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Abnormal Insula Functional Network is Associated with Episodic Memory Decline in Amnestic Mild Cognitive Impairment

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Abstract

Abnormalities of functional connectivity in the default mode network (DMN) recently have been reported in patients with amnestic mild cognitive impairment (aMCI), Alzheimer's disease (AD) or other psychiatric diseases. As such, these abnormalities may be epiphenomena instead of playing a causal role in AD progression. To date, few studies have investigated specific brain networks, which extend beyond DMN involved in the early AD stages, especially in aMCI. The insula is one site affected by early pathological changes in AD and is a crucial hub of the human brain networks. Currently, we explored the contribution of the insula networks to cognitive performance in aMCI patients. Thirty aMCI and 26 cognitively normal (CN) subjects participated in this study. Intrinsic connectivity of the insula networks was measured, using the resting-state functional connectivity fMRI approach. We examined the differential connectivity of insula

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networks between groups, and the neural correlation between the altered insula networks connectivity and the cognitive performance in aMCI patients and CN subjects, respectively. Insula subregional volumes were also investigated. AMCI subjects, when compared to CN subjects, showed significantly reduced right posterior insula volumes, cognitive deficits and disrupted intrinsic connectivity of the insula networks. Specifically, decreased intrinsic connectivity was primarily located in the frontal-parietal network and the cingulo-opercular network, including the anterior prefrontal cortex (aPFC), anterior cingulate cortex, operculum, inferior parietal cortex and precuneus. Increased intrinsic connectivity was primarily situated in the visual-auditory pathway, which included the posterior superior temporal gyrus and middle occipital gyrus. Conjunction analysis was performed; and significantly decreased intrinsic connectivity in the overlapping regions of the anterior and posterior insula networks, including the bilateral aPFC, left dorsolateral prefrontal cortex, dorsomedial prefrontal cortex, and anterior temporal pole was found. Furthermore, the disrupted intrinsic connectivity was associated with episodic memory (EM) deficits in the aMCI patients and not in the CN subjects. These findings demonstrated that the functional integration of the insula networks plays an important role in the EM process. They provided new insight into the neural mechanism underlying the memory deficits in aMCI patients.

Keywords

insula network; episodic memory; cognition; amnestic mild cognitive impairment; functional connectivity fMRI

1. Introduction

Disconnected neural network connectivity between brain regions increasingly has been proposed as a key characteristic underlying memory deficits in amnestic mild cognitive impairment (aMCI), which has a high risk of conversion to Alzheimer's disease (AD) (Bai et al., 2009a; Bai et al., 2009b; Sorg et al., 2007). These disconnections within the neural circuits of the hippocampus, amygdala, medial temporal lobe and default mode network (DMN) were recently thought to be recruited in the progress of aMCI converting to AD (Bai et al., 2009a; Bai et al., 2009b; Binnewijzend et al., 2011; Greicius et al., 2004; Li et al., 2002; Xie et al., 2012; Zhang et al., 2010). Also, functional integration abnormalities between brain areas, which are primarily reflected by the intrinsic functional connectivity, were demonstrated to be the neural basis of impaired cognition in aMCI patients (Bai et al., 2009a; Bai et al., 2009b; Sorg et al., 2007; Xie et al., 2012). Specifically, DMN functional connectivity abnormalities recently have been reported when distinguishing AD subjects from the normal population (Greicius et al., 2004), monitoring the development of aMCI (Bai et al., 2011) and tracking clinical deterioration in AD (Damoiseaux et al., 2012). However, it is noteworthy that this altered functional connectivity is evident in other psychiatric diseases (Broyd et al., 2009). As such, there is a possibility that DMN functional connectivity abnormalities may be epiphenomena instead of playing a causal role in AD progression. Other neural networks, which extend beyond the DMN, may be involved in AD progression (Agosta et al., 2012). To date, specific brain regions or networks involved in the early stages of AD, especially in aMCI, still remain unclear. Further, the neural mechanism

underlying the disease process in which the disrupted neural networks influence the cognitive decline has not been fully understood.

