Default mode network as revealed with multiple methods for resting-state functional MRI analysis

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\textbf{A B S T R A C T}

Recently, human brain activity during a resting-state has attracted increasing attention. Several studies have found that there are two networks: the default mode network and its anti-correlation network. Some studies have subsequently showed that the functions of brain areas within the default mode network are crucial in human mental activity. To further discern the brain default mode network as well as its anti-correlation network during resting-state, we used three methods to analyze resting-state functional magnetic resonance imaging (fMRI) data; regional homogeneity analysis, linear correlation and independent component analysis, on four groups of dataset. Our results showed the existence of these two networks prominently and consistently during a resting- and conscious-state across the three methods. This consistency was exhibited in four independent groups of normal adults. Moreover, the current results provided evidences that the brain areas within the two anti-correlated networks are highly integrated at both the intra- and inter-regional level.

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1. Introduction

In the field of functional neuroimaging studies, many researchers have frequently encountered task-induced signal attenuation compared to a control state (Shulman et al., 1997; Mazoyer et al., 2001). Such relative decrease is often referred to as deactivation. But not all kinds of attenuation are ‘deactivation’ (Gusnard and Raichle, 2001). Among the brain areas with decreased brain activity, the posterior cingulate cortex (PCC), precuneus (PCu), medial prefrontal cortex (MPFC), left inferior temporal cortex (ITC) and bilateral inferior parietal cortex (IPC) often exhibit decreased brain activity from a baseline state other than from an activated state (Raichle et al., 2001) in terms of an index of oxygen extraction fraction (OEF). Based on three groups of positron emission tomography (PET) data, Raichle et al. (2001) proposed that some midline areas including PCC/PCu and MPFC constitute the main elements of a default mode network. During rest but awake state, the areas within this default mode network have cerebral blood flow (CBF) and cerebral metabolism rate for oxygen (CMRO\textsubscript{2}) levels significantly above the global mean, but have OEF level similar to the global mean (Raichle et al., 2001).

Subsequently, a large set of studies has showed the existence of a default mode network and its anti-correlation network which showed negative linear correlation with the default mode network (Fransson, 2005; Fox et al., 2005) during a resting-state using functional magnetic resonance imaging (fMRI). Among those studies, linear correlation analysis for depicting functional connectivity (i.e., measuring the signal synchronization between remote brain areas) is the method most commonly used. Independent component analysis (ICA) is another method that has been used for depicting functional connectivity between bilateral visual areas (Kiviniemi et al., 2003; Van De Ven et al., 2004) as well as the functional connectivity within the default mode network (Greicius et al., 2004; Beckmann et al., 2005; De Luca et al., 2006; Sharon et al., 2006; Damoiseaux et al., 2006) in resting-state fMRI studies. It has been found that during resting but awake state, the low frequency fluctuation (LFF) (0.01–0.08 Hz) in the PCC is highly correlated with that in MPFC, bilateral angular gyrus and left ITC (Greicius et al., 2003; Fox et al., 2005; Raichle et al., 2001; Shulman et al., 1997).
Fransson (2005), and was negatively correlated with that in bilateral dorsolateral prefrontal cortex (DLPFC), bilateral medial temporal cortex (MTC), bilateral parietal lobe (IPL), bilateral insula and supplementary motor area (SMA) (Fransson, 2005; Fox et al., 2005). The latter areas have been regarded as a task-positive network (Fox et al., 2005). In addition to the aforementioned global connectivity studies, the default mode network has also been detected by methods focusing on regional spontaneous activity. Fransson (2005) found an increased level of LFFs in the default mode network by using a set of 120 cosine regressors. Using a method called regional homogeneity (ReHo) which measures the temporal similarity of voxels within a given cluster in a voxel-wise fashion (Zang et al., 2004), it was found that the brain areas with the highest ReHo were located within the default mode network (He et al., 2004).

