Altered functional connectivity in the brain default-mode network of earthquake survivors persists after 2 years despite recovery from anxiety symptoms

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Although acute impact of traumatic experiences on brain function in disaster survivors is similar to that observed in post-traumatic stress disorders (PTSD), little is known about the long-term impact of this experience. We have used structural and functional magnetic resonance imaging to investigate resting-state functional connectivity and gray and white matter (WM) changes occurring in the brains of healthy Wenchuan earthquake survivors both 3 weeks and 2 years after the disaster. Results show that while functional connectivity changes 3 weeks after the disaster involved both frontal-limbic striatal and default-mode networks (DMN), at the 2-year follow-up only changes in the latter persisted, despite complete recovery from high initial levels of anxiety. No gray or WM volume changes were found at either time point. Taken together, our findings provide important new evidence that while altered functional connectivity in the frontal-limbic-striatal network may underlie the post-trauma anxiety experienced by survivors, parallel changes in the DMN persist despite the apparent absence of anxiety symptoms. This suggests that long-term changes occur in neural networks involved in core aspects of self-processing, cognitive and emotional functioning in disaster survivors which are independent of anxiety symptoms and which may also confer increased risk of subsequent development of PTSD.

Keywords: default-mode network; functional magnetic resonance imaging; graph theory; trauma; stress

INTRODUCTION

What are the long-term consequences for humans experiencing severe emotional distress following large-scale natural disasters such as earthquakes? Is apparent recovery from initial symptoms of anxiety or depression indicative of similar recovery in functional connectivity and structural changes in the brain, or are there persistent underlying changes which may reflect heightened risk of future mental dysfunction? These questions are becoming of increasing concern for us to address as more and more individuals around the world are experiencing the consequences of exposure to extreme traumatic events, including terrorist attacks, natural disasters, accidents, physical assaults or active military combat, inherent in modern life (Satcher et al., 2007). We have previously reported an acute impact of the severe distress experienced by survivors of the Wenchuan earthquake in China in May 2008 which claimed the lives of 69,146 people (with 17,516 also reported missing) and seriously injured a further 374,131, on both functional connectivity in the brain (Lui et al., 2009) and white matter (WM) microstructure (Chen et al., 2013). Specifically, we found evidence for ‘hyperactivity’ in prefrontal-limbic-striatal brain systems known to be important for emotion processing and ‘dysfunctional connectivity’ in limbic-striatal and default-mode networks (DMN) (Lui et al., 2009). These changes were significantly associated with the increased anxiety and depression symptoms experienced by healthy survivors (Lui et al., 2009). Other recent studies have also provided evidence for the acute impact of severe stress on brain anatomy and function, including decreased gray matter (GM) volume in the left orbitofrontal cortex (Sekiguchi et al., 2013) and altered functional connectivity within temporal-limbic regions (Zhou et al., 2012), related to the occurrence of post-traumatic stress disorder (PTSD). Similarly, Papagni et al. (2011) observed that the number of stressful life events experienced was associated with decreased GM volume in the anterior cingulate, hippocampus, and parahippocampal gyrus within a period as short as 3 months in a group of clinically healthy adults. Overall, these findings suggest that major traumatic experiences have an acute impact on brain function in trauma survivors which may predispose individuals to acute stress disorders and PTSD. However, the extent to which changes in brain function observed a short-period after the experience of a trauma may persist and potentially expose individuals to the development of PTSD even long after the original event has not been established by any longitudinal studies.

Two cross-sectional studies of survivors 3 years after the 9 November 2001 terrorist attack on the World Trade Center showed reduced GM volume in insula, anterior cingulate and medial prefrontal cortex (Ganzel et al., 2008) and higher bilateral amygdala activity in response to viewing fearful compared to neutral expression faces (Ganzel et al., 2007). These findings indicate a potential long-term...
impact of acute stress on the brain in these healthy survivors although in these studies individuals were not examined longitudinally to establish the progression of altered neural network function caused by the trauma.

In recent years, an increasing number of resting-state neuroimaging studies have explored functional connectivity in brain networks using seed-based methods (Larson-Prior et al., 2009), independent component analysis (van den Heuvel and Hulshoff Pol, 2010) and graph theory analysis (Bullmore and Bassett, 2011). Graph theory analysis in particular provides a way to measure both the local and global organization of functional brain networks (Sporns et al., 2004; Stam and Reijneveld, 2007; Bullmore and Sporns, 2009) and has identified consistent topological properties of brain networks in human being including ‘small-worldness’, which suggests that the human brain segregates and integrates information with high efficiency (Achard et al., 2006; Hagmann et al., 2007; He et al., 2007). Graph theory also uses nodal characteristics to identify cortical hubs which interact with many other brain regions, facilitate functional integration and play a key role in network resilience to insult (Rubinov and Sporns, 2010). Indeed, such topological properties of brain functional networks have been found to be disrupted in various brain disorders, such as Alzheimer’s disease (Stam et al., 2007; Sané-Arigità et al., 2010), epilepsy (Liao et al., 2010; Zhang et al., 2011c), major depressive disorder (MDD) (Zhang et al., 2011a), obsessive–compulsive disorder (Zhang et al., 2011b) and schizophrenia (Liu et al., 2008; Yu et al., 2011). However, to date no study has investigated the impact of the severe stress on the topological properties of the functional brain networks in healthy survivors.

