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White Matter Microstructural Changes as Vulnerability Factors and Acquired Signs of Post-Earthquake Distress

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Abstract

Many survivors of severe disasters need psychological support, even those not suffering post-traumatic stress disorder (PTSD). The critical issue in understanding the psychological response after experiencing severe disasters is to distinguish neurological microstructural underpinnings as vulnerability factors from signs of emotional distress acquired soon after the stressful life event. We collected diffusion-tensor magnetic resonance imaging (DTI) data from a group of healthy adolescents before the Great East Japan Earthquake and re-examined the DTIs and anxiety levels of 30 non-PTSD subjects from this group 3–4 months after the earthquake using voxel-based analyses in a longitudinal DTI study before and after the earthquake. We found that the state anxiety level after the earthquake was negatively associated with fractional anisotropy (FA) in the right anterior cingulum (Cg) before the earthquake ($r = -0.61$, voxel level $p<0.0025$, cluster level $p<0.05$ corrected), and positively associated with increased FA changes from before to after the earthquake in the left anterior Cg ($r = 0.70$, voxel level $p<0.0025$, cluster level $p<0.05$ corrected) and uncinate fasciculus (Uf) ($r = 0.65$, voxel level $p<0.0025$, cluster level $p<0.05$ corrected). The results demonstrated that lower FA in the right anterior Cg was a vulnerability factor and increased FA in the left anterior Cg and Uf was an acquired sign of state anxiety after the earthquake. We postulate that subjects with dysfunctions in processing fear and anxiety before the disaster were likely to have higher anxiety levels requiring frequent emotional regulation after the disaster. These findings provide new evidence of psychophysiological responses at the neural network level soon after a stressful life event and might contribute to the development of effective methods to prevent PTSD.

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Introduction

The Japanese earthquake, a severe earthquake with a magnitude of 9.0, hit Japan on March 11, 2011. Many survivors maintain high anxiety levels due to the earthquake aftermath including frequent aftershocks and dispersed radioactive material leaking from nuclear plants[1]. Therefore, even those without posttraumatic stress disorder (PTSD) often require psychological support [2].

The neurological characteristics of subjects with PTSD [3] or stressful life events [4], [5] have been well characterized. Recently, diffusion-tensor magnetic resonance imaging (DTI) [6] was used to investigate white matter structural changes in patients with PTSD [7–10], also in healthy survivors of a disaster [11], suggesting the white matter integrity (WMI) changes in the anterior cingulum (Cg). However, a causal relationship with stressful life events remains unclear because of the cross-sectional designs. Detecting neurological underpinnings as a vulnerability factor and the acquired signs of emotional distress soon after stressful life events might contribute to a better understanding of psychological responses to stressful life events and early detection and prevention of PTSD for normal population. A previous study from our lab demonstrated longitudinal changes in grey matter volume from before to after the earthquake [12], suggesting that the reduced volume in the right anterior cingulate cortex before the earthquake was a pre-existing vulnerability factor, and the decreased volume in the left orbitofrontal cortex from before to after the earthquake was an acquired sign of post-earthquake stress. Beyond grey matter volume changes, investigating longitudinal FA changes before and after an earthquake can provide detailed evidence of microstructural abnormalities, particularly in structural connectivity related to emotional distress, after stressful life events.

This study attempted to identify WMI changes representing vulnerability factors and acquired signs of survivors' reports of emotional distress based on a longitudinal study of DTI data obtained from normal subjects before and after the earthquake. In fact, multiple studies performed in our laboratory collected DTI data from a group of healthy subjects before the earthquake. Therefore, this tragedy provided a rare opportunity to investigate WMI changes associated with such a disaster. Thirty subjects were recruited from this group to examine DTI 3~4 months after the earthquake. Anxiety levels were assessed as a measure of emotional distress following the disaster using the Japanese version of the State–Trait Anxiety Inventory (STAI) [13],[14]. State anxiety represents a psychological response to a stressful event, whereas trait anxiety represents a stable feature of one's personality. Therefore, we assumed that state anxiety scores were more appropriate than trait anxiety scores for assessing the psychological distress experienced soon after the earthquake, and we used state anxiety scores as a measure of the psychological distress experienced soon after the earthquake. We hypothesized that (a) vulnerability factors for anxiety levels after the earthquake could be detected by a significant association between state anxiety and FA before the earthquake (Pre FA) around brain regions previously implicated in PTSD and (b) the acquired signs could be detected by a significant association between state anxiety and WMI changes from before to after the earthquake (Post – Pre FA).