The existence of these two networks was suggested to perform opposite activity or competing representations during resting-state (Fox et al., 2005). In addition, several previous studies indicated that the default mode network consistently showed decreased resting activity in early Alzheimer’s disease (AD) (Reiman et al., 1996; Minoshima et al., 1997; Greicius et al., 2004; He et al., 2007a,b). The network anti-correlated to the default mode network has been suggested to be breakdown in spatial neglect after stroke (He et al., 2007a,b). Therefore, the two anti-correlated networks are of great importance in both physiology and pathology.

The main purpose of the current study was to validate the default mode network as well as its anti-correlated network with resting-state fMRI on four groups of datasets obtained with different scanners. Furthermore, a multi-method approach using ReHo, linear correlation and ICA was used to investigate these two networks in order to appreciate the resting-state brain network more comprehensively.

2. Materials and methods

2.1. Subjects and scanning

A total of 75 subjects from four centers (13, 17, 23 and 22 for group 1–4, respectively, see below) participated in the study. All subjects were recruited from the universities in the neighborhoods of the scanning centers. All subjects were right-handed and had no history of neurological or psychiatric disorder. The data from 10 subjects were discarded due to excessive head motion (see data preprocessing) and hence 40 subjects were included with 10 subjects from each center matched for age and gender. Prior to the study, the written informed consent was obtained from each subject and the study was approved by the regional ethics committee of each institution. In two groups, there were additional task fMRI sessions after the resting-state fMRI scan but these data were not included in the current study. Parameters of anatomical scanning for location, T1 and 3D images were also omitted for the same reason. During the resting-state fMRI scan, subjects lay supine in the scanner with their head fixed with foam pads and a belt. Subjects were instructed to keep their eyes closed, remain motionless as much as possible and not to think of anything in particular. After scanning, all subjects reported they had not fallen asleep during the resting-state fMRI scanning.

**Group One**: The resting-state functional images were acquired from 10 subjects (4 females) aged 18–34 (mean = 25.5 years) on a Siemens 1.5T scanner at the Beijing Xuanwu Hospital with the following parameters: 20 axial slices, thickness/gap = 5/2 mm, TR/TE = 2000/60 ms, FA = 90°, in-plane resolution = 64 × 64, FOV = 220 mm × 220 mm, 180 volumes.

**Group Two**: The resting-state functional images were acquired from 10 subjects (4 females) aged 21–31 (mean = 24.3 years) on a Siemens TRIO 3T scanner at the Institute of Biophysics, Chinese Academy of Sciences with the following parameters: 32 axial slices, thickness/gap = 3.0 mm/0.75 mm, TR/TE = 2000 ms/30 ms, FA = 90°, in-plane resolution = 64 × 64, FOV = 220 mm × 220 mm. Four subjects had 208 volumes and six subjects had 240 volumes.

**Group Three**: The resting-state functional images were acquired from 10 subjects (4 females) aged 23–28 (mean = 25.2 years) on a GE 1.5T scanner at the Peking University First Hospital with the following parameters: 20 axial slices, thickness/gap = 5/1 mm, TR/TE = 2000/40 ms, FA = 90°, in-plane resolution = 64 × 64, FOV = 220 mm × 220 mm, 190 volumes.

**Group Four**: The resting-state functional images were acquired from 10 subjects (4 females) aged 21–25 (mean = 23.3 years) on a GE 3T scanner at the Sichuan Huaxi Hospital with the following parameters: 30 axial slices, thickness = 5 mm with no gap, TR/TE = 2000/30 ms, FA = 90°, in-plane resolution = 64 × 64, FOV = 220 mm × 220 mm, 200 volumes.

