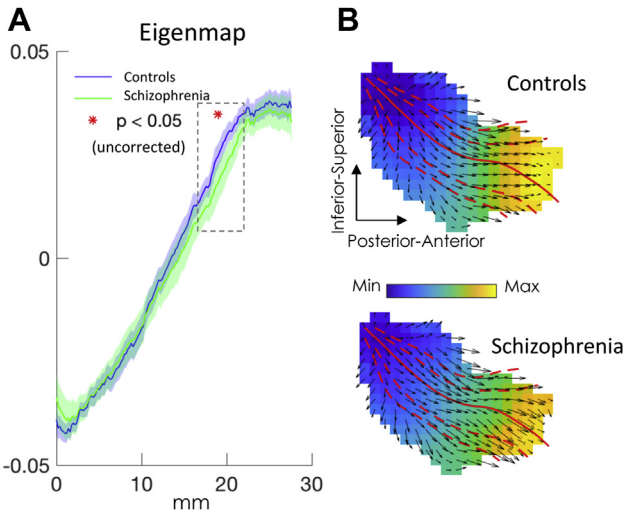

Figure 3. Diversity curves for the insular cortex in schizophrenia. **(A)** Group-averaged insula diversity curves for the schizophrenia (green curve) and healthy comparison (blue) groups. Dashed box indicates the portion of the diversity curve showing a significant reduction in the schizophrenia group ($p < .05$, uncorrected). Shading denotes 95% confidence intervals. **(B)** Group-consensus eigenmap values were mapped to the voxels of the insula to enable anatomical visualization of variation in connectional diversity for the schizophrenia (lower panel) and healthy comparison (upper) groups. The streamlines superposed onto the anatomical images were previously computed as part of a previous study (40). Diversity curves were mapped by projecting each individual's eigenmap onto the longest streamline (solid red line). Arrows indicate the gradient direction estimated for each voxel, and the lengths convey gradient magnitude. Max, maximum; Min, minimal.

Clinical Associations

Canonical correlation analysis identified a significant mode of covariance between interindividual variation in nine measures of disorder severity and two measures of insula functional connectivity ($r = .64$, $p = .03$, familywise error corrected) (Figure 4A). The canonical coefficients for each of the two functional connectivity estimates (Figure 4B) quantify the extent to which each of two connectivity estimates contributed to the significant mode of covariance. Figure 4C shows that IQ, GAF, and SOFAS are positively correlated with the significant mode of covariance, whereas SANS, PANSS-Negative, and duration of illness are negative. Therefore, higher insula functional connectivity with the ACC/SMA and STG was associated with higher IQ and psychosocial functioning (i.e., GAF and SOFAS) as well as less severity of psychotic symptoms (SANS and PANSS-Negative) and shorter duration of illness. Conversely, individuals with lower insula functional connectivity with the ACC/SMA and STG are more likely to show poorer cognitive performance and psychosocial functioning as well as more severe psychotic symptoms and longer illness duration.

In supplementary analyses, an exploratory canonical correlation analysis was conducted on the other six regions found to show significant functional connectivity reductions with the insula in the schizophrenia group (Supplemental Table S2). The exploratory canonical correlation analysis did not identify any significant associations between interindividual variation in symptoms and insula functional connectivity among this broader set of regions.

Interindividual variation in age, sex, and antipsychotic dose (chlorpromazine equivalent dose) did not associate with functional connectivity between the insula and eight clusters (Supplemental Table S2) found to show significantly reduced connectivity with the insula in the schizophrenia group. Supplemental Table S3 shows test statistics and p values for each of these 3 (age, sex, CPZ) × 8 (clusters) = 24 tests.