Methods and Materials

Recruitment and selection of participants

Eligible right-handed participants with no history of neuropsychiatric disorders were recruited from the undergraduate and postgraduate student population of the Tohoku University community. All candidates had participated in previous Magnetic Resonance Imaging (MRI) experiments conducted in our laboratory, had undergone DTI in the 2 years before the earthquake, and had agreed in advance to re-analyses of MRI scans taken before the earthquake. Because all candidates lived near the city of Sendai, which was seriously affected by the earthquake, control subjects with no experience with the earthquake were not recruited. Screening for neuropsychiatric disorders was conducted using the Mini-International Neuropsychiatric Interview (M.I.N.I.) [15], [16]. Handedness was assessed using the Edinburgh Handedness Inventory [17]. Among the numerous candidates in our database of past experiments, 30 could be contacted. All candidates enrolled in this study were part of a previous study conducted in our lab investigating grey matter volume before and after the earthquake [12]. Among the participants in this study [12], those who did not undergo DTI were excluded from the current investigation. All candidates met the above eligibility criteria and provided written informed consent before participating in the study. The M.I.N.I. confirmed that no subject had a history of psychiatric illness, including PTSD. Additionally, no subjects were taking medications for psychiatric symptoms according to a self-report questionnaire written both before and after the earthquake. This study and all previous studies were approved by the Ethics Committee of Tohoku University School of Medicine.

Psychological evaluation

All participants were evaluated for levels of anxiety using the STAI [13], [14]. The STAI measures state anxiety levels by asking subjects about their feelings "right now," whereas it measures trait anxiety levels by asking about their "usual" feelings. Levels of depression were assessed by the Center for Epidemiologic Studies Depression scale (CESD) [18], [19]. Coping styles used in daily life were assessed using the Stress Coping Inventory (SCI) [20]; Japanese version developed by the Japanese Institute of Health [21]. The SCI includes two major factors: 1) cognitive coping strategy, and 2) emotional coping strategy.

All participants were also interviewed by trained psychologists using the Japanese version of the Clinician-Administered PTSD Scale (CAPS) structured interview [22], [23]. In accordance with the M.I.N.I., no subject was diagnosed with PTSD. As for criterion A in CAPS, seven subjects experienced the earthquake as a supra-threshold psychological trauma. Although they did not experience direct life-threatening events due to the earthquake or tsunami, some of them thought that the houses or buildings that they were in at the time of the earthquake might collapse, and some of them thought that the leakage of radioactive materials from nuclear plants might be life threatening. As a result, these seven subjects were assessed to have satisfied criterion A. Actually, four of the seven subjects who met criterion A did not have any PTSD symptoms. As for criteria B, C and D in CAPS, of the 30 participants, seven met more than one criterion but none met all criteria for the three clusters of PTSD symptoms, which include re-experiencing the event, avoidance, and hyperarousal. Four of the seven subjects who had more than one PTSD symptom also did not experience life-threatening events due to the earthquake or tsunami. Specifically, the PTSD symptoms of these four subjects were mainly caused not by the earthquake directly but by the leakage of radioactive materials from nuclear plants or differences in interpersonal relationships after the earthquake. We believe that psychological stress from this kind of disaster comes not only from the disaster itself but also from continuous stressful events after the disaster. Additionally, the highest total CAPS score was 39, which is categorized as subthreshold PTSD [24]. Therefore, all subjects were regarded as "non-PTSD." The structured diagnostic interview and MRI analysis were conducted 3~4 months after the earthquake.

All psychological measurements were evaluated after the earthquake. The demographic characteristics of the subjects are presented in Table 1.

Image acquisition

All MRI data were acquired with a 3-T Philips Intera Achieva scanner. The diffusion-weighted data were acquired using a spin-echo echo-planar imaging (EPI) sequence (TR = 10,293 ms, TE = 55 ms, big delta (Δ) = 26.3 ms, little delta (δ) = 12.2 ms, FOV = 22.4 cm, $2\times2\times2$ mm^3 voxels, 60 slices, SENSE reduction factor = 2, number of acquisitions = 1). The diffusion weighting was isotropically distributed along 32 directions (b value = 1,000 s/mm^2). Additionally, a dataset with no diffusion weighting (b value = 0 s/mm^2; b0 image) was acquired. The total scan time was 7 min 17 s. Then, FA values were calculated from the collected images. This information is of particular interest when making inferences regarding white matter microstructural properties, as diffusion is faster along axons than in the perpendicular direction. Consequently, diffusion in white matter is anisotropic (*i.e.*, diffusion rates in different directions are unequal). By contrast, isotropic diffusion is equally fast in all directions. FA in each voxel was used as a measure of the degree of diffusion anisotropy. FA varies between 0 and 1, with 0 representing isotropic diffusion and 1 representing diffusion occurring entirely in one direction. After DTI image acquisition, FA map were calculated from DTI data using the software that was pre-installed on the Philips MR console.