2.2. Data preprocessing

The first 10 volumes (20 s) were discarded to remove the initial transient effects and to allow subjects to get used to the scanner noise. For each group, 170 volumes were kept and the remaining volumes were discarded. Further preprocessing included slice-timing, head motion correction, spatial normalization to the MNI (Montreal Neurological Institute) template and spatial resampling (3 mm × 3 mm × 3 mm) by using SPM2 (http://www.fil.ion.ucl.ac.uk/spm). Subjects with maximum head motion exceeding 3 mm in translation or 1° in rotation were excluded from further data analysis. Further process was performed using AFNI (Analysis of Functional Neuroimaging) software (Cox, 1996). Data was temporally hand-pass filtered (0.01–0.08 Hz) to reduce physiological noise (Lowe et al., 1998; Greicius et al., 2003), and the linear trend was removed.

The preprocessed data was analyzed using the following methods.

2.3. ReHo analysis

Kendall coefficient of concordance (KCC) (Kendall and Gibbons, 1990) was used for measuring the similarity of the time series within a functional cluster based on the regional homogeneity hypothesis (Zang et al., 2004). In the current study, 27 nearest neighboring voxels were defined as a cluster and a KCC value (range 0–1) was given to the voxel at the center of this cluster (Zang et al., 2004). The individual ReHo map was generated in a voxel-wise fashion with the free ReHoMRI software (by Dr. H.E. Yong, http://www.bic.mni.mcgiil.ca/users/yonghe). The ReHo maps were spatially smoothed with 6 mm of full width at half maximum (FWHM). For standardization purposes, non-brain tissue in the anatomical images was removed and a brain mask was created using the MRCro software (by Chris Rorden, http://www.psychology.nottingham.ac.uk/staff/cr1/mrcro.html, see Smith, 2002). Each individual ReHo map was divided by the subject’s global mean KCC value within the brain mask. This is a similar standardization procedure to that of PET studies (Raichle et al., 2001). One-sided one-sample t-tests were performed within each group to show where in the brain the standardized KCC value was larger than one. The t-map of each group was corrected for multiple comparisons using the AlphaSim command in AFNI (Cox, 1996; B.D. Ward, http://afni.nih.gov/afni/docpdf/AlphaSim.pdf) and the corrected threshold was set at P < 0.05.

2.4. Functional connectivity analysis

A spherical region of interest (ROI) (radius = 10 mm) was centered at the given coordinates (−5, −49, 40) within PCC.
For each subject, the mean time series within this ROI was considered the reference time course. Then a seed correlation analysis (SCA) was performed in a voxel-wise way with the global trend and the six head motion parameters as covariates. Individual $r$-maps were normalized to $Z$-maps by using Fisher's $Z$ transformation (Press et al., 1992). All $Z$-maps were spatially smoothed (FWHM = 6 mm) and entered into a two-sided one-sample $t$-test to determine the regions showing significant functional connectivity with PCC. The result of each group was corrected for multiple comparisons (Monte Carlo simulation, implemented by using the AlphaSim command in AFNI (Cox, 1996; B.D. Ward, http://afni.nih.gov/afni/docpdf/AlphaSim.pdf) with corrected $P < 0.05$. In more detail, the threshold for ReHo analysis was a combination of $P < 0.01$ for single voxel and a cluster size of at least $864 \text{ mm}^3$. The threshold for SCA and ICA was the combination of $P < 0.05$ for single voxel and a cluster size of at least $3618 \text{ mm}^3$.

