Selective aberrant functional connectivity of resting state networks in social anxiety disorder

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A R T I C L E   I N F O

Article history:
Received 30 January 2010
Revised 4 May 2010
Accepted 5 May 2010
Available online 11 May 2010

Keywords:
Functional connectivity
Resting state network
fMRI
Social anxiety disorder

A B S T R A C T

Several functional MRI (fMRI) activation studies have highlighted specific differences in brain response in social anxiety disorder (SAD) patients. Little is known, so far, about the changes in the functional architecture of resting state networks (RSNs) in SAD during resting state. We investigated statistical differences in RSNs on 20 SAD and 20 controls using independent component analysis. A diffuse impact on widely distributed RSNs and selective changes of RSN intrinsic functional connectivity were observed in SAD. Functional connectivity was decreased in the somato-motor (primary and motor cortices) and visual (primary visual cortex) networks, increased in a network including medial prefrontal cortex which is thought to be involved in self-referential processes, and increased or decreased in the default mode network (posterior cingulate cortex/precuneus, bilateral inferior parietal gyrus, angular gyrus, middle temporal gyrus, and superior and medial frontal gyrus) which has been suggested to be involved in episodic memory, and self-projection, the dorsal attention network (middle and superior occipital gyrus, inferior and superior parietal gyrus, and middle and superior frontal gyrus) which is thought to mediate goal-directed top-down processing, the core network (insula-cingulate cortices) which is associated with task control function, and the central-executive network (fronto-parietal cortices). A relationship between functional connectivity and disease severity was found in specific regions of RSNs, including medial and lateral prefrontal cortex, as well as parietal and occipital regions. Our results might supply a novel way to look into neuro-pathophysiological mechanisms in SAD patients.

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Introduction

Social anxiety disorder (SAD) is a common mental disorder (Stein and Stein, 2008), which is thought to involve emotional hyperactivity, cognitive distortions, and ineffective emotion regulation (Goldin et al., 2009). Epidemiological studies conducted on general population have pointed out that the lifetime prevalence of SAD ranges between 4.0% and 16% (Ohayon and Schatzberg, 2010). Findings from neurophysiological and brain-imaging studies (Damsa et al., 2009; Engel et al., 2009) have shown that patients with SAD exhibit greater activity than healthy subjects in several areas related to emotional process as the amygdala and insula during social fear and anxiety conditioning (Etkin and Wager, 2007; Phan et al., 2006; Stein et al., 2002; Tillfors et al., 2001), suggesting the presence in SAD of impaired cortico-limbic circuit that mainly involves medial prefrontal cortex and limbic regions (Phan et al., 2006; Stein et al., 2002; Tillfors et al., 2001). Moreover, abnormal activation in the fusiform gyrus, striatal regions, superior temporal gyrus, orbital prefrontal cortex, and inferior frontal gyrus (Gentili et al., 2008; Sareen et al., 2007; Tillfors et al., 2002) were also observed in this disease. These widely distributed differences in brain activity during tasks suggest that brain functional abnormalities are not related to a single region dysfunction but to a wider network dysfunctions. As a matter of fact, however, traditional activation paradigms and multiple regression analysis cannot assess network connectivity and its dysfunction. Thus, a better understanding of the neurobiology of SAD would require investigations at different brain network levels, even during a resting state condition (Etkin et al., 2009). These would allow to assess possible differences in the crosstalk among brain regions that could represent the baseline antecedent of abnormal activations in response to given tasks.
Resting state brain networks were often shown to present abnormalities in many neuropsychiatric disorders (Calhoun et al., 2008; 2009; Garrity et al., 2007; Greicius et al., 2004; 2007; Mohammadi et al., 2009; Seeley et al., 2009; Sorg et al., 2007). These abnormalities mostly relate to the alterations of coherent intrinsic neuronal activity of blood oxygen level-dependent (BOLD) fluctuations observed in the resting state by functional magnetic resonance imaging (fMRI) (Fox et al., 2005). Functional connectivity investigations documented that intrinsic brain activity in the resting state is spatially organized in a set of specific coherent patterns (Beckmann et al., 2005; Damoiseaux et al., 2006; 2008; De Luca et al., 2006; Jafri et al., 2008; Liao et al., 2010; Mantini et al., 2007). Interestingly, such patterns, namely resting state networks (RSNs), recapitulate the functional architecture of somato-motor, visual, auditory, attention, language, and memory networks that are commonly modulated during active behavioral task (Corbetta and Shulman, 2002; Fox et al., 2006; Mantini et al., 2009). Among them, the default mode network (DMN) would be of considerable interest, as it consistently increased activity during rest than cognitive tasks (Raichle et al., 2001). A previous study in anxiety patients showed a reduced deactivation of the medial prefrontal cortex (MPFC) and an increased deactivation of the posterior cingulate cortex (PCC) during listening to threat-related words as compared to resting condition (Zhao et al., 2007). These two brain regions are critical in the DMN (Broyd et al., 2009; Buckner et al., 2008). In addition, findings from fMRI and single photon emission computed tomography (SPECT) studies consistently implicate alterations of critical regions in DMN in patients with SAD (Gentili et al., 2009; Warwick et al., 2006). More recently, a pioneering resting state functional connectivity study has demonstrated that increased connectivity of a fronto-parietal network (namely executive control network) and decreased connectivity of insula-cingulate network (namely salience network) in generalized anxiety disorder (Etkin et al., 2009).