We hypothesize that the insula and its network could play an important role in the progression to AD. Previous studies showed that the insula was affected in AD development, and insula atrophy effectively discriminated AD patients from the normal population (Fan et al., 2008). As a crucial hub of the human brain network, the insula is anatomically connected to a wide range of cortical, limbic and paralimbic structures through widely afferent and efferent projections. It is functionally implicated in higher-order cognition, emotion, autonomic and sensory processes at the system level (Allen et al., 2008; Naqvi et al., 2007). Accumulating evidence also has shown that the insula subregions mediate the diverse information processes usually linked to emotion or the regulation of the body's homeostasis via afferent input and efferent output from the frontal-parietal-temporal lobes and limbic systems (Sridharan et al., 2008; Taylor et al., 2009). Specifically, the anterior insula (aIns) often aids the global representation of the body state through integrated homeostatic afferent signals from the posterior insula with emotional salience (Seeley et al., 2007). It also is responsible for the transmission of information between the DMN and the cognitive control networks, which are coupled in competitive interaction in cognitive information processing (Sridharan et al., 2008). The posterior insula (pIns) is thought to be primarily associated with the physical information of the body, including pain, thirst, and hunger (Taylor et al., 2009). In addition, it was recently noted that the insula subregional networks in the human brain presented different connectivity patterns and appeared to be implicated across a variety of cognitive and emotional domains (Peltz et al., 2011; Taylor et al., 2009). Nevertheless, in the aMCI patients, the role of the insula networks, and therefore, the insula itself, has received much less attention than other brain structures affected in the episodic memory (EM) process. Based on these findings, we hypothesized that disrupted insula network connectivity would remarkably deteriorate EM performance in aMCI patients, and thus, the level of functional integration of the insula network could predict the progression of aMCI to AD.

In the current study, therefore, the specific objectives were to examine the differential connectivity pattern of the insula subregions in CN subjects and aMCI patients. Specifically, we analyzed whether the breakdown connectivity of the insula networks was implicated in the cognitive decline of aMCI subjects.

2. Methods and Materials

2.1 Participants

Forty-three aMCI patients and 33 CN healthy subjects were recruited through community health screening and newspaper advertisements (all subjects were Chinese and right handed). The Research Ethics Committee of Affiliated ZhongDa Hospital, Southeast University, approved the study and written informed consent was obtained from all participants. Thirteen aMCI and seven CN subjects were excluded because of excessive motion artifacts (i.e., exceeding more than 1-mm translational movement or more than 1° rotational movement) and/or incomplete image scans. This left 30 aMCI and 26 controls for further analysis.

All subjects underwent comprehensive neuropsychological assessments, including the Mini-Mental State Examination (MMSE), Rey Auditory-Verbal Learning Test-Delayed Recall (AVLT-DR), Rey-Osterrieth Complex Figure Test-Immediately Recall (ROCFT-IR), Trail-Making Tests A and B, Digit Span Test and Symbol Digit Modalities Test. All aMCI patients met the diagnostic criteria described previously (Bai et al., 2009a; Petersen, 2004). Exclusion criteria were: regular use of drugs or medications, a history of neurological or psychiatric illness, and contraindications to MRI scanning. Control subjects were required to have a Clinical Dementia Rating of 0, MMSE score ≥ 26, AVLT-DR score > 4, and eight or more years of education. The inclusion and exclusion assessment was performed by two experienced neuropsychiatric physicians who administered a structured interview to subjects and their informants.

2.2 MRI Acquisition

Imaging was performed, using a General Electric 1.5 Tesla scanner (General Electric Medical Systems, USA) with a homogeneous birdcage head coil. High-resolution spoiled gradient-recalled echo (SPGR) 3D axial images were acquired for anatomical reference. The parameters were: repetition time (TR)/echo time (TE) = 9.9ms/2.1ms; flip angle (FA) = 15°; acquisition matrix = 256 × 192; field of view (FOV) = 240 × 240 mm^2; thickness = 1.0 mm. Axial resting-state (no cognitive tasks were performed, eyes were closed, and ears were occluded) functional connectivity MRI (R-fMRI) datasets were obtained in seven minutes and six seconds with a single-shot gradient-recalled echo-planar imaging (GRE-EPI) pulse sequence. The R-fMRI imaging parameters were: TR/TE = 3s/40ms; FA = 90°; acquisition matrix = 64 × 64; FOV = 240 × 240 mm; thickness = 4.0 mm.

2.3 Functional Image Analysis

2.3.1 Imaging Preprocessing—R-fMRI data analysis was carried out, using Analysis of Functional NeuroImages (AFNI) software (http://afni.nimh.nih.gov/afni). Briefly, motion correction was performed by volume registration of the R-fMRI data (3dvolreg). Then, detrending was carried out with Legendre polynomials (3dDetrend). Possible contamination from the signals in white matter, CSF, global signal and six-motion vectors was regressed out from the whole brain. Then, a band-pass filter was applied to keep only low-frequency fluctuations within the frequency range of 0.015 Hz and 0.1 Hz.