Therefore, in this investigation, we have applied graph theory analysis of resting-state functional magnetic resonance imaging (fMRI) to explore longitudinally the progressive impact of severe stress on whole brain functional networks in physically healthy survivors of the Wenchuan earthquake within 25 days and 2 years after the earthquake. We have also carried out structural MRI analysis to investigate any potential links between these and observed functional connectivity changes. Here, we hypothesized that: (i) the healthy survivors would show altered topological properties of whole-brain functional networks shortly after the earthquake, especially in brain regions known to be important for stress and emotion processing and (ii) some of the alterations in brain functional networks would recover 2 years after the trauma exposure in the survivors, consistent with reduced anxiety and depression, but that other changes might persist in brain networks associated with PTSD.

MATERIAL AND METHODS

Participants
Survivors of the Wenchuan earthquake were recruited from the hospital and from communities near the hospital that were the most affected geographic regions, where peak seismic intensity ranged from 9 to 11 on the Mercalli intensity scale. In these regions, thousands of individuals were buried and died under collapsed buildings, and the community remained in fear of intense aftershocks. All survivors were recruited by poster advertisement in which a free magnetic resonance imaging (MRI) examination of the brain was offered. The inclusion criteria for the survivors included: (i) physically experienced the earthquake; (ii) without any personal medical injury; (iii) had personally witnessed death, serious injury, or the collapse of buildings. Finally, a total of 44 healthy trauma survivors were recruited within 25 days (range 13–25 days) of the disaster (Table 1), who have been enrolled in our previous studies (Lui et al., 2009; Chen et al., 2013; Lui et al., 2013). All the 44 participants had personally witnessed serious injury and the collapse of buildings. Among them 30 had personally witnessed death. All of them were born in the earthquake regions and have lived there ever since. A structured clinical interview based on the diagnostic and statistical manual of mental disorders-IV (SCID-IV) was given to the participants to rule out the possibility of a current psychiatric disorder. Before structural and resting-state MRI scanning, levels of anxiety and depression in participants were also evaluated by using the self-rating anxiety scale (SAS) (Zung, 1971) and the self-rating depression scale (SDS) (Zung et al., 1965). The follow-up evaluation took place about 2 years after the earthquake (range 22.5–26.9 months; mean ± s.d.: 24.1 ± 1.6) and also included a structured clinical interview, measurement of levels of anxiety and depression (SAS and SDS) and structural and resting-state fMRI scans. However, only 21 among them were able to finish the follow-up, others were lost due to reasons such as the immigration or suffering from the physical diseases such as chest tumor, hypertension and diabetes which met our exclusion criteria. During the past 2 years’ follow-up, all the 21 survivors did not experience any non-earthquake stressors or major life changes. At the time of the follow-up evaluation, all the earthquake trauma survivors were free of mental and physical disorders and were not taking antidepressant or anxiolytic medication.

A total of 21 age-, education duration- and sex-matched healthy subjects (P > 0.05; Table 1) were used as controls. These latter subjects were recruited from the same local areas and scanned just a little earlier before the earthquake occurred for another project on schizophrenia (Table 1). Actually, we have contacted the 21 controls, but they were unwilling to come back to the finish the follow-up MRI scan. Although we could not use this same healthy control group for a follow-up MRI scan, we did use a further group of 34 healthy control subjects in an independent pilot study where two MRI structural and ‘resting state’ scans were carried out on the same individuals at either short-term (6 weeks) or long-term (6 and 12 months) intervals. This allowed us to test for the stability of structural and resting-state MRI data in healthy controls over time. Regrettfully, these controls were also unwilling to come back to finish the 2-year follow-up MRI scan. The 34 individuals were scanned after the earthquake. To minimize the confounding factors, we did not use them as the control sample. We found a high intraclass correlation coefficient (ICC) (0.743 ≤ ICC ≤ 0.879) in the blood oxygenation level-dependent (BOLD) signals obtained in either short term or long-term follow up scans in these control subjects and did not find significant alterations in functional connectivity (P > 0.05, paired t-test). Furthermore, we found a moderate to high ICC (0.2 ≤ ICC ≤ 0.677) for the nodal characteristics across time. A voxel-based morphometry (VBM) analysis also did not find evidence for altered GM or WM volume in the different scans (for the details, see supplementary material). The exclusion criteria for all participants in the study included: the existence of any current psychiatric disorder, organic brain disorders, alcohol or drug abuse or chronic serious illness (e.g. tumor, epilepsy, diabetes). This study was approved by the local ethics committee, and all participants signed informed consent.