Little is known, so far, about the changes in the functional architecture of RSNs in SAD during resting state. In previous task-related studies, single regions devoted to perception, attention, meaning attribution, self-focused attention, social cognition and emotions those functions were found differentially activated in SAD patients as compared to healthy controls. These differential activations could be explained just in terms of aberrant regional responses, but alternatively they may reflect ‘normal responses within a dysfunctional network’. In this regard, investigations on RSNs in SAD patients could provide valuable data to validate the network impairment hypothesis. In the present study, we assessed the topological differences of the RSNs between SAD patients and healthy controls, using independent component analysis (ICA) on resting state fMRI data. The primary aims of the present work are to test: 1) whether the functional connectivity of any RSNs might be aberrant in SAD patients and 2) if so, whether these changes are related to the measured clinical severity. The results of this study may supply a novel way to look into the neuro-pathophysiological mechanisms of SAD.

### Methods and materials

#### Subjects

A first study group was composed of 20 patients (22.90 ± 2.99 yrs, all right-handed) who were recruited through the Mental Health Center of the Huaxi Hospital, Chengdu, China (Table 1). Diagnosis of SAD was determined by consensus between the two attending psychiatrists and a trained interviewer using the Structured Clinical Interview DSM-IV (SCID)-Patients Version. Other inclusion/exclusion criteria were: no history of neurological and psychiatric disease and no diagnosis of other mental disorders except SAD. SAD patients did not receive psychotherapy and psychiatric medications. A second group was composed of 20 age-, sex-, education-matched healthy controls (HC) (21.65 ± 3.57 yrs, all right-handed) was recruited and screened using the SCID-Patients Version to confirm the current absence of psychiatric and neurological illness. Additionally, healthy controls were interviewed to confirm that there was no history of psychiatric illness among their first-degree relatives. All participants of the two groups were evaluated with the Liebowitz Social Anxiety Scale (LSAS), Hamilton Anxiety Rating Scale (HAMD), Hamilton Depression Rating Scale (STAI), State-Trait Anxiety Inventory. The p value was obtained by Kruskal–Wallis test. The other p values were obtained by two-sample two-tailed t-test.

<table>
<thead>
<tr>
<th></th>
<th>SAD (n=20)</th>
<th>HC (n=19)</th>
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<tbody>
<tr>
<td>M±SD</td>
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<tr>
<td>Gender (n: male/female)</td>
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</tr>
<tr>
<td>Duration (mths)</td>
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<td>-</td>
</tr>
<tr>
<td>LSAS Total score</td>
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<td>19.21±7.68</td>
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</tr>
<tr>
<td>Fear factor</td>
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<td>SATI-S</td>
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<tr>
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<tr>
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<td>Head motion</td>
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Data from questionnaires are presented in terms of mean score (M) and standard deviation (SD) in SAD and HC groups. Statistical comparisons between the two groups are also provided.

SAD, social anxiety disorder; HC, healthy controls; LSAS, Liebowitz Social Anxiety Scale; HAMA, Hamilton Anxiety Rating Scale; HAMD, Hamilton Depression Rating Scale; STAI, State-Trait Anxiety Inventory.