2.3.2 Identification of Seed Regions—Four subdivisions of the insula cortex were manually drawn on the coronal slices, with reference to the sagittal and axial slices, on the structural images of individual subject, using AFNI software. The boundaries of each subdivision were defined in the references previously described (Allen et al., 2008; Kim et al., 2003). Briefly, the insula is enclosed by three circular sulci: the superior circular insular sulcus, the inferior circular insular sulcus and the orbitoinsular sulcus. On the coronal view, the anterior boundary of the insula adjoins the superior circular insular sulcus and the orbitoinsular sulcus. The posterior boundary of the insula was defined by the superior and inferior circular insular sulci. The mesial boundary of the insula was defined by an arbitrary line, which linked the deepest extents of the two ends of the superior and orbitoinsular circular sulci. The lateral surface of the insula is the operculum, which is formed from parts of the enclosing frontal, temporal and parietal lobes. The anterior and posterior part of insula

were defined by the central sulcus of the insula, which was identified and marked on the contiguous coronal and sagittal slices of the brain through the insula region. Operationally, a mouse-controlled cursor traced relevant coronal slices. Boundaries were displayed in real time on these orthogonal MRI slices with AFNI software. The insula was successfully traced, as demonstrated in Fig. 1A, which shows a sagittal view of the traced insula.

2.3.3 Intrinsic Functional Connectivity Analysis—The four subinsula regions of interest (ROIs) were used to construct insula subregional networks, as illustrated in Fig. 1A. They were employed as "seeds" for functional connectivity analysis. For each subject, the average time course of each seed was extracted from the functional EPI images, and then, was correlated with the time courses of whole-brain voxels, using Pearson cross-correlation. Because the spatial resolutions of the SPGR images (1*1*1 mm) and EPI images (3.75*3.75*4 mm) were different, only those voxels in the EPI images that were occupied at least 50% by ROI voxels masked on the 3D SPGR images were included in the voxel time course analysis (Xie et al., 2011). Next, the correlation map was converted to a *z*-value map [Fisher r-to-z transformation, $m = 0.5*\ln(1+r)/(1-r)$]. Finally, the data was spatially normalized to the Talairach template image, resampled to 2-mm isotropic voxels and smoothed with a 6-mm Gaussian kernel. The individual connectivity map was obtained.

2.4 Statistical Analysis

2.4.1 Behavioral Data—The composite score analysis is widely used in data processing to increase statistical power by reducing random variability, and floor and ceiling effects (Schneider et al., 2007; Wilson et al., 2010). The composite scores for cognitive measure were obtained in a two-step process: First, the raw scores from each test for each subject were transformed to *z* scores with reference to the means and standard deviations of each test for all subjects. Second, the composite scores were calculated by averaging the *z* scores of the individual tests related to the following: EM (two tests, including the AVLT-DR and ROCFT-IR), Executive Function (two tests, including Trail Making Tests A and B); Perceptual Speed (Symbol Digit Modalities Test) and Working Memory (Digit Span Test). The composite scores of aMCI and CN subjects were compared, using a Student's *t* test. MMSE was performed for description and diagnostic classification, but not used in the composite measures. We used the Kolmogorov-Smirnov Test to confirm that our data has a normal distribution ($p = 0.149 > 0.05$).

2.4.2 Volumetric Comparison of the Insula Subregions—Optimized voxel-based morphometry (VBM) analysis was performed, using the VBM8 toolbox in SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8) to identify the gray matter (GM) atrophy of insula in aMCI subjects relative to the CN group. The individual T1-weighted images for all subjects were segmented into GM, white matter (WM) and cerebral spinal fluid (CSF). The summation of these volumes equals the intracranial volume. The segmented GM was then normalized and smoothed at 6-mm full-width half maximum. The individual volumes of the insula subregions corresponding to the GM volume (modulated images) were extracted to determine the GM volume of each insula subregion, and then, were entered into a 2×2 analysis of variance (ANOVA) for group comparison in commercially available statistical software (SPSS 16.0; SPSS Inc, Chicago, Illinois) after controlling the intracranial volumes.

2.4.3 Group-Level Intrinsic Connectivity Analysis and Behavioral Significance

—Individual connectivity maps of insula subregions for each subject were analyzed with a random-effects one-sample *t* test to identify voxels. This showed a significant positive or negative correlation with the seed time series, and the pattern of each insula subregional network for CN and aMCI groups was obtained, respectively ($p < .01$, corrected with AlphaSim, cluster size >1400 mm^3). To examine the group difference of the aIns and pIns network connectivity across all subjects, a voxelwise 2×2 ANOVA (group × side) analysis was performed (3dRegAna, AFNI) ($p < .05$, corrected with AlphaSim, cluster size > 4048 mm^3). Then, conjunction analysis (the main effects of the group on the aIns network overlapped with the main effects of the group on the pIns network from ANOVA analysis) was employed to find the overlapping regions, which were connected with bilateral aIns and pIns regions. Next, the average functional connectivity strength in the distinct overlapping region that was connected to these four seeds was extracted from each individual subject, using masks generated from the conjunction analysis.