Table 1. Demographic characteristics of the non-PTSD survivors.

Number of subjects (male/female)	30 (24/6)
Age (years)	21.0±1.6
Number of previous lifetime traumas	1.97±1.0
Period between pre- and post-earthquake MR imaging (days)	271.4±122.9
CAPS	
Total	7.0±11.5
Re-experience	1.7±2.7
Avoidance	2.5±5.1
Hyperarousal	2.8±5.1
CESD score	11.3±10.0
STAI scores	
State	42.9±11.2
Trait	42.5±9.1
SCI scores	
Co	32.7±14.2
Em	30.7±9.8

Values are shown as means ± standard deviations.
CAPS, clinician-administered PTSD scale; CESD, Center for Epidemiologic Studies Depression scale; STAI, State–Trait Anxiety Inventory; SCI, Stress Coping Inventory; Co, cognitive coping strategy; Em, emotional coping strategy.
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Pre-processing of diffusion imaging data and statistical analysis

Pre-processing and data analysis were performed using statistical Parametric Mapping software (SPM5; Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (MathWorks, Natick, MA, USA). First, our original b0 image template was created as follows. Using the affine and nonlinear spatial normalization algorithm, the b0 images from the pre-earthquake scans of all subjects in this study were spatially normalized to the SPM5 T2 template, which is based on averages taken from 152 brains from the Montreal Neurological Institute database. Then, we calculated a mean image of the normalized b0 images as our original b0 image template. Using the affine and nonlinear spatial normalization algorithm, the b0 image of each participant was normalized to our original b0 image template. Before normalization of the FA map, the post-earthquake FA maps were co-registered with the pre-earthquake FA maps from each subject. Then, using the parameter for this affine and nonlinear normalization procedure, an FA map of each participant was spatially normalized to yield images with 2 × 2 × 2-mm voxels and spatially smoothed using a Gaussian kernel of 10 mm FWHM. The resulting maps representing the FA were then subjected to the group regression analysis described below.

Statistical analyses

The group-level analysis tested for a relationship between individual state anxiety as measured by the STAI and regional FA. Voxel-by-voxel multiple regression analyses were performed using the state anxiety for Pre FA and Post–Pre FA in VBM5 on SPM5. The analysis was performed with sex and period between the pre- and post-earthquake MRI data acquisition as additional covariates. According to the relevant guidelines[25], this study, which had a sample size of 30, should be limited to two variables. Therefore, we made it a priority to control the effects of sex and period, and age was not included in the analysis as a covariate. All tests of FA were performed using an absolute threshold of FA >0.2 [26], such that if a voxel anywhere in the brain had an FA value >0.2 in all subjects, that voxel was included in the analysis. This measure was used because FA is more susceptible to errors arising from partial volumes [27], and this FA cut-off value allowed us to dissociate white matter structure from other tissue [28].

Significant regions were inferred using cluster-level statistics [29]. In this procedure, the null hypothesis was rejected when the clusters had a large spatial extent. The distribution of cluster sizes was found by parametric methods based on the theory of Gaussian random fields, which accounts for image volume, smoothness, and the cluster-defining threshold. At the cluster level, inference is determined according to the cluster size; that is, the probability that any cluster is larger than the critical cluster size is controlled. Only clusters with a p-value <0.05 after correction for multiple comparisons related to cluster size and an uncorrected voxel-level cluster-determining threshold of $p<0.0025$ were considered statistically significant in this analysis [30]. Next, to evaluate the strength of the association between white matter structural changes and state anxiety levels, we performed structural equation modeling (SEM) using the state anxiety scores from the STAI, Pre FA, and Post–Pre FA at peak voxels in each cluster as observed variables. Finally, we performed *post hoc* correlation analyses between the FA values in the regions of interest (ROIs) found in the aforementioned whole-brain analyses for trait anxiety scores, CAPS, and the two main factors of the SCI.