MR data acquisition
MR images sensitized to changes in BOLD signal levels were obtained by using a 3-T MR imaging system (EXCITE; General Electric) with a gradient-echo echo-planar imaging sequence: repetition time/echo time (TR/TE): 2000/30 ms; flip angle (FA): 90°; slice thickness: 5 mm (no slice gap); matrix: 64 × 64; field of view (FOV): 240 × 240 mm², and voxel size: 3.75 × 3.75 × 5 mm³. Each brain volume comprised 30 axial slices, and each functional run contained 200 image volumes preceded by five dummy volumes, resulting in a total scan time of 410 s. All participants were instructed not to focus their thoughts on anything in particular and to keep their eyes closed during the resting-state MR acquisition. All participants reported that they complied with

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#### Table 1 Demographic information for physically healthy trauma survivors and healthy controls

| Characteristics                  | Survivors (baseline) means ± s.d. (n = 21) | Survivors (follow-up) means ± s.d. (n = 21) | Controls Means ± s.d. (n = 21) | P value  |
|----------------------------------|-------------------------------------------|-------------------------------------------|--------------------------------|----------|
| Female to male, n                | 8:13                                      | 8:13                                      | 8:13                           |          |
| Mean age, years                  | 40.8 ± 11.3                               | 42.8 ± 11.3                               | 37.4 ± 10.8                    | 0.74     |
| Years of education               | 9.4 ± 4.7                                 | 9.4 ± 4.7                                 | 10.9 ± 3.5                     |          |
| Time after earthquake            | 21.8 ± 3.7 (days)                         | 24.1 ± 1.6 (months)                       |                                |          |
| SAS scores                       | 44.9 ± 12.3                               | 36.4 ± 11.4                               | <0.001                         |          |
| SDS scores                       | 47.8 ± 10.5                               | 38 ± 9.7                                  | 0.003                          |          |

Notes: SAS, self-rating anxiety scale; SDS, self-rating depression scale; s.d., standard deviation; Y, year.

these instructions. High resolution three-dimensional T1-weighted (3D-T1) images were acquired with a spoiled gradient recalled sequence (TR/TE = 8.5/3.4 ms, FA = 12°, 156 axial slices with thickness = 1 mm, axial FOV = 24 × 24 cm² and data matrix = 256 × 256). Daily quality assurance scans were also performed to ensure the quality and consistency of imaging data obtained.

### Structural MR data preprocessing

VBM analysis was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and MATLAB 7.9 (Math-Works, Natick, MA, USA). Structural MRI images were preprocessed by diffeomorphic anatomical registration using the exponentiated LIE ALGEBRA (DARTEL) toolbox (Ashburner, 2007) as implemented in SPM8. This approach involves the creation of a study-specific template and the segmentation of each individual image using such template, with the aim of maximizing accuracy and sensitivity (Yassa and Stark, 2009). The following steps were followed: (i) checking for scanner artifacts and gross anatomical abnormalities for each subject; (ii) setting the image origin to the anterior commissure; (iii) using the DARTEL toolbox to produce a high-dimensional normalization protocol; (iv) checking for homogeneity across the sample; and (v) conducting an 8-mm full width half-maximum Gaussian kernel for spatial smoothing transformation. After spatial preprocessing, the smoothed, modulated, normalized GM and WM datasets were used for statistical analysis.

### fMRI data preprocessing

Functional image preprocessing was carried out using the DPARSF (http://www.restfmri.net) and SPM8 (http://www.fil.ion.ucl.ac.uk/spm) toolkit. Functional images, after exclusion of the first 10 images to ensure steady-state longitudinal magnetization, were initially corrected for temporal differences and head motion. No translation or rotation parameters in any given data set exceeded ±2 mm or ±2° and no group differences were found in respect to head translation and rotation (both P > 0.05). Functional images were warped into a standard stereotaxic space at a 3 × 3 × 3 mm³ resolution, using the Montreal Neurological Institute (MNI) echoplanar imaging template, and then were spatially smoothed with an 8-mm full-width half-maximum (FWHM) isotropic Gaussian kernel. No spatial smoothing was applied for functional connectivity network (FCN) analysis, in order to avoid introducing artificial local spatial correlations in accordance with previous suggestions (Salvador et al., 2005; Zhang et al., 2011c; Zuo et al., 2012). For each subject, representative time series in each region of interest (ROI) were obtained by averaging the functional MRI time series across all voxels in the ROI. To remove spurious sources of variance, time series were preprocessed as follows: first, six head motion parameters, averaged signals from CSF and WM, and global brain signal were regressed (Fox et al., 2005; Fox et al., 2009); next, the time series were band-pass filtered (0.01–0.08 Hz).