To investigate the behavioral significance of altered connectivity of the insula networks, a multivariate regression analysis was employed (*3dRegAna*, AFNI) between the averaged connectivity in each overlapping region and the EM scores in the aMCI subjects and CN subjects, respectively. The following is the equation (Goveas et al., 2011; Xie et al., 2012):

$$m_i = \beta_0 + \beta_1 * \mathrm{EM} + \beta_2 * \mathrm{Volume}_{\mathrm{GM}} + \beta_3 * \mathrm{Gender} + \beta_4 * \mathrm{Education} + \beta_5 * \mathrm{Age} \quad [1]$$

Where m_i is the *m* value of *i*th voxel across group subjects, β_0 is the intercept of the straight line fitting in the model; β_1 and β_2 are the effects of the EM scores and gray matter volumes of the right posterior insula subregion (decreased gray matter volumes in this region were identified in aMCI subjects; see below), respectively; β_3, β_4, and β_5 are the effects of gender, education, and age in the aMCI group subjects, as covariates of no interest in the above linear regression model. Similarly, we calculated the correlation between Executive Function, Perceptual Speed, Working Memory and *m* values on each overlapping region, using the above approach.

3. Results

3.1 Subject Characterization

Demographic information and clinical evaluations for all participants are shown in Table 1. No significant difference in age or gender was noted between the aMCI and CN groups. Significant declines in EM, Executive Function, Perceptual Speed and Working Memory were found in aMCI patients, compared to CN subjects.

3.2 Volumetric Comparison of Insula Subregions

ANOVA analysis revealed a significant difference of volumes in the posterior insula subregions ($F = 2.22$, $p = .027$) but no difference in the anterior insula subregional volumes. Post-hoc analysis further identified only decreased volumes in the right posterior insula subregion in aMCI subjects, compared to those in the CN group ($p < .05$), as shown in

Figure 1B. In addition, whole-brain voxelwise VBM analysis also identified the GM atrophy in the right posterior insula subregion in aMCI subjects relative to the CN group.

3.3 Intrinsic Connectivity of the Insula Subregional Networks in aMCI Subjects and CN Subjects

Each insula subregional network is composed of a positive network and an anticorrelated (negative) network. Specifically, for the CN subjects, the left aIns showed significantly positive connectivity with the bilateral operculum, dorsolateral prefrontal cortex (DLPFC), inferior frontal gyrus (IFG), presomatomotor area (preSMA), anterior cingulate cortex (ACC), inferior parietal cortex (IPC), caudate, putamen, thalamus, and hippocampus. Significantly anticorrelated connectivity was evident as related to the bilateral ventral-medial prefrontal cortex (vmPFC), posterior cingulate cortex (PCC), precuneus, and cerebellum. The positive right aIns network consisted of the bilateral operculum, IFG, preSMA, dorsal ACC (dACC), middle cingulate cortex (MCC), IPC, caudate, putamen, thalamus, and hippocampus. The anticorrelated right aIns network included the bilateral PCC, precuneus and the cerebellum. The positive left pIns network consisted of the bilateral operculum, IFG, preSMA, IPC and subcortical regions, including the caudate, putamen, thalamus, and hippocampus. The anticorrelated left pIns network included the bilateral superior frontal gyrus (SFG), right DLPFC, PCC, precuneus and the cerebellum. The positive right pIns network was seen in the bilateral operculum, IFG, preSMA, thalamus and hippocampus. Anticorrelated regions were not found in the right pIns network after correction, as shown in Figure 2A. For aMCI subjects, the patterns of the insula subregional networks were presented in Figure 2B.

3.4 Differential Intrinsic Connectivity in Anterior and Posterior Insula Networks, Overlapping Regions and Behavioral Significance

We examined the group differences of connectivity specificity in the aIns and pIns networks. As shown in Figures 3A and 3B, several regions consistently showed abnormal connectivity in aMCI patients. In the aIns network, decreased positive connectivity in aMCI patients was found in the frontal-parietal-occipital-temporal system, including the bilateral anterior prefrontal cortex (aPFC), VMPFC, DLPFC, IFG, cuneus, and anterior temporal pole (aTP). This also was evident in the cingulate-opercula network, including the dACC and operculum, and subcortical regions, including the bilateral caudate and thalamus. Decreased anticorrelated (negative) connectivity was seen in the bilateral posterior superior temporal gyrus (STG), PCC, and middle occipital gyrus (MOG) in aMCI patients. In the pIns network, decreased connectivity was also seen in aMCI patients in the brain regions related to the bilateral aPFC, IFG, cuneus, left dorsomedial prefrontal cortex (DMPFC), angular gyrus and aTP. Increased connectivity in aMCI patients was located in the dorsal frontal cortex (dFC), preSMA, and right anterior MCC. Conjunction analysis found overlapping regions with decreased connectivity in the bilateral aPFC and DMPFC, left DLPFC and aTP. No increased connectivity was evident in the overlapping regions (Figure 3C). Additionally, no significant interaction between the group and the side, as related to the insula subregional functional connectivity, was found.