Results

The demographic characteristics of subjects are presented in Table 1. The distribution of anxiety levels is illustrated in Table 2. The state anxiety scores show significant positive correlations with both trait anxiety ($r = 0.66$, $p = 0.0001$) and CAPS scores ($r = 0.50$, $p = 0.005$). We also found a significant negative correlation between the state anxiety scores and the factor representing emotional coping strategy on the SCI ($r = -0.54$, $p = 0.002$).

After controlling for sex and the period between pre- and post-earthquake MRI data acquisition, state anxiety scores were negatively associated with Pre FA in the right Cg (Montreal Neurological Institute [MNI] coordinates, x = 20, y = 36, z = 0; Fig. 1a, Table 3) and positively associated with Post–Pre FA in the left anterior Cg (MNI coordinates, x = −22, y = 34, z = 18; Fig. 1b; Table 3) and with a cluster including both of the left uncinate fasciculus (Uf; MNI coordinates, x = −18, y = 26, z = −8; Fig. 1b, Table 3) and the anterior commissure (Ac; MNI coordinates, x = −10, y = 18, z = −8; Table 3). Furthermore, SEM data showed that Pre FA in the right anterior Cg and Post–Pre FA in the left anterior Cg and the left Uf accounted for 60% of the score variance in state anxiety ($R2 = 0.60$; Fig. 2). Additionally, the *post hoc* correlation analysis revealed that the Post–Pre FA in the left Uf was negatively correlated with the factor of cognitive coping strategy ($r = -0.40$, $p = 0.029$) and emotional coping strategy on

Table 2. Distribution of anxiety levels.

	Extremely low	Low	Normal	High	Extremely high
State	0	5	10	10	5
Trait	0	3	16	6	5

STAI, State–Trait Anxiety Inventory.
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the SCI ($r = -0.44$, $p = 0.015$). Also, the trait anxiety scores were negatively correlated with the Pre FA in the right Cg ($r = -0.47$, $p = 0.010$) and positively correlated with the Post – Pre FA in the left Cg ($r = 0.42$, $p = 0.022$) and the left Uf ($r = 0.36$, $p = 0.049$). No significant correlations were observed between the FA values in each ROI and CAPS scores.

Discussion

State anxiety scores were negatively associated with Pre FA in the right anterior Cg and positively associated with Post–Pre FA in the left anterior Cg, the left Uf, and the left Ac. According to our hypothesis, lower WMI in the right anterior Cg and increased WMI in the left anterior Cg, the left Uf, and the left Ac were white matter structural changes that respectively represented vulnerability factors and acquired signs of anxiety level after the earthquake.

Several lines of evidence support the notion that lower WMI in the right anterior Cg is a vulnerability factor for anxiety after stressful events. The anterior Cg bundle is a part of the principal white matter tract in the Papez circuit, which includes the ACC and the amygdala [31]. Decreased anterior Cg WMI in patients with PTSD has been frequently reported [8–10]. Also, smaller dorsal and ventral ACC volumes are reported in patients with PTSD [32], [33] and in normal subjects after stressful life events [4]. Regarding vulnerability factors, a previous study from our lab found that a smaller right ventral ACC volume was a pre-trauma vulnerability factor for PTSD symptoms [12], which is congruent with the present findings. On the other hand, investigations of monozygotic twin pairs with PTSD have found that smaller hippocampal volume was a vulnerability factor [34] and that a smaller rostral ACC was an acquired sign of PTSD [35]. Although there are apparent discrepancies between our findings and the monozygotic twin studies, it is postulated that the discrepant findings result from fundamental differences in study designs. Monozygotic twin studies cannot distinguish acquired signs of PTSD from acquired signs from birth to trauma because of the cross-sectional design of the study, which occurs after the traumatic events [35]. Based on our findings, the lower WMI in the right anterior Cg is a pre-trauma vulnerability factor for anxiety levels after a stressful event. This may have been identified as an acquired sign in the monozygotic twin study.

The functional roles of the anterior Cg and Uf indicate that psychological responses of survivors occur soon after the earthquake. The Uf, also involved in the emotional processing [36], is a principal white matter tract that connects the orbitofrontal cortex (OFC) and limbic regions including the amygdala and the anterior temporal cortices [37] [38]. In fact, neural responses in the OFC are preferentially enhanced with those in the amygdala during extinction [39] and this relationship is crucial to the voluntary regulation of emotion [40]. Given our finding that scores for