### FCN analysis

The whole brain was divided into 90 functional ROI (http://findlab.stanford.edu/research) to carry out a large-scale analysis of resting-state brain networks (Shierer et al., 2012). The regional mean BOLD time-series was obtained by averaging voxel time series in each ROI. We then obtained a temporal correlation matrix (90 × 90) whose elements (rij) were Pearson correlation coefficients between every pair of ROI. Individual elements of rij were subjected to statistical testing for constructing weighted FCNs, and the values of rij that did not pass the false discovery rate (FDR) correction (P < 0.05) were set to zero. Graph theoretical analyses were carried out on each of the FCNs using the Brain Connectivity Toolbox (http://www.brain-connectivity-toolbox.net) (Rubinov and Sporns, 2010). We calculated both overall topology and nodal characteristics. The overall topologies included small-world properties (σ) related to weight clustering coefficient (Cw), weight characteristic shortest path length (Lw), normalized weight clustering coefficient (γ) and normalized weight characteristic shortest path length (λ). The nodal characteristics included the nodal degree (Si), the nodal efficiency (Ei), and the betweenness centrality (Bi). See below for detailed definition.

### Graph theoretical analyses

#### Small-world properties

Small-world properties were originally proposed by Watts and Strogatz (1998). Here, we investigated small-world properties of each of the weighted FCNs (Achard et al., 2006; Zhang et al., 2011c). The weighted clustering coefficient of a node i, Cw, which expresses the likelihood that the neighborhoods of node i are connected (Omnella et al., 2005), is defined as follows: $C_w = \sum_{k,l \in N_i} w_{kl} w_{hl} / k_{i(k-1)}$, where wij is the weight between nodes i and j in the network and ki is the degree of node i. The clustering coefficient is zero, Cw = 0, if the nodes are isolated or with just one connection. The overall clustering coefficient, Cw, was computed as the average of Cw across all nodes in the network: $C^{net}_w = 1/N \sum_{i \in N} C^{net}_w$, extent measure of the local interconnectivity or cliquishness of the network.

The path length between nodes i and j was defined as the sum of the edge lengths along the path, where each edge’s length was obtained by computing the reciprocal of the edge weight, 1/wij. The shortest path length $L_{ij}$ between nodes i and j was defined as the one with the shortest length between the two nodes. The weight characteristic shortest path length $L^{net}_{ij}$ of a network was measured by a ‘harmonic mean’ length between pairs (Newman, 2003), to overcome the problem of possible disconnected network components. Formally, $L^{net}_{ij}$ is the reciprocal of the average of the reciprocals: $L^{net}_{ij} = 1 / \sum_{i \in N} \sum_{j \in N} L_{ij}$, where N is the number of nodes. The weight characteristic shortest...
path length quantifies the ability for information propagation in parallel.

To examine small-world properties related to $C_{net}^i$ and $L_{net}^i$, brain networks were compared to random networks. A small-world network has a similar path length but higher clustering than a random network, that is $\gamma = C_{net}^i / C_{random} > 1$, $\lambda = L_{net}^i / L_{random} \approx 1$ (Watts and Strogatz, 1998). These two conditions can also be summarized into a scalar quantitative measurement, the small-worldliness, $\sigma = \gamma / \lambda$, that is typically larger than one in case of small-world organization (Achard et al., 2006). For each individual brain network a set of 100 comparable random networks with similar degree sequence and symmetric adjacency matrix were formed, and $C_{random}^i$ and $L_{random}^i$ were defined as the average weighted clustering coefficient and weighted path length.