Further, the multivariate regression analysis was performed to demonstrate that the degree of intrinsic connectivity strength in the overlapping regions was positively correlated with EM scores in aMCI patients, as shown in Figure 4. However, no correlation was evident with respect to Executive Function, Perceptual Speed and Working Memory in aMCI patients. Additionally, we did not find a significant correlation between the intrinsic connectivity strength in the overlapping regions with EM, Executive Function, Perceptual Speed, and Working Memory in CN subjects.

4. Discussion

The current study results demonstrate that EM impairment in aMCI patients is associated with insula network disruption. These findings strongly support our hypothesis that disrupted insula network connectivity would significantly contribute to EM impairment in aMCI patients. They provide new insight into the neural mechanism underlying the memory deficits in aMCI patients.

4.1 Gray Matter Atrophy of Right Posterior Insula in aMCI Subjects

In the current study, we found GM atrophy in the right pIns in aMCI subjects, compared to CN subjects. Insula GM atrophy, except for atrophy in the medial temporal lobe structures, was frequently reported in previous studies that focused on aMCI or AD patients (Caroli et al., 2010; Davatzikos et al., 2011; Fan et al., 2008; Hamalainen et al., 2007; Karas et al., 2004; Spulber et al., 2012). Importantly, GM atrophy in cerebral regions is associated with a higher risk of subsequent progression to AD (Caroli et al., 2010; Davatzikos et al., 2011; Fan et al., 2008; Karas et al., 2004; Spulber et al., 2012). More recently, a two-year longitudinal study that used the VBM approach demonstrated that insula atrophy is primarily evident in progressive MCI subjects, but not in stable MCI subjects (Spulber et al., 2012). These findings indicate that insula atrophy may be a biomarker to differentiate progressive MCI and stable MCI in the early stages of AD, and to monitor the progression of aMCI converted to AD (Spulber et al., 2012).

4.2 Disrupted Intrinsic Connectivity of the Insula Network in aMCI Patients Compared to CN Subjects

In CN subjects, the insula networks, as expected, presented similar patterns with previous findings (Ebisch et al., 2011; Seeley et al., 2007; Taylor et al., 2009). However, in aMCI subjects, the disrupted intrinsic connectivity of the insula networks was found in the operculum, frontal, parietal, and temporal occipital lobes, and subcortical regions. Previous anatomical studies have shown that structurally reciprocal projections exist between the insula and the regions indicated above (Butti and Hof, 2010), which functionally encompass brain networks that are implicated in human perception, cognition and attention processing (Craig, 2010). In addition, the recruitment of the insula networks also is associated with signal processes reflecting the homeostasis and interoceptive awareness through subsequent emotional arousal evaluation (Butti and Hof, 2010; Menon and Uddin, 2010). This view was already supported by the well-known predictions of somatic marker hypothesis and the James-Lange theory for emotional awareness: the insula, as an important component of the neural circuits underlying the emotional awareness, plays a crucial role in transmitting the

homeostatic information to subjective feelings (Craig, 2002; Ebisch et al., 2011). Therefore, the disrupted insula network connectivity in aMCI patients may reflect the decoupling of functional brain networks underlying interoception, self-awareness and appropriate emotional and cognitive responses (Craig, 2010; Goldstein et al., 2009). Furthermore, previous studies have shown that brain regions in the frontal-parietal cortex and cingulate-operculum system are central to the cognitive control process (Dosenbach et al., 2008; Olsson and Ochsner, 2008). The differential connectivity in the neural circuits within the insula networks may indicate that symptomatic characteristics such as memory deficits occur in the early stages of AD. Currently, we also found decreased anticorrelated connectivity in the visual-auditory pathway (posterior STG, MOG) in aMCI patients. Such abnormal connectivity may alter semantic memory network, as recently described (Binder et al., 2009; Etkin et al., 2009) and this could be a result from the compensatory mechanism. The neuroanatomical and functional demonstration of the insula networks is associated with high-order cognitive processes, thereby indicating the important role of the insula networks on the neural mechanism underlying cognitive impairment in aMCI patients.