#### Image acquisition

Experiments were performed on a 3.0-T GE-Signa MRI scanner (EXCITE, General Electric, Milwaukee, USA) in Huaxi MR Research Center. Foam padding was used to minimize head motion for all subjects. Functional images were acquired using a single-shot, gradient-recalled echo-planar imaging sequence (TR = 2000 ms, TE = 30 ms and flip angle = 90°). Thirty transverse slices (FOV = 24 cm, in-plane matrix = 64 × 64, slice thickness = 5 mm, without gap, voxel size = 3.75 × 3.75 × 5), aligned along the anterior commissure-posterior commissure (AC–PC) line were acquired. For each subject, a total of 205 volumes were acquired, resulting in a total scan time of 410s. Subjects were instructed simply to rest with their eyes closed, not to think of anything in particular, and not to fall asleep. Subsequently, for spatial normalization and localization, a set of high-resolution T1-weighted anatomical images was acquired in axial orientation using a 3D spoiled gradient-recalled (SPGR) sequence (TR = 8.5 ms, TE = 3.4 ms, flip angle = 12°, matrix

Table 1

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size = $512 \times 512 \times 156$ and voxel size = $0.47 \times 0.47 \times 1 \text{mm}^3$) on each subject.

Data preprocessing

Data preprocessing was carried out using SPM2 software (http://www.fil.ion.ucl.ac.uk/spm). The first five volumes were discarded to ensure steady-state longitudinal magnetization. The remaining 200 volumes were first corrected for the spatial difference and head-motion correction. Data of one out of 20 subjects in HC group were excluded because translation and rotation exceeded ±1.5 mm or ±1.5°, respectively. We also evaluated the group differences in translation and rotation of head motion according to the following formula:

$$\text{Motion/Rotation} = \frac{1}{I} \sum_{i=2}^{I} \sqrt{|x_i-x_{i-1}|^2 + |y_i-y_{i-1}|^2 + |z_i-z_{i-1}|^2}$$

where $I$ is the length of the time series ($I=200$ in this study), $x_i, y_i, z_i$ are translations/rotations at the $i$th time point in the $x, y$ and $z$ directions, respectively. The functional images were realigned with the corresponding T1-volume and warped into a standard stereotactic space at a resolution of $3 \times 3 \times 3 \text{ mm}^3$, using the Montreal Neurological Institute (MNI) echo-planar imaging template in SPM2. Then, they were spatially smoothed by convolution with an isotropic Gaussian kernel (FWHM = 8 mm).

ICA and identification of RSNs

Group spatial ICA was conducted using the GIFT software (http://icatb.sourceforge.net/, version 1.3e) (Calhoun et al., 2001). To determine the number of independent components (ICs), dimension estimation on the data sets of the two groups was performed using the minimum description length (MDL) criterion (Jafri et al., 2008). Then, fMRI data from all subjects in each group were concatenated and the temporal dimension of the aggregate data set was reduced by means of principal component analysis (PCA), respectively, followed by an IC (with time-courses and spatial maps) estimation using the FastICA algorithm. Two separate groups spatial ICA were both conducted on the SAD and HC groups, with 43 and 46 ICs respectively, ensuring that the RSNs had similar spatial pattern in the two groups (Jafri et al., 2008). IC time-courses and spatial maps for each subject were back-reconstructed, using the aggregated components and the results from the data reduction step (Calhoun et al., 2001; Jafri et al., 2008). For each IC, the time-course corresponds to the waveform of a specific pattern of coherent brain activity, and the intensity of this pattern of brain activity across the voxels is expressed in the associated spatial map (Mantini et al., 2007). To display the voxels that contributed most strongly to a particular IC, the intensity values in each spatial map were converted to $Z$ values, the intensity values between ICs with RSN templates, please see Supplementary Figs. S1 and S2 for HC and SAD groups, respectively.