**Nodal characteristics analysis**

A nodal topological characteristic, nodal degree ($S^i$), was used. The nodal degree ($S^i$) was computed as the sum of the weights of all the connections of node $i$, that is $S^i = \sum_{j \in N} w_{ij}$. The degree $S^i$ quantifies the extent to which a node is relevant to the graph (Rubinov and Sporns, 2010). The total connection strength $S_{net}^i$ of the network was computed as the sum of $S^i$ for all nodes $N$: $S_{net}^i = \sum_{j \in N} S^j$. Nodes with high weighted degree $S^i$ can be considered as centers for information integration. The nodal efficiency of a given node is defined as the inverse of the mean harmonic shortest path length between this node and all other nodes in the network (Achard et al., 2006), according to the formula: $E_{net}^i = \frac{1}{N-1} \sum_{j \in N} \frac{1}{L_{ij}}$. $E_{net}^i$ quantifies the importance of the nodes for the communication within the network. The betweenness centrality $B_{net}^i$ of a node considers the fraction of all shortest paths in the network that pass through the node (Freeman, 1977). Here, we computed the normalized betweenness. The betweenness centrality captures the influence of a node over information flow between other nodes in the network.

**Statistical analysis of functional MR data**

We applied a matching strategy prior to comparison between the earthquake survivor and healthy control groups, in which the same network cost value ensured each network having the same number of edges under the small-worldliness range ($0.06 < \sigma < 0.37$). In the precisely defined threshold range, brain functional networks of both the healthy survivors, whether <25 days or 2 years post trauma, and control groups showed a small-world organization ($\sigma > 1$), with higher clustering coefficients ($\gamma > 1$) but almost identical characteristic path lengths ($\lambda \approx 1$), compared with comparable random networks. Despite common small-world architecture, statistical analyses revealed significant differences in the

**Correlation between topological measures and clinical variables**

Once significant between-group differences were observed in any network metrics, we further investigated the underlying relationships between the alteration of the reported levels of emotional distress (SAS and SDS) and the alteration of topological properties (AUC of each graph characteristics), performed by Pearson’s correlation analysis ($P<0.05$), to investigate the emotional relevance of altered brain network topologies in survivors.

**Statistical analysis of structural MR data**

Voxel-by-voxel-based comparisons of GM and WM volume were performed using two-sample t tests to examine differences between the control group and survivors, whether shortly after the earthquake (<25 days) or 2-year afterwards (at follow-up). To examine the GM and WM volume difference between the survivors at baseline and follow-up, we used a voxel-wise paired t-test. Results were assessed using the family-wise error (FEW) threshold of $P_{FEW} < 0.05$, corrected for multiple comparisons.

**RESULTS**

**Alterations in small-world properties**

The topological properties of brain networks depend on the choices of thresholds. In this study, we applied a matching strategy in which the same network cost value ensured each network having the same number of edges under the small-worldliness range ($0.06 < \sigma < 0.37$). In the precisely defined threshold range, brain functional networks of both the healthy survivors, whether <25 days or 2 years post trauma, and control groups showed a small-world organization ($\sigma > 1$), with higher clustering coefficients ($\gamma > 1$) but almost identical characteristic path lengths ($\lambda \approx 1$), compared with comparable random networks. Despite common small-world architecture, statistical analyses revealed significant differences in the

**Fig. 1** Differences in global topological properties of functional brain networks between earthquake survivors and healthy controls. Significant differences were found in normalized weight clustering coefficient ($\gamma$), normalized weight characteristic shortest path length ($\lambda$) and small-worldliness ($\sigma$) between survivors and controls at 21–25 days after the earthquake. After 2 years, no significant differences in $\gamma$, $\lambda$ and $\sigma$ values were observed between survivors and controls. Error bars denote standard deviations. NC, normal controls; HS, healthy survivors; HS-FU, healthy survivors at follow-up.
small-world parameters between survivors and control subjects (Figure 1). Shortly after the trauma, the survivors group showed significantly lower values in both normalized clustering coefficient $\gamma$ ($P = 0.02$) and small-worldness $\sigma$ ($P = 0.01$) and higher normalized characteristic path lengths $\lambda$ ($P = 0.02$) compared with controls (Figure 1). Two years after the trauma, there was no statistically significant group difference in the three small-world parameters $\lambda$ ($P = 0.19$), $\sigma$ ($P = 0.05$) and $\gamma$ ($P = 0.06$) between the survivors and controls (Figure 1). However, a direct comparison of survivors between <25 days and the 2-year follow-up revealed no statistically significant differences in $\gamma$ ($P = 0.2$), $\lambda$ ($P = 0.14$) and $\sigma$ ($P = 0.33$).