2.5. Group independent component analysis (ICA)

The preprocessed fMRI data were spatially smoothed (FWHM = 6 mm) before ICA procedure. Group ICA (Calhoun et al., 2001b) was performed with the GIFT software (http://icatb.sourceforge.com, Correa et al., 2005) and 20 components were predefined from each group’s dataset (Calhoun et al., 2001a; Garrity et al., 2007). For each group, the fMRI data of all subjects were firstly concatenated. Then the data reduction, ICA decomposition and back reconstruction for each individual subject were performed (Calhoun et al., 2001a,b; Sorg et al., 2007). Finally, 20 ICs for each subject were obtained. Each IC included a spatial map and a time course. The value within the spatial map was the $z$ score that represents the degree of correlation of a given voxel's fMRI signal with the time course of the component (Greicius et al., 2007). To automatically select the default mode component, the PCC ROI defined in the functional connectivity analysis stage was used as a template. The difference (i.e., goodness-of-fit) of mean $z$-value in voxels within the template minus that of voxels outside the template was calculated for each component (Greicius et al., 2004). Among the 20 components from each group, the component with the greatest goodness-of-fit was identified as the default mode component. Individual default mode components were then placed into a two-sided one-sample $t$-test within each group. The result of each group was corrected for multiple comparisons (Monte Carlo simulation, implemented by using the AlphaSim command in AFNI (http://afni.nih.gov/afni/docpdf/AlphaSim.pdf) with corrected $P < 0.05$.

2.6. Similarity analysis across methods

A conjunction analysis was performed for the similarity analysis. First, each voxel’s absolute $t$-value above the threshold was set to one and the resultant binary maps from the three methods were combined within each group. An intersection map was created if the voxel had the value of one for all three methods.

All within-group statistical maps and conjunction maps obtained from the above steps were transformed to Talairach and Tournoux coordinates (Talairach and Tournoux, 1988) and superimposed on the anatomical template available in MRicro (http://www.sph.sc.edu/comd/rorden/mricro.html) for presentation purposes.

3. Results

3.1. ReHo maps

One-sided one-sample $t$-test for each group showed that the default mode network which included PCC/precuneus, MPFC, bilateral angular gyrus and bilateral ITC, exhibited significantly higher ReHo than the global mean. This pattern was consistent across all four groups (Fig. 1a–d, $P$-value < 0.05, corrected). Other areas that showed higher ReHo, including SMA, bilateral DLPFC, bilateral

Fig. 1. One-sample $t$-test on the normalized KCC value for each group (a: Siemens 1.5 T, b: Siemens 3 T, c: GE 1.5 T, d: GE 3 T). The threshold was a combination of $P < 0.01$ for single voxel and a cluster size of at least $864 \text{ mm}^3$ ($P < 0.05$ after corrected). $Z$ axial coordinates in the Talairach and Tournoux space are from −14 mm to 46 mm in steps of 12 mm. (L: left; R: right. A: posterior cingulate cortex; B: medial prefrontal cortex; C: angular gyrus; D: supplementary motor area; E: inferior parietal lobe; F: insula; G: medial temporal cortex; H: inferior temporal cortex; I: dorsolateral prefrontal cortex; J: lingual/fusiform gyrus; K: parahippocampal gyrus).
insula, bilateral IPL and bilateral MTC, located in the task-positive network as described elsewhere (Fransson, 2005; Fox et al., 2005). In addition, some brain areas including lingual gyrus, fusiform gyrus and parahippocampal gyrus which were not within the two networks also showed higher ReHo in all four groups (Fig. 1).

### 3.2. Functional connectivity analysis

Linear correlation analysis showed that MPFC, bilateral angular gyrus, bilateral ITC had significantly positive correlation with PCC (Fig. 2a–d). Bilateral insula, bilateral inferior parietal lobe, SMA, bilateral middle temporal cortex and bilateral DLPFC showed negative correlation with PCC. These patterns were very similar across the four groups with the same threshold ($P$-value < 0.05, corrected). These brain areas have previously been reported to be involved in the two anti-correlated brain networks (Fransson, 2005; Fox et al., 2005).