Second-level analysis of the RSNs

The ICs corresponding to eight RSNs were also extracted from all subjects. The spatial maps of each RSNs were then gathered in each group for a random-effect analysis using one-sample $t$-tests. Thresholds were set at $p<.01$ (multiple comparison using the false discovery rate (FDR) criterion) (Genovese et al., 2002). For visualization purposes, group-level RSN maps were overlaid on a surface-based representation of the MNI canonical brain using the SPM Surfrend toolbox (written by I. Kahn; http://spmsurfrend.sourceforge.net). The surfaces were then rendered using FreeSurfer (CorTechs Labs, Inc., Charlestown, MA) (Dale et al., 1999; Fischl et al., 1999). To qualitatively and quantitatively compare the RSNs between SAD and HC, two-sample $t$-tests were calculated ($p<.01$, FDR corrected). The group comparisons were restricted (masked) to the voxels within corresponding RSNs. The mask was created by combining the regions of corresponding RSNs in both SAD and HC, which were obtained from one-sample $t$-tests results ($p<.01$, FDR corrected) (Mohammadi et al., 2009; Sorg et al., 2007).

Post-hoc correlation analyses

We performed a post-hoc Pearson correlation analysis in order to investigate the relationship between $Z$ values in network maps and clinical severity. The voxels in corresponding RSNs showing significantly different (increased or decreased) $Z$ values between SAD and HC groups were extracted as a mask consisting of several regions of interest (ROIs). These averaged masks (ROIs) (at group level) were applied to all subjects. The mean $Z$ values of each individual within these ROIs were correlated to the LSAS (including total score, fear factor and avoidance factor, respectively) and then thresholded at a significance level of $p<.05$, corrected for multiple comparison using the Bonferroni correction for the number of brain regions that showed aberrant functional connectivity. In addition, considering these analyses were exploratory in nature, a statistical significance level of $p<.05$ uncorrected and $p<.01$ uncorrected, were also used.

Results

Psychological or behavioral data

Group demographic characteristics and psychological or behavioral scores are showed in Table 1. Compared with HC, SAD patients showed significantly impaired performance of LSAS, HAMD, HAMA, and higher levels of anxiety assessed by the STAI-T and pre-scanning STAI-S, whereas others showed no significant differences between groups.

Spatial pattern of RSNs in each group

The one-sample $t$-tests revealed a typically spatial pattern in each RSN in both SAD and HC group. Our procedure for IC classification produced consistent RSNs (Beckmann et al., 2005; Damoiseaux et al., 2006; Damoiseaux et al., 2008; De Luca et al., 2006; Jafri et al., 2008; Liao et al., 2010; Mantini et al., 2007; Mohammadi et al., 2009; Sorg et al., 2007), which are illustrated in Fig. 2. Supplementary Tables S1 and S2 provide a list of the brain regions in each RSN, along with the MNI coordinates of the peak foci and the associated Brodmann areas (BA), for HC and SAD groups respectively.

DAN is thought to mediate goal-directed top-down processing (Corbetta and Shulman, 2002). This network, which is left-lateralized,
Fig. 1. Selection of the eight brain networks among the independent components (ICs) obtained with ICA. The map of each component (only three components all illustrated in the figure) is spatially correlated with a network template (Step 1). Next, the ICs are sorted according their spatial correlation coefficients. The IC with the largest spatial correlation coefficient is selected (Step 2). The eight resting state network (RSN) templates that include the dorsal attention (DAN), central-executive (CEN), default mode (DMN), core (CN), self-referential (SRN), somato-motor (SMN), visual (VN), and auditory (AN) networks from our previous studies (Mantini et al., 2007, 2009), are shown.
primarily involves middle and superior occipital gyrus, parietal gyrus, inferior and superior parietal gyrus, and middle and superior frontal gyrus. CEN (Koechlin and Summerfield, 2007; Sridharan et al., 2008), the key regions of which include the dorsal lateral prefrontal cortices and the posterior parietal cortices. DMN (Fox et al., 2005; Greicius et al., 2004; Raichle et al., 2001) has been suggested to be involved in episodic memory (Vincent et al., 2006), and self-projection (Buckner and Carroll, 2007). It involves PCC/PCUN, bilateral inferior parietal gyrus, angular gyrus, middle temporal gyrus, superior frontal gyrus and medial frontal gyrus. CN is associated with task control function (Dosenbach et al., 2006; 2007; Mantini et al., 2009) and includes the anterior cingulate, the bilateral insular and dorso-lateral prefrontal cortices. SRN (D’Argembeau et al., 2005; Mantini et al., 2007) comprises the ventromedial prefrontal cortex (vmPFC), medial orbital prefrontal cortex (MOPFC), gyrus rectus, and pregenual anterior cingulate gyrus (PACC). SMN includes pre- and post-central gyrus, the primary sensory-motor cortices and the supplementary motor area. VN includes the inferior, middle and superior occipital gyrus, the temporal–occipital regions along with superior parietal gyrus. AN primarily encompasses the bilateral middle and superior temporal gyrus, Heschl gyrus, and temporal pole.