DISCUSSION

This study comprehensively characterized the functional connectivity architecture of the insular cortex in individuals with schizophrenia. In contrast to previous functional connectivity studies of the insula in schizophrenia, rather than treating the insula as a single, homogeneous region or considering only its anterior aspect, we specifically focused on investigating how the insula's connectivity with the rest of the cortex varied across its topography (i.e., across its spatial extent). The insular cortex is a connectionally diverse region, in that its most dorsoposterior extremity connects with markedly different cortical and subcortical areas compared with its ventroanterior aspect (11). In this study, we aimed to determine whether this connectional diversity was aberrant in

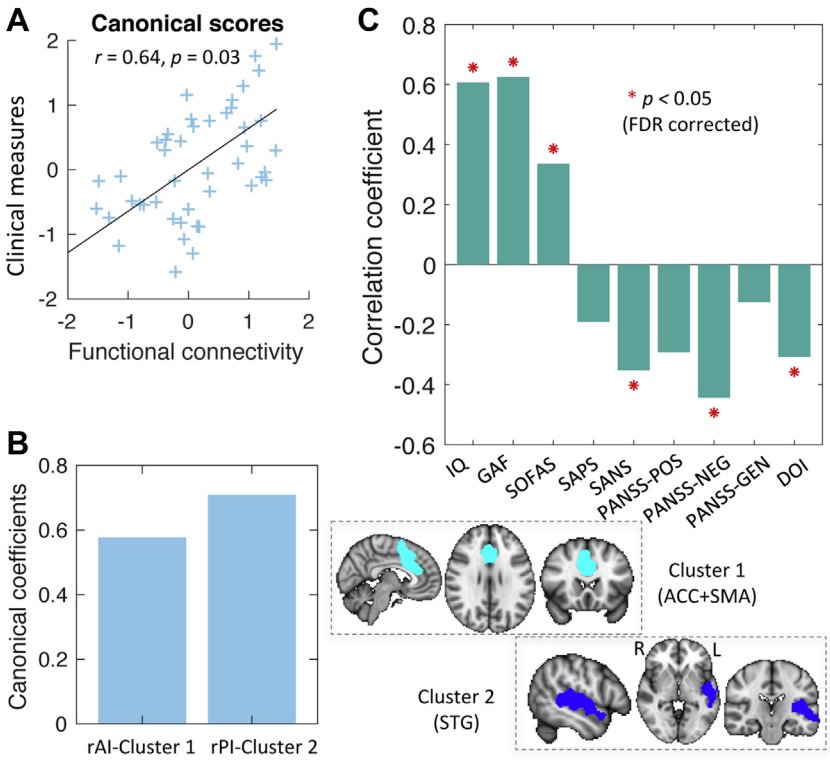

Figure 4. Association between insula functional connectivity and interindividual variation in clinical characteristics. Canonical correlation analysis identified a significant mode of covariance between interindividual variation in two measures of insula functional connectivity and nine measures of disorder severity, including measures of cognition, psychosocial functioning, psychotic symptoms, and duration of illness (DOI). **(A)** Scatter plot of canonical scores for the functional connectivity measures (horizontal axis) and measures of disorder severity (vertical axis). The mode of covariance remained significant after correction for multiple comparisons (r = .64, p = .03; familywise error corrected across all canonical correlation analysis modes with permutation testing, n = 10,000). **(B)** Bar plot showing canonical coefficients for the functional connectivity strengths corresponding to right anterior insula (rAI) and anterior cingulate cortex/supplementary motor area (ACC/SMA) (cyan cluster, .57) and right posterior insula (rPI) and superior temporal gyrus (STG) (blue cluster, .71). **(C)** Bar plot showing correlation coefficients between the nine measures of disorder severity and the significant canonical correlation analysis mode. Measures with positive canonical coefficients are positively correlated with the canonical correlation analysis mode of variance. Therefore, increased insula functional connectivity is associated with increased IQ, Global Assessment of Functioning (GAF), and Social and Occupational Functioning Assessment Scale (SOFAS) but decreased Scale for Assessment of Negative Symptoms (SANS), Positive and Negative Syndrome Scale–Negative (PANSS-NEG), and DOI. Red asterisks indicate measures that are significantly correlated with the canonical score (false discovery rate [FDR] corrected). PANSS-GEN, Positive and Negative Syndrome Scale–General Psychopathology; PANSS-POS, Positive and Negative Syndrome Scale–Positive; SAPS, Scale for Assesment of Positive Symptoms.

schizophrenia. We found that the connectivity profiles of the anterior and posterior insula were less well differentiated in schizophrenia and that the extent of reduction in connectivity between insula subregions and key regions of the salience network and auditory cortex were associated with interindividual variation in symptom severity.