To investigate whether or not the brain areas showing anti-correlation with PCC were positively correlated to each other, three spherical (radius = 3 mm) ROIs, i.e., SMA (5, 2, 51), left insula (−35, 2, 8) and left inferior parietal lobe (−52, −26, 42), respectively, were picked from the common brain areas across the four groups within the task-positive network. Pair-wise linear correlation analysis was performed among the averaged time courses. One-sample $t$-tests were performed on the $z$ score after Fisher $Z$ transformation (Press et al., 1992) for each group. The results showed that almost all these brain areas were significantly positively correlated to each other across four groups ($T > 2.25$, $P < 0.05$). Only two groups showed less significant positive correlation between SMA and left inferior parietal lobe (Siemens 1.5 T group: $T = 1.72$, $P = 0.12$; GE 1.5 T group: $T = 1.89$, $P = 0.09$). This result suggested that the nodes within the task-positive network were positively correlated, although that the positive correlation among brain areas within task-positive networks seemed less powerful than that within the task-negative network.

### 3.3. Group ICA

The default mode network was represented in each of the four groups by Group ICA (Fig. 3a–d) ($P$-value < 0.05, corrected). Meanwhile, a network including SMA, bilateral insula and bilateral IPL was found showing negative projection of the default mode network component in all the four groups (Fig. 3a–d), being consistent with the anti-correlation network previously reported (Fransson, 2005; Fox et al., 2005).

### 3.4. Common areas across three methods

In the conjunction analysis, several brain areas were consistently presented across the three methods in each group (Fig. 4) including the default mode network nodes of PCC, MPFC and bilateral AG (Raichle et al., 2001). Anti-correlation brain network nodes including insula and DLPFC were also revealed across the three methods (Fransson, 2005; Fox et al., 2005).

### 4. Discussion

Our study provided a cross-validation of the default mode brain network and its anti-correlated network by employing multi-method analysis and fMRI data from four groups. As Raichle et al. represented the identical default mode network patterns across three groups regardless of the variation of different scanners (Raichle et al., 2001), we also focused on the similar patterns across four groups and methods in the present study. In using an identical
Fig. 3. Group t-test maps of the specific independent component for each group (a: Siemens 1.5 T, b: Siemens 3 T, c: GE 1.5 T, d: GE 3 T). The threshold was the combination of $P < 0.05$ for single voxel and a cluster size of at least 3618 mm$^3$ ($P < 0.05$ after corrected). Z axial coordinates in the Talairach and Tournoux space are from −14 mm to 46 mm in steps of 12 mm. The red-yellow color indicates positive $t$-value and blue-light blue color indicates negative $t$-value. (L: left; R: right. A: posterior cingulate cortex; B: medial prefrontal cortex; C: angular gyrus; D: inferior parietal lobe; E: insula; F: supplementary motor area; G: medial temporal cortex; H: inferior temporal cortex; I: dorsolateral prefrontal cortex). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.

Fig. 4. The common brain areas were revealed across three methods from four groups (a: Siemens 1.5 T, b: Siemens 3 T, c: GE 1.5 T, d: GE 3 T). Z axial coordinates in the Talairach and Tournoux space are from −14 mm to 46 mm in steps of 12 mm. The red color indicates high ReHo and positive correlation with the default mode network. The blue color indicates high ReHo and negative correlation with the default mode network. (L: left; R: right. A: posterior cingulate cortex; B: medial prefrontal cortex; C: angular gyrus; D: inferior temporal cortex; E: dorsolateral prefrontal cortex; F: insula). For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.
preprocessing procedure, the patterns of both default mode network (i.e., PCC, MPFC and bilateral inferior parietal cortex (IPC)) as well as its anti-correlated network (i.e., SMA, bilateral insula and bilateral DLPFC) were found in each data group and by each method (Figs. 1–3). The results suggest that spontaneous brain activities within the two networks have strong intra- as well as inter-regional synchronization. The current results provide further evidence for the existence of two anti-correlated brain networks (Fransson, 2005; Fox et al., 2005) which categorizes the human brain into dynamic and ongoing activity (Fox et al., 2005).