Aberrant RSNs in patients with SAD

The two-sample t-tests revealed the differences in functional connectivity between the two groups (Fig. 3; Supplementary Table S3). Compared to HC, SAD patients showed specifically decreased \( p < .01, \text{FDR corrected} \) functional connectivity in the SMN and the VN. Conversely, SAD patients displayed specifically increased \( p < .01, \text{FDR corrected} \) functional connectivity in the SRN. SAD patients illustrated both increased and decreased \( p < .01, \text{FDR corrected} \) functional connectivity in the DAN, CEN, DMN and CN. AN did not reveal significant group difference at \( p < .001, \text{uncorrected for multiple comparisons (clustering extent threshold, 15 voxels)} \). Supplementary...
Table S3 summarizes for the aberrant functional connectivity brain regions of each RSN and the MNI coordinates of the peak foci, as well as the associated Brodmann areas (BA).

Correlation of regions of RSNs with anxiety scale

LSAS values in SAD patients showed a significant (p < .05) positive correlation with mean Z values of the right orbital inferior frontal gyrus in DAN, the left medial superior frontal gyrus in DMN, the right anterior cingulate gyrus in CN, and negative correlation with mean Z values of the left superior parietal gyrus in DAN, the left superior frontal gyrus in CEN), the left inferior occipital gyrus in VN (Fig. 3 and Table 2). Bonferroni correction was used for the multiple comparisons when performing correlation analysis (13, 23, 8, 14, 3, 3 and 3 brain regions showed aberrant functional connectivity in DAN, CEN, DMN, CN, SRN, SMN and VN, respectively). In addition, the other brain regions with aberrant functional connectivity showed no significant correlation with LSAS.

Discussion

In the present study, we aimed at verifying whether the intrinsic functional connectivity of RSNs might be aberrant in patients with SAD and at assessing the presence of a link between these changes and disease severity.

It is important to note that our RSNs were also chosen according to the available knowledge on psychopathology of SAD and its neuronal correlates. In particular, SAD, among other anxiety disorders, is supposed to be related to an attentional bias and on self-focused attention (see for instance Clark and Wells, 1995), which relies on DAN, SRN, CN and DMN. Since neurobiological studies showed differences between SAD patients and HC during perceptive tasks as well as during public speaking (e.g. Gentili et al., 2008; Lorberbaum et al., 2004; Stein et al., 2002), we also chose the VN, the AN, the SMN and the CEN. Different regions belonging to these networks have already showed abnormal brain activations in SAD patients as compared to HC.

Our analysis showed that eight RSNs were spatially consistent across patients and controls. These findings were consistent with previous fMRI studies on RSNs (Beckmann et al., 2005; Damoiseaux et al., 2006; 2008; De Luca et al., 2006; Jafri et al., 2008; Liao et al., 2010; Mantini et al., 2007; Mohammadi et al., 2009; Seeley et al., 2007). However, among eight RSNs, functional connectivity (including both increased and decreased) was significantly different in DAN, CEN, DMN and CN; decreased functional connectivity was found in the SMN and the VN; increased functional connectivity was found in the SRN in SAD patients (see Supplementary Table S3, Fig. 3). These results are consistent with the literature on differential activations of specific cerebral regions in SAD patients as compared to healthy controls. If activation studies provided evidence for specific alterations in regions devoted to executive as well as emotional functions, the current RSN results suggest that these aberrant responses may be underpinned by specific network dysfunctions.

The pattern of dorsal attention network (DAN) obtained in the present study was largely consistent with those mapped with the specific task-induced activation (Corbetta and Shulman, 2002) or with seed-based functional connectivity (Fox et al., 2006) or ICA (Beckmann et al., 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Liao et al., 2010; Mantini et al., 2007). DAN is involved in voluntary (top-down) orienting and shows activity increases after presentation of cues indicating where, when, or to what subjects should direct their attention. It is also involved in many higher-order cognitive tasks (Corbetta and Shulman, 2002). Considering the cognitive models of SAD, which emphasized the role of emotional hyperactivity (Clark and McManus, 2002), a failure of emotion regulation is thought to be another key feature of SAD (Hofmann, 2004). Difficulties with emotion regulation have been also postulated as a core mechanism underlying mood and anxiety disorders. In addition, we found in VLPFC increased functional connectivity to be related to LSAS (Fig. 3 and Table 2). This suggests that brain regions belonging to DAN could play a role in emotional regulation and in the higher state of vigilance and awareness, which is typical of SAD patients.