4.3 Underlying the Mechanism of Cognitive Decline Implicated in the Insula Networks

It is widely accepted that high-order cognition is not attributed to isolated brain regions. Rather, it results from the dynamic interaction of distributed brain areas operating at the large-scale neural-network level (Mesulam, 2009; Pessoa, 2008). In the current study, the fact that the intrinsic connectivity of the insula networks was positively correlated with the EM scores suggests intrinsic connectivity of the insula networks can predict EM performanc*e* in aMCI patients. Understanding the underlying mechanism of this fact is critical for gaining new insight into the neural basis of the conversion of aMCI to AD. On the one hand, studies frequently reported that MCI affected many aspects of emotion processing, which resulted in the high occurrence of negative emotional symptoms, especially depression, in MCI (Henry et al., 2009; Steffens et al., 2006; Xie et al., 2012). Moreover, high-order cognitive brain function interacts in a complicated manner with emotion through dynamic coalitions of neural networks among brain regions (Pessoa, 2008; Xie et al., 2012). Thus, the disturbance of emotional awareness regulation in aMCI patients will have a negative impact on the process of cognition, and ultimately lead to cognitive decline. On the other hand, the operculum, as a core component of the cingulate-opercula control network, is responsible for maintaining task sets (Dosenbach et al., 2008) and switching information between the executive control network and the DMN (Seeley et al., 2007). Another important region, the aPFC, which is well developed in humans, is particularly sensitive to tasks that are primarily involved in the process of emotion and intentional states, and monitors the information transfer, which is involved in metacognitive reflective awareness regulation (Olsson and Ochsner, 2008). Other overlapping regions, including the DLPFC, DMPFC and aTP are primarily involved in the process of cognition and memory (Dosenbach et al., 2008; Olsson and Ochsner, 2008). Therefore, impaired functional connectivity of the neural circuits between the insula and these regions is likely to interfere with information maintenance, transfer, and feedback and this further result in cognitive decline. Furthermore, our results in Figure 4 show that the more severe the EM impairment, the more negative connectivity between insula and these overlapping regions in aMCI patients. Additionally, the voxelwise linear regression analyses were separately

performed for the anterior and posterior insula networks correlated with episodic memory scores. It was found that the correlation maps were very similar with Figures 3A and 3B, indicating that the functionally disrupted regions in both anterior and posterior networks had behavioral significance. In addition, both networks not only correlated with the episodic memory scores individually, but they also correlated interactively through the common regions of the bilateral aPFC, DMPFC, left DLPFC and aTP, as indicated through the conjunction analysis shown in Figure 3C. These findings directly suggest that the functional integration of the insula networks engages in the process of episodic memory in aMCI patients.

Several limitations of this study should be noted. First, we did not directly measure subjective emotional feelings for our study subjects. It would be useful to examine subjective emotional feelings in aMCI patients and comparison subjects in future studies. Second, the cardiac and respiratory signals were not recorded. These may have limited the generalization of our findings. However, results in several studies were reported without the cardiac and respiratory signal removal. This did not significantly influence the R-fMRI findings (Fox et al., 2009; Sheline et al., 2010). Moreover, the low-frequency respiratory volume and cardiac rate displayed a significant shared variance with the global signal. Implementing the global signal regression proved to be a practical way to overcome this limitation (Chang and Glover, 2009).

5. Conclusion

In conclusion, the intrinsic connectivity of the insula network was disrupted in aMCI patients. This disrupted intrinsic connectivity was associated with EM scores. These findings strongly suggest that insula networks play an important role in the functional integration of the EM process. They provide new insight into the neural mechanism underlying the memory deficits in aMCI patients.

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Highlights

- Reduced cognitive performances and right posterior insula volumes;
- Disrupted intrinsic connectivity of insula subregional networks;
- Overlapping regions with decreased insula subregional network connectivity;
- Disrupted connectivity strengths were correlated with episodic memory deficits;
- Disrupted connectivity strengths were not correlated with other cognitive deficits.

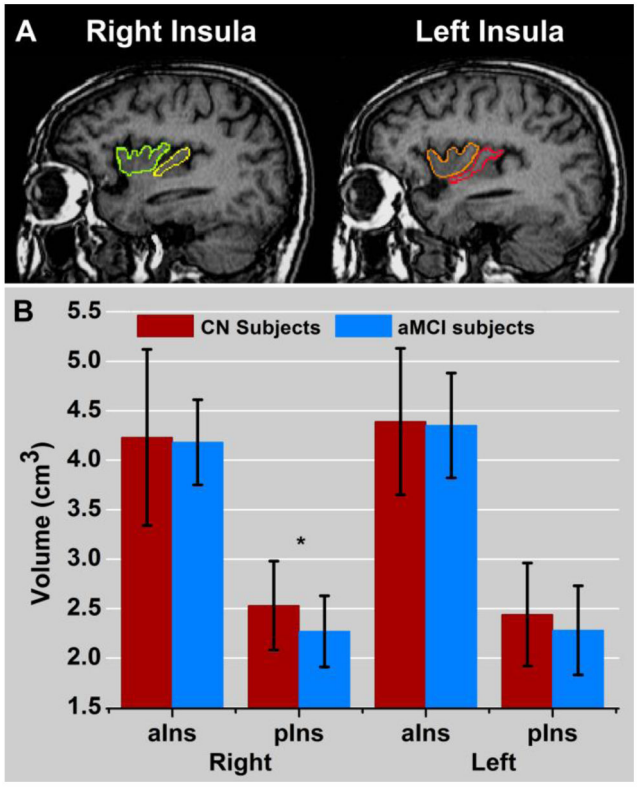

Figure 1. Volumetric Comparison of Insula Subregions between aMCI and CN Groups

A) Slice view of the insula subregions. Green color: right anterior insula; Yellow color: right posterior insula; Orange color: left anterior insula; Red color: left posterior insula color. **B**) Decreased volume in the right posterior insula subregion in aMCI subjects compared to those the CN group. *$p < 0.05$. Bars represent mean values; error bar, standard deviation of the mean. Abbreviations: aMCI, amnesic mild cognitive impairment; CN, cognitively normal; aIns, anterior insula; pIns, posterior insula.