### Alterations in nodal characteristics

We identified the brain regions showing significant between-group differences in at least one nodal metric ($P < 0.05$) (Zhang et al., 2011a). Shortly after the trauma, compared with normal control subjects, the healthy survivors exhibited significantly increased nodal characteristics in bilateral insula, left angular gyrus, left retrosplenial cortex/posterior insular cortex (PCC), right supplementary motor area (SMA) and precuneus. Significantly decreased nodal characteristics were found in left posterior insula/putamen and left frontal operculum/inferior frontal gyrus (Table 2). Two years after the trauma event, there was no statistically significant group difference in the nodal characteristics in the bilateral insula, left posterior insula/putamen, left angular gyrus and right SMA between the survivors and controls; the altered nodal characteristics in precuneus, left retrosplenial cortex/PCC and left frontal operculum/inferior frontal gyrus, mainly implicated in DMN, continued to show a significant difference between survivors and controls (Table 2). Direct comparisons between <25 days and the 2-year follow-up in survivors revealed significantly decreased nodal characteristics in the bilateral insula (Table 2) and no statistically significant differences in left angular gyrus ($P = 0.11$), right SMA ($P = 0.29$) and left posterior insula/putamen ($P = 0.35$).

### Table 2 Alterations of nodal characteristics in brain regions in healthy survivors after trauma and controls

| Brain regions | P value ($P < 0.05$) | Coordinates (MNI) |
|---------------|---------------------|-------------------|
|               |                     | Nodal Efficiency | Betweenness centrality | Nodal Degree |
| Baseline:     |                     |                  | X | Y | Z |
| Survivors > controls | | | | | |
| Left insula   |                     | 0.03             | 42 | 14 | 4 |
| Right insula  |                     | 0.02             | 42 | 16 | 2 |
| Left angular gyrus | | | 0.01* | 48 | 68 | 34 |
| Precuneus     | 0.01*               | 0.01*            | 2 | 72 | 40 |
| Right supplementary motor area | | | 0.00* | 2 | 12 | 60 |
| Left retrosplenial cortex/posterior cingulate cortex | | | 0.01* | 12 | 58 | 56 |
| Survivors < controls | | | | | |
| Left posterior insula/putamen | | | 0.03 | 36 | 14 | 6 |
| Left frontal operculum/inferior frontal gyrus | | | 0.02 | 48 | 14 | 26 |
| Follow-up:   |                     | 0.04             | 0.02 | 2 | 72 | 40 |
| Survivors > controls | | | | | |
| Precuneus     | 0.04                | 0.02             | 2 | 72 | 40 |
| Left retrosplenial cortex/posterior cingulate cortex | | | 0.02 | 12 | 58 | 56 |
| Left middle frontal gyrus/superior frontal gyrus/precentral gyrus | | | 0.02 | 26 | 0 | 54 |
| Survivors < controls | | | | | |
| Right parahippocampal gyrus | | | 0.01* | 28 | 34 | 20 |
| Left frontal operculum/inferior frontal gyrus | | | 0.01* | 48 | 14 | 26 |
| Direct comparison between baseline and follow-up in survivors: | | | | | |
| Survivors (baseline) > survivors (follow-up) | | | | | |
| Left insula   |                     | 0.02             | 42 | 14 | 4 |
| Right insula  |                     | 0.03             | 42 | 16 | 2 |
| Survivors (baseline) < survivors (follow-up) | | | | | |
| Left precuneus | | | 0.02 | 8 | 52 | 62 |
| Left middle frontal gyrus/superior frontal gyrus/precentral gyrus | | | 0.01* | 26 | 0 | 54 |

*Note: Regions still survived after correcting for multiple comparisons using a false-positive adjustment.

### Relationships between network measures and clinical variables

The pre-post alterations of nodal characteristics in the right insula and right SMA were positively ($P = 0.01$, $r = 0.56$; $P = 0.01$, $r = 0.56$, respectively) correlated with pre-post alterations of reported levels of anxiety (SAS scores) (Figure 2). There were no significant ($P > 0.05$) correlations between the alterations of the global and nodal network metrics and those of SDS scores.

### Group difference in gray matter and white matter volume

A whole-brain VBM analysis revealed no significant GM or WM volume differences between survivors and controls or within survivors at the two post-trauma time points. We then extracted the Eigen values of mean GM volume within the three regions in which altered nodal characteristics did not normalize at the 2-year follow-up (Figure 2) using Marsbar, an automated tool (http://marsbar.sourceforge.net). Two-sample t-tests were performed to examine differences between the control group and survivors whether shortly after the earthquake (<25 days) or 2-year afterwards (at follow-up) and paired t-tests were conducted to examine the difference between the survivors at baseline and follow-up in SPSS 16.0 with $P < 0.05$ deemed to be significant. The statistical comparisons revealed no significant GM volume differences within the three regions in which altered nodal characteristics did not normalize at the 2-year follow-up between survivors and controls or within survivors at the two post-trauma time points (see supplementary Table S1).