A lateral fronto-parietal central-executive network (CEN) (Koechlin and Summerfield, 2007; Seeley et al., 2007; Vincent et al., 2008), whose key regions include the dorsal lateral prefrontal cortices and the posterior parietal cortices, was also identified in many studies of cognitive control over both emotional and non-emotional material (Duncan and Owen, 2000; Miller and Cohen, 2001; Ochsner and Gross, 2005). CEN also falters in most neurodegenerative diseases as degeneration spreads beyond the sites of initial injury into widely interconnected supervisory neocortical systems (Seeley et al., 2007). Specifically, Etkin et al. (Etkin et al., 2009) suggested that amygdalo-fronto-parietal coupling in anxiety patients reflected the habitual engagement of a cognitive control system to regulate excessive anxiety. Importantly, our findings reflected selective changes of functional connectivity of dorsal-lateral prefrontal (DLPFC), and further negatively correlated with severity of SAD symptoms. The neural substrates underlying executive functioning, e.g. the DLPFC, modulate the activation of amygdala and the extended limbic cortex (Nomura et al., 2004). This result is in line with other papers about the topic (Gentili et al., 2008; Lorberbaum et al., 2004), in which lower activation of DLPFC was found during a socially relevant task. Such result was put in relationship with the prevalence of emotions over the cognitive control system. This finding may thus reflect the functional impairment of CEN in the cognitive control of social anxiety.

Core network (CN) was found to be associated with task control function (Dosenbach et al., 2006; 2007; Mantini et al., 2009). This network had also previously identified during resting state, and implicated in “salience” processing (Seeley et al., 2007). It further had an important role in cognitive control related to switching between the DMN and task-related networks (Sridharan et al., 2008). Most of the previous studies highlighted a hyperactivity of the insula of anxiety patients during the processing of negative emotion (Etkin and Wager, 2007; Etkin et al., 2009; Gentili et al., 2008; Phan et al., 2006; Tillfors et al., 2001). We found decreased functional connectivity in insula. We argue that persistent hyperactivity of the insula of anxiety patients when the neural correlates of a current fear or anxiety state or a trait vulnerability for excessive fear responses (Etkin et al., 2009; Stein et al., 2007) would disrupt functional connectivity from insula to other brain systems. On the contrary, the other brain regions in CN, such as dorsal anterior cingulate and mid-cingulate gyri showed increased functional connectivity in SAD. The ACC is involved in affective processing of negative information in SAD patients (Amir et al., 2005). Moreover the cingulate cortex is well connected both with both amygdalar subregions and with the insula, which has afferent and efferent connections to anterior cingulate. We therefore speculate that cingulate increased connectivity in the CN network is effective in balancing limbic hyperactivity. Alternatively, the increased connectivity in ACC may be due to a higher vigilance and alertness in SAD patients. This latter interpretation is supported by the fact that the

Fig. 3. 3D renders of a two-sample t-test each RSNs in the SAD vs. HC (p < .01, FDR corrected). The warm and cold colors indicate the brain regions with significantly increased and decreased functional connectivity in SAD, respectively. Scatter plots in red and blue boxes show significant (p < .05) positive and negative correlations, respectively, between mean Z values of ROIs and the LSAS scores in SAD patients.
correlation with the LSAS was mostly significant with the avoidance factor.

Brain activity at rest is known to involve functional processes of different kinds, including monitoring of external environment and body state, stimulus-independent thought, planning and problem exclusive, planning future actions (Raichle et al., 2001). Among all these processes, self-referential mental activity may play an important role (D’Argembeau et al., 2005; Mantini et al., 2007). Self-referential mental activity processing has been assumed to filter, select, and provide those stimuli which are relevant for the self of a particular person, so that it can be regarded rather as intermediary between sensory and higher-order processing. The SRN has already shown peculiar physiological characteristics with a high level of neural activity during resting conditions (Raichle et al., 2001). In the present study, patients with SAD were found to have significant increased functional connectivity in ventral medial prefrontal cortex (VMPFC) in SRN compared to HC (see Supplementary Table S3, Fig. 3).