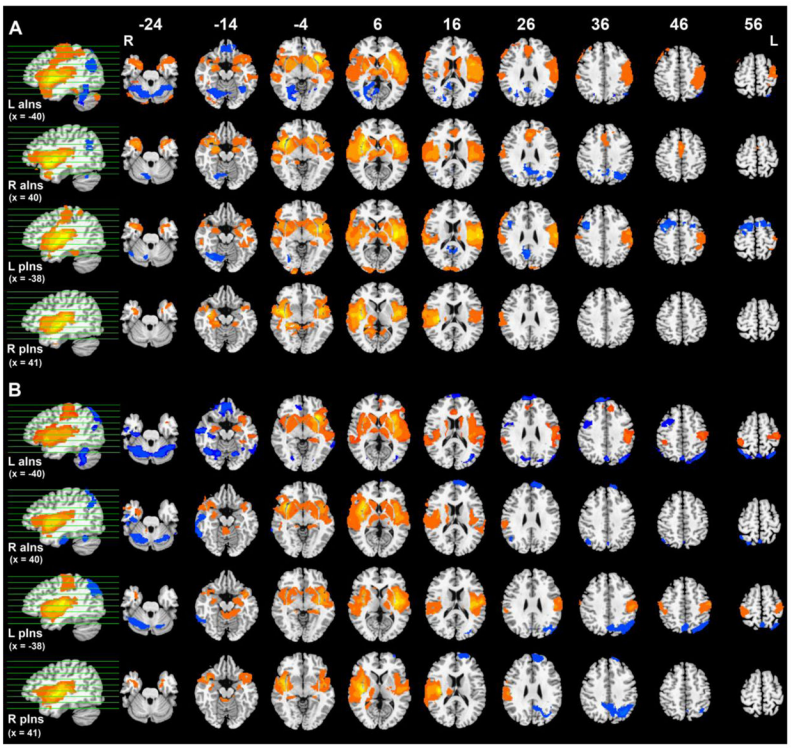

Figure 2. Resting-State Functional Connectivity Pattern between the Distinct Insula Subregions and the Rest of Brain ($p_{corrected} < 0.01$) in aMCI and CN Groups

This result shows the different connectivity patterns of insula subregional networks. A: CN group; B: aMCI group. Warm color indicates positive correlation and blue color indicates negative correlation. Abbreviation: L aIns, left anterior insula; R aIns, right anterior insula; L pIns, left posterior insula; R pIns, right posterior insula.

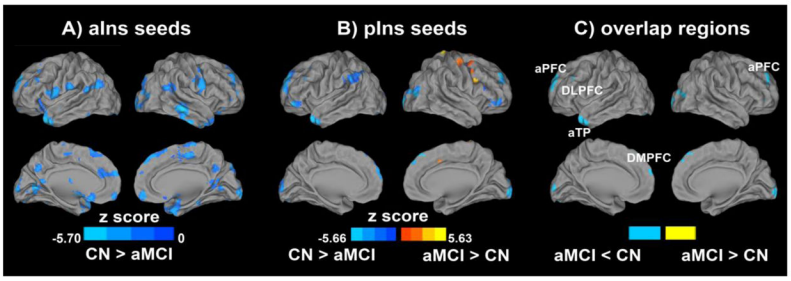

Figure 3. Group-Level Difference in Functional Connectivity of the Anterior Insula (aIns) and Posterior Insula (pIns) Subregional Networks

A) Group-Level difference in functional connectivity of the aIns network across all subjects; **B**) Group-Level difference in functional connectivity of the pIns network across all subjects. Warm colors indicate increased connectivity and blue colors indicate decreased connectivity in aMCI subjects compared to CN subjects. Deceased regions were mainly located in the frontal, temporal, occipital lobe and frontal-operculum areas. Increased connectivity was seen in the dorsal frontal cortex (dFC), presomatomotor area, and right anterior middle cingulate cortex. C) Overlapping regions from the conjunction analysis of the differential connectivity between the aIns network (A) and pIns network (B). Significantly decreased connectivity of the aIns and pIns networks were found in the aMCI group relative to the CN group. Color bar is presented with *z* score.