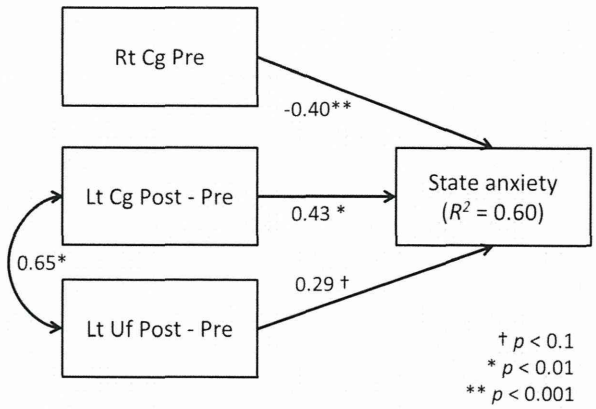

Figure 2. SEM implemented on a path diagram. The strength of the path coefficients between state anxiety scores and Pre FA in the right anterior Cg (−0.40, $p<0.001$) and between state anxiety scores and Post–Pre FA in the left anterior Cg (0.43, $p<0.01$) and the left Uf (0.29, $p = 0.06$) are shown. The path coefficient strength between Post–Pre FA in the left anterior Cg and the left Uf (0.65, $p<0.005$) are shown as well. The brain regions that predict state anxiety level evaluated by STAI are shown on the left. FA, fractional anisotropy; Rt, right; Lt, left; Cg, cingulum; Uf, uncinate fasciculus; STAI, State–Trait Anxiety Inventory.
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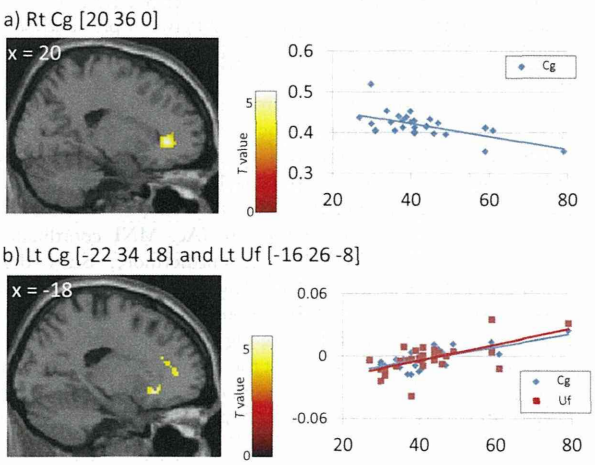

Figure 1. Relationship between state anxiety and FA. State anxiety scores were negatively associated with Pre FA in the right anterior Cg (a, $r = -0.61$, $p = 0.0004$) and Post–Pre FA in the left anterior Cg (*b*, $r = 0.70$, $p = 0.00002$) and the left Uf (*b*, $r = 0.65$, $p = 0.0001$), as illustrated by the scatter plots on the right. Vertical axes represent FA values at peak voxels in each cluster and horizontal axes indicate total state anxiety scores. The left Uf and the Ac were included in the same cluster. FA, fractional anisotropy; Rt, right; Lt, left; Cg, cingulum; Uf, uncinate fasciculus.
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Table 3. MNI coordinates, voxel sizes, *z*-scores, and *P*-values for results of the SPM analyses.

Brain region	MNI coordinates			k (voxels)	*z*-scores	*p*-values
	x	y	z			(cluster level)
Pre						
Rt Cg	20	36	0	310	4.62	0.010
Post–Pre						
Lt Cg	−22	34	18	128	4.34	0.026
Lt Uf	−18	26	−8	161	4.12	0.013
Ac*	−10	18	−8		3.83	

MNI, Montreal Neurological Institute; Rt, right; Lt, left.
Cg, cingulum; Uf, uncinate fasciculus; Ac, anterior commissure.
*The Ac is included in the same cluster as the Lt Uf.
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cognitive coping strategy were negatively correlated with scores for increased FA in Uf, those who were unlikely to have a cognitive coping strategy in daily life may have increased their WMI in Uf soon after the earthquake; this may have been induced by frequent reliance on emotional regulation due to post-earthquake stress. Based on previous cognitive training studies suggesting that the integrity of the white matter related to trained cognitive functions increases [41], [42], this would be expected to strengthen the integrity of the white matter. Therefore, subjects with the increased WMI in the left Uf and Cg would be required to regulate their emotions more frequently, but failed to regulate. Then they had higher state anxiety levels than subjects with decreased WMI in the left Uf and Cg.