Although previous SAD studies showed lateral frontal cortex altered both in social anxiety provocation (Kilts et al., 2006) and in resting condition (Warwick et al., 2008). Medial prefrontal cortex was noted in the inhibition or extinction of excessive cortico-limbic activity in anxiety disorders (LeDoux, 1998). Moreover, alterations in neural information processing in brain regions related to self-reference were found in a PET study (Kelley et al., 2002). Our findings were also consistent with the prominent cognitive behavioral models of SAD, which hypothesized a tendency towards increased self-awareness and monitoring as a response to a fear of being observed and evaluated by others ( Rapee and Heimberg, 1997).

The default mode network (DMN) exhibits high levels of activity during resting state and decreases the activity for processes of externally oriented mental activity, induced by a wide range of sensory and cognitive tasks (Broyd et al., 2009; Buckner et al., 2008; Raichle et al., 2001). The present findings showed that the right PCUN had decreased functional connectivity in SAD patients as compared to HC (Fig. 3 and Supplementary Table S3). PCC/PCUN is sometime referred to as a pivotal hub of the DMN in social cognition and in theory of mind (Gentili et al., 2009). Our findings are consistent with a previous fMRI study on SAD patients showing a lower deactivation in the PCC/PCUN during task condition (Carey et al., 2004; Gentili et al., 2009). Moreover, Warwick et al. reported a decreased perfusion of the right PCUN in SAD patients during resting state (Warwick et al., 2008), suggesting that the decreased functional connectivity in PCUN may be associated with the psychobiological mechanism underlying SAD (Warwick et al., 2008). As a matter of fact, our findings sustain the hypothesis that PCUN would be able to suspend functional connectivity within the DMN, being related to perception of socially relevant emotional state and self-related mental representations (Buckner et al., 2008).

The aberrant functional connectivity of DMN in SAD patients is not only represented by decreased functional connectivity in the PCC/PCUN, but also by the increased functional connectivity in the DMPFC. Moreover, the mean Z value of DMPFC positively correlated with LSAS (Fear factor). The DMPFC, the second hub of DMN, is supposed to provide information from prior experiences in the form of memories during the construction of self-relevant mental simulation (Buckner et al., 2008). The DMPFC has been recognized as increasingly important for the regulation of emotion in general, and anxiety-related processing in particular (Fossati et al., 2004; Kelley et al., 2002). These data, along with our findings on SRN, suggest that medial prefrontal cortex play a role in the prominent cognitive behavioral models of SAD. It has been shown that DMPFC and PCC/PCUN activities were also linked to the DMN role in social cognition and particularly in the relationship between the self and the social environment. In this regard, Mitchell argued that “when left to its own devices, the human brain appears naturally to engage in social-cognitive thought” (Mitchell, 2006). Studies on autistic patients showed DMN alterations in these two areas (Kennedy et al., 2006; Kennedy and Courchesne, 2008a,b). Furthermore, the activity during rest of these DMN areas were found in healthy volunteers to be modulated by the degree of self-relatedness of affective pictures presented just before (Schneider et al., 2008).

In a common sense, the perceptual systems representing the sensory system, including the visual, auditory and somato-motor networks, can be considered at the lower-order of the cognitive processing hierarchy. According to our results, the AN did not demonstrate different functional connectivity between SAD and HC, which was consistent with a previous fMRI study (Etkin et al., 2009). Moreover, significantly decreased functional connectivity in the visual processing network (VN) (lingual and inferior occipital gyrus) and sensory processing (SMN) (pre- and post-central gyrus) would contribute to perceptual impairments in SAD patients. Previous emotional fMRI study suggested that, for social threat, VN and SMN showed significantly greater BOLD responses in SAD (Goldin et al., 2009).

Moreover, in the social anxiety imagery task, the social anxiety-related neural responses in VN and SMN were changed prior to and after treatment (Kilts et al., 2006). The negative correlation between functional connectivity in inferior occipital gyrus and LSAS (all factors) further provided evidence for perceptual impairments in SAD patients during resting state.