Our initial clustering analysis suggested that the insula was most parsimoniously parcellated into two subregions in the majority of individuals with schizophrenia and healthy comparison individuals. Volumetrically, we found that the posterior insula subregion was significantly larger in the schizophrenia group, whereas the anterior insula was significantly reduced, relative to the healthy comparison group. It is important not to confuse these volumetric differences with previous studies reporting that schizophrenia is associated with reduced insula gray matter volume, particularly the anterior aspect of the insula (52–56). We used a common mask to delineate the insula in all individuals, and thus we did not model any potential interindividual variation in the entire insula volume. The volumetric differences found in the present study relate to segregation of functional connectivity (57–59) and are not necessarily related to gray matter morphology. In particular, the increased volume of the posterior insula subregion suggests that the characteristic connectivity profile of the posterior insula encompasses a larger share of the insula's entire volume in individuals with schizophrenia. This is at the expense of the anterior insula occupying a smaller volume.

We found that the differentiation between the anterior and posterior insula subregions was significantly reduced in the schizophrenia group relative to the healthy comparison group as quantified by the modularity Q value. This suggests that the connectional diversity of the insula is reduced in schizophrenia; namely, the network of areas with which the anterior insula connects is less well differentiated from the network of areas with which the posterior insula connects. In fact, differentiation between the anterior and posterior insula was only marginally higher than chance level in the schizophrenia group ($Q \approx 0.1$). Specifically, the anterior insula subregion preferentially connects to the frontolimbic and paralimbic areas and cerebellar nonmotor regions, whereas the posterior subregion shows extensive connectivity with the parietotemporo-occipital lobe and motor regions of cerebellum. This anterior-posterior subdivision is consistent with previous functional/diffusional parcellation studies in humans (10,12,13,15,60,61) and axonal tracing studies in macaques (62,63).

We found that functional connectivity between the insula and multiple cortical and subcortical areas as well as the cerebellum was significantly reduced in the schizophrenia group. Many of the areas affected comprise cognitive networks (20,21) and are associated with a wide range of cognitive, affective, and sensorimotor functions that are disturbed in schizophrenia (64–66). We found a significant interaction in the connectivity profiles between diagnosis (schizophrenia vs. healthy comparison group) and insula subregion (anterior vs. posterior). In the schizophrenia group, the anterior insula

connected with increased strength to regions that are normally connected with the posterior insula (i.e., the precentral/postcentral gyrus, occipital/parietal cortex, and anterior cerebellar lobe). Conversely, the posterior insula showed increased connectivity in schizophrenia with regions that are preferentially connected with the anterior insula in healthy individuals (i.e., prefrontal cortex, thalamus, and posterior cerebellar lobe). This interaction effect suggests that the connectivity profiles of the anterior and posterior insula are less well differentiated in schizophrenia, consistent with lack of insula connectional diversity in the disorder.

Using the recently developed concept of a diversity curve (11), we found that the insula's connectional diversity varied gradually along a rostrocaudal axis in both the schizophrenia and the healthy comparison groups. Furthermore, we found no evidence of a discrete boundary between putative insula subregions, which would have been evidenced by an abrupt change in the diversity curve slope. It is important to note that while we did not find any evidence of discrete insula subregions, modeling the insula's connectional diversity with subregions can nevertheless provide meaningful insight. Discrete approximations can simplify continuous systems, which, in turn, facilitates inference and more intuitive interpretations. For example, mapping of neural connectomes mandates the delineation of discrete subregions to serve as network nodes (67). Anterior and posterior insula subregions can thus represent distinct nodes in connectomic studies. Insula diversity curves for the schizophrenia group were reduced within an anterior portion of the insula; however, this between-group difference did not survive correction for multiple comparisons. Within the anterior portion of the insula implicated (dashed box in Figure 3A), the slope of the diversity curve showed a trend toward reduction in the schizophrenia group, which may suggest that this portion of the insula is abnormally homogeneous in the disorder.