In contrast, there is an apparent discrepancy with previous DTI studies demonstrating lower WMI in the anterior Cg and/or the Uf in patients with anxiety disorders such as PTSD [8–10], social anxiety disorder (SAD) [43], and generalized anxiety disorder (GAD) [44] and in healthy subjects with high anxiety levels [45], [46] [11]. It was suggested that lower WMI in the Cg and/or Uf represents a dysfunction of emotion regulation in patients with anxiety disorders [8–10], [43], [44]. We believe that this discrepancy between increased and lower WMI could be explained by a difference in early stage and long lasting anxiety levels. High anxiety levels soon after a stressful life event would be associated with frequent access to the anterior Cg and the Uf cognitive functions, which are involved in emotional processing and emotional regulation, respectively. Conversely, long lasting high anxiety levels, which is also common in the aforementioned anxiety disorders, induces cognitive dysfunction, which is associated with lower WMI in the anterior Cg and Uf. This interpretation is consistent with that of diffusional anisotropy elevation caused by temporary activation of the Cg in PTSD [7], which is also supported by our findings that state anxiety scores, which represent possibly temporary anxiety levels experienced soon after the earthquake, were more strongly correlated with increased WMI in these regions than were trait anxiety and CAPS scores. Together, these findings indicate that increased WMI in the anterior Cg and the Uf represents early-stage psychological responses to a stressful life event, and decreased WMI represents the late stage, which is reflected in the development of anxiety disorders (*e.g.*, PTSD, SAD, and GAD).

In addition, these findings indicate asymmetrical characteristics of anterior Cg psychological responses to a stressful life event in normal subjects. Previous neuroimaging studies investigating patients with PTSD revealed right hemisphere predominance [47], left hemisphere dysfunction [48], and asymmetrical WMI reduction in the anterior Cg [9]. Asymmetrical functional connectivity in the cognitive division of the ACC exists in healthy subjects as well [49]. A possible interpretation of the current results is that those with low right anterior Cg function are likely to become anxious but are protected against the development of PTSD by the maintenance of left anterior Cg function.

Some limitations should be considered when interpreting our results. First, psychological data related to emotional distress, such as anxiety levels before the earthquake, were not available. This was a predetermined limitation of the study because pre-earthquake dataset were not obtained to address the emotional issues. Additionally, because the STAI is designed to assess general anxiety in a non-specific manner, we could not determine whether the anxiety levels were caused by the earthquake. However, all subjects had no history of psychiatric diseases, suggesting their anxiety levels before the earthquake were within normal levels. Also, the significant correlation between state anxiety levels and CAPS scores indicates that the anxiety levels were raised by the earthquake, because CAPS scores were definitely results of the earthquake. Furthermore, the SEM analysis supports the model in which the direction from brain structural changes to anxiety levels was suggested. The results of these analyses complement the lack of psychological data before the earthquake. Second, this study did not include subjects with supra-threshold PTSD symptoms, because most candidates in our pre-earthquake database were assumed to have been affected by the earthquake to some extent but not to have been exposed to life-threatening experiences. Therefore, the scope of the current study was the neural correlates of individual differences in state anxiety levels in the normal population after experiencing the disaster, regardless of psychological trauma. We believe that investigation of subjects with subclinical PTSD symptoms can provide sufficient evidence, an assumption that has been made in previous studies [4], [5], [11], [50], [51], [52], which would namely contribute to early detection and prevention of PTSD. In any case, a further longitudinal study of patients with supra-threshold PTSD symptoms caused by traumatic events is necessary to examine whether the neural microstructural connectivity changes observed in the current investigation are applicable to such individuals. Third, the majority of our subjects were males. To deal with this issue, sex was treated as an additional covariate. However, the possibility that the unbalanced sex distribution of subjects distorted the results remains. Fourth, the participants in the present study were limited to university students. Thus, the results may not generalize to older populations.

Despite these limitations, this is the first longitudinal study distinguished WMI changes that represent a vulnerability factor from structural changes that represent an acquired sign of high state anxiety. Additionally, the results demonstrating increased FA in the left anterior Cg and the Uf provide new evidence of temporal FA elevation in the early-stage response to stressful life events before anxiety disorders (*e.g.*, PTSD, SAD, and GAD) develop. Such disorders are characterized by decreased FA in these areas. These findings may be helpful for discriminating between survivors with and without emotional distress soon after a stressful life event, and between survivors who will and will not, in future, experience anxiety after a stressful life event, even in the normal population. These findings provide a better understanding of psychophysiological responses to a stressful life event at the neural network level and may contribute to the development of effective methods to prevent stress-related disorders, namely PTSD, in the normal population.