Several limitations of the current study are deserved to be mentioned. First, our ICA method was not able to provide information on the functional connectivity in the limbic system, although the latter was found in few resting state studies (Damoiseaux et al., 2008).

From this standpoint, the correlation of the fMRI BOLD signals with the mean time-courses from “seed” regions in the limbic system will be needed to explore this issue, in accordance to previous studies on generalized anxiety disorder (Etkin et al., 2009). In addition, although the RSNs represent a finite set of spatiotemporal basis function from which task-networks are then dynamically assembled and modulated during different behavioral states, their neurophysiological meaning still remains unclear. Furthermore, although a large body of literature consistently showed that spontaneous brain activity (Fon and Raichle, 2007) is organized into RSNs (Beckmann et al., 2005; Damoiseaux et al., 2006; De Luca et al., 2006; Liao et al., 2010; Mantini et al., 2007), the RSNs documented so far do not provide a complete description of brain functional architecture. In fact, they do not cover the whole cortex. On the other hand, the eight RSNs in the present study have been selected on a neurophysiological basis, and therefore provide a

### Table 2

<table>
<thead>
<tr>
<th>Anatomical locations</th>
<th>FC</th>
<th>Hem</th>
<th>Total score</th>
<th>Fear</th>
<th>Avoidance</th>
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<tbody>
<tr>
<td>DAN</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Inferior frontal</td>
<td>R</td>
<td>†</td>
<td>.428 *</td>
<td>.618 ***</td>
<td>.143</td>
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<td>gyrus, orbital</td>
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<tr>
<td>Superior parietal</td>
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<td>‡</td>
<td>- .533 *</td>
<td>- .639 ***</td>
<td>- .315</td>
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<tr>
<td>CEN</td>
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<tr>
<td>Superior frontal</td>
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<td>†</td>
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<td>- .488 *</td>
<td>- .551 *</td>
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<td>†</td>
<td>.311</td>
<td>.447 *</td>
<td>.123</td>
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<tr>
<td>gyrus, medial</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>CN</td>
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<tr>
<td>Anterior cingulate</td>
<td>R</td>
<td>†</td>
<td>.421 *</td>
<td>.286</td>
<td>.444 *</td>
</tr>
<tr>
<td>gyrus</td>
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<tr>
<td>VN</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Inferior occipital</td>
<td>L</td>
<td>‡</td>
<td>- .621 ***</td>
<td>- .562 ***</td>
<td>- .531 ***</td>
</tr>
</tbody>
</table>

Hem, hemisphere; R, right; FC, functional connectivity; †, increased; ‡, decreased; * denotes p < .05 uncorrected; ** denotes p < .01 uncorrected and *** denotes p < .05 Bonferroni corrected.
valuable framework through which alterations of functional connectiv-ity in SAD can be assessed.

Conclusion

In summary, the results of our resting state study indicate a diffuse impact on widely distributed RSNs and selective changes of RSN intrinsic functional connectivity in SAD patients. Decreased functional connectivity negatively correlating with disease severity provides evidence for RSN impairments, especially in processing self-related representations. Increased functional connectivity positively corre-lating with disease severity within the medial prefrontal cortex suggests an association between increased self-reference and mon-itoring, as a response to a fear of being observed and evaluated by others. This is likely to prevent limbic overactivity in SAD patients. It is particularly worth noting that insula showed differences in the RSN connectivity between the two groups. Insula almost consistently presents a differential activation, namely a hyperactivation, in SAD patients across different activation studies. Accordingly, we claim that such differences could represent not a primary feature of the disorder but a not specific arousal response to a stimulus considered as a threat. The primary psychobiological alteration in SAD patients might be explained, in this concern, by functional connectivity alterations contributing to the ill-evaluation of a given stimulus (e.g. a face) still considered a threat. Future studies might permit to clarify specific relationship between specific RSN abnormalities and core phenomena of SAD.

Acknowledgments

This research was partly supported by the Natural Science Foundation of China (grant nos. 90820006, 30770590 and 30625024 to HC, GQ), by the 863 Program (grant no. 2008AA02Z408 to HC, GQ), by the 973 Project (grant no. 2008CB517407 to WZ) and the Flanders Research Foundation (FWO grant to DM).

Appendix A Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2010.05.010.

References