From the perspective of methodology, the three methods could be classified by two categories: intra- and inter-regional features. In the intra-regional perspective, ReHo method measures the regional similarity with the hypothesis that the time series in a functional cluster should be highly synchronous (Zang et al., 2004). But ReHo does not provide information of the synchronization among remote areas. For functional connectivity analysis, seed correlation analysis was used to calculate the temporal correlation between the time course of a given area (e.g., PCC) and the time course from other brain areas (Greicius et al., 2003). ICA can separate independent “sources” from mixed BOLD signals (Calhoun et al., 2001b), and the brain areas of the same component map were considered an approximate synchronized network. Both the SCA and ICA methods investigate inter-regional synchronization. In previous studies, this synchronization was suggested to efficiently organize the information processing amongst remote brain areas (Buzsáki and Draguhn, 2004; Fox et al., 2005). In the present study, the brain areas within default mode network and a few brain areas within the task-positive network showed significantly higher ReHo than the rest of the brain (Fig. 1). The patterns of the two anti-correlated networks were similar between SCA and ICA (Figs. 2 and 3). When combining the three methods (Fig. 4), it suggests that some brain areas (e.g., PCC, MPFC, bilateral AG, MTC and insula) within the two networks have high intra- as well as inter-regional synchronization of spontaneous activity. These characteristics could be regarded as a model to increase the efficiency of transferring information both within nodes and across nodes in the network.

In spite of the consistency in the two anti-correlated networks revealed by the three methods, some inconsistency was exhibited due to the essential differences of the three methods. Firstly, the current study found that, while SCA and ICA yielded consistent pattern in the task-positive network, the pattern by ReHo method was less consistent with that obtained by the two other methods (Fig. 4). It suggested that ReHo method is more sensitive to the activity within the task-negative network than the task-positive network. Secondly, some regions including the fusiform gyrus, lingual gyrus and parahippocampal gyrus had higher ReHo (Fig. 1), but they were not included in the two anti-correlated networks found by SCA and ICA. This result may indicate that these areas were also active during resting-state even though they were not within the two anti-correlated networks. The function of these brain areas during resting-state would be further investigated. Thirdly, inconsistent results were also evidenced between ICA and SCA. For example, the bilateral postcentral gyrus was negatively correlated with PCC by SCA method but not by ICA in any of the three groups (Figs. 2 and 3). It may be due to that ICA measures the integrated synchronization among the networks while SCA measures the correlation of a specific area with others. In this context, different methods may reflect different aspects of comprehensive human brain function. ReHo measures the local signal coherence, SCA measures long-range linear correlation, and ICA provides more integrated coherence within the entire network. However, none of the three methods can directly reflect the amplitude or intensity of spontaneous activity. Regardless of the current limitations of comparing multiple methods, the combination of local coherence analysis and global integration analysis will further reveal the comprehensive mechanisms of human brain function.

Although the four groups showed similar patterns for the two anti-correlated networks, some limitations such as different scanner manufacturers, strength field, scanning parameters, and subject groups preclude the direct comparison of subtle differences among groups. It is therefore necessary to control these factors in future multi-center studies when comparing the difference between scanners. It should also be noted that, although the corrected P value in within-group results was the same for the three methods, the threshold for single voxel before multiple comparison correction was different (i.e., P < 0.01 for ReHo method while P < 0.05 for SCA and ICA). Therefore, it should be cautious to compare the ReHo results with the other two methods. Another limitation was that the band-pass filtering applied in the present study could not completely eliminate physiological noises such as cardiac and respiratory signals according to the Nyquist sampling theorem. These signals should be simultaneously recorded during the data acquisition and thus could be regressed out in future studies (Low et al., 1998; Birn et al., 2006).

In conclusion, our study provided a cross-validation of the default mode of brain network and its anti-correlated network based on multi-center fMRI data and multi-method analysis despite of non-controlled scanning parameters or subjects. Our results suggest that the spontaneous brain activities within the two networks have strong intra- as well as inter-regional synchronization and this would help to understand the human brain function mechanism more comprehensively.

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