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Author Contributions

Conceived and designed the experiments: A. Sekiguchi MS YT YK TA SH SN CMM A. Sakuma RK. Performed the experiments: A. Sekiguchi MS YK TA SH SN CMM. Analyzed the data: A. Sekiguchi YK. Contributed reagents/materials/analysis tools: A. Sekiguchi MS YT YK RN HT AT. Wrote the paper: A. Sekiguchi. Revised the article: A. Sekiguchi MS YT RN HT RK.

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ORIGINAL ARTICLE

Brain structural changes as vulnerability factors and acquired signs of post-earthquake stress

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Many survivors of severe disasters, even those without posttraumatic stress disorder (PTSD), need psychological support. To understand the pathogenesis of PTSD symptoms and prevent the development of PTSD, the critical issue is to distinguish neurological abnormalities as vulnerability factors from acquired signs of PTSD symptoms in the early stage of adaptation to the trauma in the normal population. The neurological underpinnings of PTSD have been well characterized, but the causal relationships with the traumatic event are still unclear. We examined 42 non-PTSD subjects to find brain morphometric changes related to the severity of PTSD symptoms in a longitudinal magnetic resonance imaging study extending through the Great East Japan Earthquake. We found that regional grey matter volume (rGMV) in the right ventral anterior cingulate cortex (ACC) before the earthquake, and decreased rGMV in the left orbitofrontal cortex (OFC) through the earthquake were negatively associated with PTSD symptoms. Our results indicate that subjects with smaller GMV in the ACC before the earthquake, and subjects with decreased GMV in the OFC through the earthquake were likely to have PTSD symptoms. As the ACC is involved in processing of fear and anxiety, our results indicate that these processing are related to vulnerability for PTSD symptoms. In addition, decreased OFC volume was induced by failing to extinct conditioned fear soon after the traumatic event. These findings provide a better understanding of posttraumatic responses in early stage of adaptation to the trauma and may contribute to the development of effective methods to prevent PTSD.

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Keywords: acquired sign; brain structure; disaster; Posttraumatic stress disorder; voxel-based morphometry; vulnerability

INTRODUCTION

The Great East Japan Earthquake, a severe magnitude 9.0 earthquake, hit Japan on 11 March 2011. The eastern half of Japan was severely affected, and the northeast coast suffered widespread destruction caused by a massive tsunami triggered by this earthquake. More than 15 000 people have been confirmed dead, and ~4000 remain missing 6 months after the earthquake. Stress-related disorders such as acute stress disorder and posttraumatic stress disorder (PTSD) are likely to occur among the large number of survivors,[1–3] but even those without PTSD will require psychological support.[4] However, survivors without PTSD are likely to hesitate to ask for psychological supports and may not receive help, in contrast to those with PTSD. Distinguishing neurological abnormalities as a vulnerability factor from the acquired signs of PTSD symptoms in the early stage of adaptation to the trauma is essential both to understand the pathogenesis of PTSD and to prevent survivors from developing PTSD. Such information may provide a better understanding of posttraumatic responses and the development of effective methods to prevent PTSD.

The neurological underpinnings of PTSD have been well characterized, but the causal relationships to the traumatic event remain unclear, because of difficulties with prospective studies.[5] Previous neuroimaging studies of patients with PTSD revealed morphological changes in several brain regions, including the hippocampus/parahippocampus,[6] amygdala,[6] anterior cingulate cortex (ACC),[7–9] insula[10] and orbitofrontal cortex (OFC),[11–13] which were also found in healthy adults after stressful life events.[14,15] Evaluation of monozygotic twin pairs with combat-related PTSD has provided evidence that smaller hippocampal volume is a vulnerability factor for PTSD,[16] and smaller pregenual ACC represents an acquired sign of PTSD.[8] However, longitudinal structural changes as a vulnerability factor and an acquired sign of PTSD symptoms remain unclear. Some longitudinal studies have examined patients with PTSD after traumatic events, but failed to find subsequent brain structural changes.[11,17,18] A recent longitudinal study revealed that decreased volumes in the ACC and hippocampus/parahippocampus were associated with the number of stressful life events, but the impacts of stress-related responses on brain structure were not examined.[14] Therefore, the significance of longitudinal structural changes caused by posttraumatic response remained unclear.