Importantly, we found that the reduced functional connectivity between anterior insula and ACC including SMA, known as the salience network, and the functional connectivity between posterior insula and the language and auditory cortex (STG, Heschl's gyrus, planum temporale, and Wernicke's area) were associated with poor clinical outcomes, including cognitive impairment and general social and occupational functioning as well as the severity of symptoms, particularly negative symptoms. Our findings support the emerging hypothesis that the salience network is significantly involved in the pathophysiology of psychosis (7,46,68,69), particularly with respect to disturbances in the integration of sensory perception facilitated by the posterior insula (68). Of note, reduced insula functional connectivity was also associated with longer illness duration, suggesting progressive deterioration of connectivity over the course of illness, although longitudinal neuroimaging would be required to confirm this suggestion. We therefore conclude that impaired functional connectivity between the insula and these key regions may be a determinant of disorder severity.

Limitations

First, our sample is moderate in size, and our fMRI data are of poorer spatial and temporal resolution compared with the data used in our previous analysis of the insula in healthy individuals (11). Second, although antipsychotic dose was not correlated with any of the functional connectivity effects reported in this study, medication cannot be excluded as a potential confound (70–72). Interestingly, analysis of functional connectivity during a salience attribution task indicated that schizophrenia-related reductions in connectivity between the insula and ACC were evident only in the subgroup of untreated individuals with first-episode psychosis and not in the medicated subgroup (72). However, these findings require replication, given the small size of the medicated subgroup. Finally, IQ was significantly reduced in the schizophrenia group, representing a potential confounding effect on the neuroimaging findings reported in our study. However, it is generally difficult to disentangle low IQ from the illness because of the widespread neurocognitive deficits evident in the majority of individuals with schizophrenia (65), and controlling for IQ as part of statistical inference may be considered inappropriate for this reason (73).

Conclusions

Using resting-state fMRI, we comprehensively characterized functional connectivity across the topography of the insular cortex in individuals with schizophrenia. We represented the insula's connectional diversity using discrete subregions (anterior and posterior) as well as a continuum of gradual variation. We found that the anterior and posterior insula subregions were connected to distinct cortical and subcortical regions in the healthy comparison individuals. However, in the schizophrenia group, this distinction in the connectivity profiles of the anterior and posterior insula was significantly reduced, suggesting a lack of segregation between these two subregions in the disorder. This was evidenced by a significant interaction between diagnosis and subregion, whereby the anterior insula showed increased connectivity with somatosensory/motor cortices, occipital/parietal cortices, and motor regions of cerebellum in schizophrenia, whereas the connectivity between the posterior insula and prefrontal cortex, thalamus, and cerebellar nonmotor region was stronger. Insula diversity curves suggested that the anterior insula may be abnormally homogeneous in the schizophrenia group. Finally, we found that impaired insula functional connectivity was associated with interindividual variation in cognitive deficits, psychotic symptoms, and psychosocial functioning. We conclude that the anterior and posterior insula show differential connectivity profiles, but this differentiation is reduced in schizophrenia, resulting in reduced connectional diversity across the insula and possibly contributing to the psychotic symptoms of schizophrenia. Future research may focus on mapping the subregional architecture of the insular cortex as a function of age using longitudinal neuroimaging data. This would establish the age range during which the connectivity profiles of the anterior and posterior insula significantly differentiate and whether this differentiation is evident before the onset of psychosis.

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ARTICLE INFORMATION

From the Melbourne Neuropsychiatry Centre (YT, AZ, CB, CP), Department of Psychiatry, The University of Melbourne and Melbourne Health, Carlton South; Department of Biomedical Engineering (AZ) and Centre for Neural Engineering (CP), The University of Melbourne; Florey Institute for Neuroscience and Mental Health (CP), Parkville; Cooperative Research Centre for Mental Health (CB, IE, CP), Carlton, Victoria, Australia; Departments of Medical Genetics (CB), Psychiatry (CB), and Physiology and Pharmacology (CB), University of Calgary, Calgary, Alberta, Canada; and Institute of Psychiatry, Psychology and Neuroscience (IE), King's College London, London, United Kingdom.

Address correspondence to Ye Tian, M.D., Melbourne Neuropsychiatry Centre, Level 3, Alan Gilbert Building, 161 Barry Street, The University of Melbourne, Carlton South, Victoria 3053, Australia; E-mail: yetianmed@gmail.com.

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