### DISCUSSION

The present longitudinal study characterizes for the first time dynamic stress-related alterations in the topological properties of functional brain networks in a cohort of healthy survivors of the Wenchuan earthquake in China within 25 days and 2 years after the initial traumatic event. The current investigation demonstrates that the healthy
survivors showed acute functional alterations involving frontal-limbic-striatal network and DMN within 25 days after the earthquake. However, the progression of the impact of the trauma on the two networks is different, with full recovery of normal resting-state function in frontal-limbic-striatal networks associated with remission of increased levels of anxiety whereas altered function in the DMN remains even after 2 years. Interestingly, no changes in GM or WM volume were found at either time-point after the earthquake.

It is not surprising that the global functioning of the brain, as reflected by its small-world functional connectivity properties, showed acute changes within 25 days of earthquake but recovered after 2 years. Shortly after the trauma, the functional brain networks of the survivors showed significantly lower $\gamma$ value in relative to control subjects, which indicate a shift of the network small-worldness to more random (He and Evans, 2010). This was further supported by the higher $\lambda$ value in the survivors in relative to controls. The findings indicated a random shift of brain network architecture relevant to acute mass stress in survivors (Liao et al., 2010), which may contribute to a less modularized information processing or fault tolerance compared with regular small-world networks in survivors (Latora and Marchiori, 2001) and are also consistent with the wide spread attenuated functional connectivity in the survivor group shortly after the trauma (Lui et al., 2009). The absence of any significant difference in small-world parameters between the survivors and controls by 2 years after the earthquake indicates that regaining control of small-world parameters, and associated information processing, in survivors is an important part of the recovery process whereby the optimal balance between local specialization and global integration is restored. Previous studies have found that trauma survivors without psychiatric disorders can successfully cope with stress by regulating their reward, fear, emotion reactivity and social behavior (Feder et al., 2009). The present findings provide new evidence that at the neural network function level healthy trauma survivors may also struggle to overcome the trauma through regulating their whole brain network function. However, it should be noted that a direct comparison of survivors between <25 days and the 2-year follow-up did not reveal statistically significant differences in the small-world parameters between the two time points. This may reflect the relative small sample size, which would limit the power of our analyses, or an incomplete recovery of whole brain network function.

One of the most interesting findings was the recovery of nodal characteristics, especially within frontal-limbic-striatal systems (Figure 2). Shortly after the traumatic event, survivors exhibited significantly increased nodal degree in bilateral insula and right SMA, implying denser connections involving insula and SMA in survivors than in controls. Nodes with high nodal degree can be considered as centers for information integration. The betweenness centrality captures the influence of a node over information flow between other nodes in the network. Therefore, decreased betweenness centrality in left posterior insula/putamen reflects attenuated function in information transport across the network (Sporns et al., 2007; Hagmann et al., 2008; Song et al., 2011). These are consistent with our previous study revealing significantly increased amplitude of low-frequency (0.01–0.08 Hz) fluctuations in the prefrontal-limbic and striatal systems as well as weakened functional connectivity involving limbic-striatal areas (Lui et al., 2009). The prefrontal-limbic and striatal systems have a key involvement in affective processing (Cardinal et al., 2002) and the prefrontal cortex plays a role in controlling negative emotions (Feder et al., 2009;
Moreover, other studies have demonstrated a critical role for insula and striatal areas in the pathogenesis of anxiety disorders, including the recollection of traumatic memories (King et al., 2009; Fonzo et al., 2010) and the processing of fear and pain (Lanius et al., 2004; Bryant et al., 2008; Strigo et al., 2010). The SMA has also been shown to be involved in altered working memory processing in PTSD (Shaw et al., 2009). The insula, mediadorsal thalamic nucleus and the ventromedial striatum all have reciprocal connections with the prefrontal and amygdala regions (Price, 2003; Price and Drevets, 2012), both of which are critically implicated in resilience after trauma and successful adaptation to stress in survivors (Feder et al., 2009; Russo et al., 2012). The altered nodal characteristics in the frontal-limbic-striatal systems suggest that these areas may play an important role in the in episodic memory and emotional processing that is typically found in survivors of traumatic events. Our findings that recovery of nodal characteristics in the right insula and SMA are significantly associated with reduced levels of anxiety after 2 years in the survivors suggests that regulation of information transmission and integration involving these regions may play a key role in successful adaptation to extreme stress.