Abbreviation: aIns, anterior insula; pIns, posterior insula; CN, cognitively normal; aMCI, amnestic mild cognitive impairment; aTP, anterior temporal pole; aPFC, anterior prefrontal cortex; DLPFC, dorsolateral prefrontal cortex; DMPFC, dorsomedial prefrontal cortex.

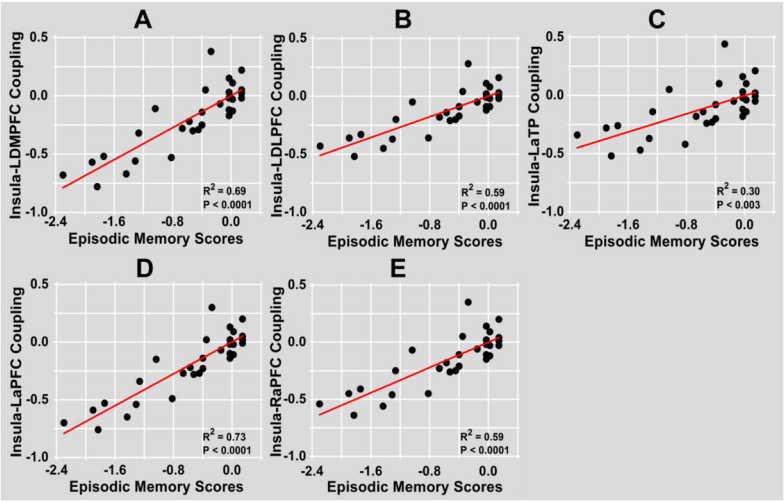

Figure 4. Behavioral Significance of Disrupted Insula Network Connectivity in aMCI Patients

The intrinsic connectivity of the insula connected to overlapping regions in aMCI patients were positively correlated with Episodic Memory (adjusted scores) after controlling the effects of age, education, gender, and right posterior insula subregional volumes. **A**) insula-LDMPFC: $R^2 = 0.69$, $p < 0.0001$; **B**) insula-LDLPFC: $R^2 = 0.59$, $p < 0.0001$; **C**) insula-LaTP: $R^2 = 0.30$, $p < 0.003$; **D**) insula-LaPFC: $R^2 = 0.73$, $p < 0.0001$; **E**) insula-RaPFC: $R^2 = 0.59$, $p < 0.0001$; and **F**) the insula connectivity index: $R^2 = 0.66$, $p < 0.0001$. Abbreviations: LDMPFC, left dorsomedial prefrontal cortex; LDLPFC, left dorsolateral prefrontal cortex; LaTP, left anterior temporal pole; LaPFC, left anterior prefrontal cortex; RaPFC, right anterior prefrontal cortex.

Table 1

Demographic Information and Psychometric Data of the Cognitive Tests and Composite Measures

Significantly decreased cognitive function related to the Episodic Memory, Executive Function, Perceptual Speed, and Working Memory was found in the aMCI group compared to the CN group.

	aMCI (n = 30)		CN(n = 26)		
Characteristic	**M**	**SD**	**M**	**SD**	***p* value**
Gender(female/male)	11/19		12/14		NS†
Age, years	72.57	4.83	70.31	4.76	.085
Education, years	14.27	3.23	14.27	3.23	.402
MMSE	27.10	1.58	28.15	1.29	.009*
Episodic Memory	−0.56	0.71	0.65	0.33	< .001*
AVMT-DR	−0.83	0.41	0.96	0.46	< .001*
ROCFT-IR	−0.29	1.24	0.34	0.44	.017*
Executive Function	0.33	0.98	−0.38	0.69	.003*
Trail Making Test-A	0.32	0.99	−0.37	0.89	.009*
Trail Making Test-B	0.33	1.15	−0.38	0.61	.006*
Perceptual Speed					
Symbol Digit Modalities Test	−0.44	0.87	0.51	0.91	< .001*
Working Memory					
Digit Span Test	−0.42	0.91	0.48	0.90	< .001*

Notes:

† *p* value was obtained by χ^2 test;

* *p* values were obtained by a multivariate analysis of variance (MANOVA). Unless otherwise indicated, data are presented as mean ± SD. Composite scores: Episodic Memory was determined by AVLT-Delayed Recall and ROCFT-Immediate Recall; Executive Function was established by the Trail Making Test-A and Trail Making Test-B; Perceptual Speed was analyzed with the Symbol Digit Modalities Test; Working Memory was determined by the Digit Span Test.

Abbreviations: aMCI, amnesic mild cognitive impairment; CN, cognitively normal; M, mean; SD, Standard Deviation; NS, no significance; MMSE, Mini-Mental State Examination; AVLT-DR, Rey Auditory-Verbal Memory Test-delayed recall; ROCFT-IR, Rey-Osterrieth Complex Figure Test-immediate recall.