Another interesting finding was the increased nodal characteristics in left retrosplenial cortex/PCC and precuneus and decreased nodal characteristics in left frontal operculum/inferior frontal gyrus in survivors either shortly or 2 years after the trauma, which indicated the altered nodal characteristics in these brain regions in survivors group did not normalize over time (Figure 2). It should be noted that both the retrosplenial cortex/PCC and precuneus belong to the DMN (Raichle et al., 2001; Buckner et al., 2008), which also showed attenuated connectivity at a very early stage in trauma survivors (Lui et al., 2009). Nodal efficiency quantifies the importance of the nodes for the communication within the network. The higher nodal efficiency in precuneus observed in survivors imply that the DMN tends to form clusters of ROI which preserve efficient communication (Caeyenberghs et al., 2012). The PCC has been identified as a hub with high betweenness centrality value, which often interacts with many other regions, facilitates functional integration and plays a key role in network resilience to insult in a previous study (Buckner et al., 2009). The increased betweenness centrality in PCC may be indicative of compensatory mechanisms in the facing of external stress in survivors. However, unlike the functional recovery of frontal-limbic-striatal system, the early altered nodal characteristics in DMN in the survivors group did not normalize over time, suggesting a long-term traumatic impact on the function of their DMN. Consistent with our findings, a recent study also found that healthy survivors either shortly or 2 years after the trauma have demonstrated a major risk factor predisposing patients to the development of PTSD (Qin et al., 2012), especially for the late-onset PTSD (Smid et al., 2012) or an adaptive response to the trauma. Therefore, our finding highlights the need for a long-term evaluation for these ‘healthy’ trauma survivors.

The absence of any significant changes in either GM or WM volumes in survivors at either <25 days or at the 2-year follow up suggests that the functional connectivity changes we have found are not due to structural abnormalities. However, a previous MR diffusion study has identified intrinsic microstructural reductions (fractional anisotropy) in survivors shortly after the earthquake in medial frontal cortex, para-hippocampal gyrus, parietal lobe and basal ganglia (Chen et al., 2013). Thus it is possible that there may be a contribution of WM microstructure reductions to functional connectivity, although the previous study found no changes in either insula or SMA. Our findings are inconsistent with a previous study (Ganzel et al., 2008) which found reduced GM volume in insula, anterior cingulated and medial frontal cortex in survivors 3 years after the 9 November 2001 terrorist attack on the World Trade Center. The small sample size in this study, different trauma types and different MRI scan time after the trauma between the two studies may contribute to the inconsistency.

Some issues should be considered when interpreting the present results. First, the healthy controls did not undergo the MRI scan 2 years after the first scan, although both two groups were scanned on the same MRI and a daily quality assurance scan was performed. Moreover, evidence has shown that resting-state functional networks in healthy subjects exhibit modest to high test-retest reliability (Shehzad et al., 2009; Wang et al., 2011). Furthermore, we did a test-retest reliability analysis of BOLD data from healthy controls in an independent pilot study to confirm the stability of the resting state fMRI data in healthy controls in the present study (see supplementary material). Secondly, the sample size in this study is relatively small and we did not use standard scales to measure individual trauma experience, such as the Impact of Event Scale, or ask the degree of life threat and amount of perceived danger (Horowitz et al., 1979; Blanchard and Hickling, 1997), due to the limited amount of time, energy, financial resources and the long follow-up time. Thirdly, some of our findings lack of significant within-subject differences. However, they have a trend for significance (0.11 ≤ P ≤ 0.35, for the details, please see the Result section). This may reflect the relative small sample size, which would limit the power of our analyses, or an incomplete recovery of whole brain network function. The follow-up time may be not long enough to detect the within-subject difference. On the other hand, although the survivors physically experienced the earthquake and had personally witnessed death, serious injury or the collapse of buildings, none of them experienced personal injury and had psychiatric illnesses. This may reflect the relatively ‘mild’ trauma characteristics of the selected sample, which may also contribute to the lack of significant within-subject differences. Finally, since none of the survivors in our study have developed PTSD two-year after the earthquake (and still have not done so after nearly 4 years), we cannot at this point confirm whether the persistent change in functional DMN connectivity is associated with an increased risk of developing PTSD. However, PTSD can occur even many years after traumatic events have been experienced.

Taken together, the present longitudinal study demonstrates a complex effect of severe emotional distress on brain network function in earthquake survivors over 2 years. This is characterized by recovery of optimal network organization in whole brain functional networks and of altered nodal characteristics, especially in frontal-limbic-striatal networks and associated with reduced anxiety. Thus there is clear evidence for functional resilience in the brains of survivor 2-years after their severe trauma experience. However, recovery is not complete, with evidence of a long-term impact on the function of DMN being observed indicating a potential relationship with late-onset developed PTSD and highlighting the need for long-term evaluation and possible intervention in these otherwise ‘healthy’ survivors. Further longitudinal studies which focus on the temporal progression of altered brain network function in trauma survivors, and changes which
predict risk for developing PTSD, are clearly necessary for providing long-term advice and care to victims of severe psychological trauma following natural or other disasters.

SUPPLEMENTARY DATA
Supplementary data are available at SCAN online.

CONFLICT OF INTEREST
None declared.

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