This study tried to identify brain structural changes representing vulnerability factors and acquired signs of PTSD symptoms in non-PTSD survivors, based on a longitudinal study of structural magnetic resonance (MR) images obtained before and after the earthquake. In fact, we had obtained extensive structural MR imaging database from a group of healthy adolescents before the

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earthquake in multiple studies performed in our laboratory. Therefore, this extremely miserable episode provided a rare opportunity for investigating brain structural changes associated with such a disaster. We recruited 42 subjects from this group to examine structural MR images at 3–4 months after the earthquake. PTSD symptoms were also assessed using the Japanese version of the clinician-administered PTSD scale (CAPS) structural interview.[19] We hypothesized that the vulnerability factors for PTSD could be detected by a significant association between the CAPS scores and smaller regional grey matter volume (rGMV) before the earthquake (Pre rGMV) in brain regions previously implicated in PTSD, and that the acquired signs could be detected by a significant association between the CAPS scores and decrease in rGMV from before to after the earthquake (Post-Pre rGMV). More specifically, based on previous monozygotic twin studies,[8,16] we could predict that smaller rGMV in the hippocampus would be identified as a pre-trauma vulnerability factor, and smaller rGMV in the ACC as an acquired sign of PTSD symptoms.

MATERIALS AND METHODS

Recruitment and selection of participants

Eligible participants were recruited from undergraduate and postgraduate students of the Tohoku University community, who met the eligibility criteria of having no history of neuropsychiatric disorders and right-handed dominance. All candidates had participated in past MR imaging experiments conducted in our laboratory, and had undergone structural MR imaging within 2 years before the earthquake, and had agreed to re-analyses of MR images obtained before the earthquake in advance. As all candidates lived around Sendai city, which was strongly affected by the earthquake, we did not plan to recruit control subjects unaffected by the earthquake. Neuropsychiatric disorders were screened using the mini international neuropsychiatric interview (M.I.N.I.).[20,21] Handedness was assessed by the Edinburgh Handedness Inventory.[22] We were able to contact 42 of the numerous candidates in our database of past experiments. All candidates satisfied the eligibility criteria and provided written informed consents before participating in the current study to examine the possible effects of psychological trauma on brain structure, in accordance with the Declaration of Helsinki Principles.[23] The M.I.N.I. confirmed that no subject had any history of psychiatric illness including PTSD, and no subjects were exposed to life-threatening experiences due to the earthquake and tsunami. This study and all previous studies were approved by the Ethics Committee of Tohoku University.

Psychological evaluations

All participants were interviewed by trained psychologists (A Omoto, N Aikawa, N Saito, Y Watanabe and YK) using the CAPS structured interview.[19,24] In accordance with the M.I.N.I., no subject was diagnosed as having PTSD. Of the 42 participants, 8 subjects filled more than one, but not all, criteria of three clusters of PTSD symptoms, including re-experiencing of the event, avoidance and hyperarousal. In addition, the highest total CAPS score was 39, which is categorized as subthreshold PTSD.[25] Therefore, we could regard all subjects as non-PTSD. The levels of anxiety and depression were evaluated using the state-trait anxiety inventory[26,27] and the Center for Epidemiologic Studies Depression Scale.[28,29] The diagnostic structured interview and MR imaging were conducted at 3–4 months after the earthquake. All psychological measurements were evaluated only after the earthquake.

Image acquisition

All MR imaging data acquisition was conducted with a 3-T Philips Intera Achieva scanner (Best, Netherlands). By using a MPRAGE sequence, high-resolution T1-weighted structural images (240 × 240 matrix, repetition time = 6.5 ms, echo time = 3 ms, field of view = 24 cm, 162 slices, 1.0-mm slice thickness) were collected.

Voxel-based morphometry analysis

To investigate the structural changes as vulnerability factors and acquired signs of PTSD symptoms in non-PTSD patients, voxel-based morphometry (VBM) was conducted. First, post-earthquake images were coregistered with pre-earthquake images in each subject on SPM2. Preprocessing of the morphological data was performed with VBM2 software,[30] an extension of SPM2. Default parameter settings were used.[30] In order to reduce the scanner-specific bias, a customized grey matter anatomical template was created from the pre-earthquake data of all participants in this study. Next, the T1-weighted structural images of each subject were segmented into grey and white matter partitions using the new grey and white matter prior probability maps. The resulting images included the extracted grey and white matter partitions in the native space. The grey matter partition was then normalized to the new grey matter probability map. The normalization parameters determined from this initial step were then applied to the native T1-weighted structural image. These normalized T1-weighted structural data were then segmented into grey and white matter partitions. The volumes of global grey matter, white matter and cerebrospinal fluid space were calculated using segmented and modulated images by adding a value derived from the voxel volume and multiplied by the value of each voxel. To facilitate optimal segmentation, normalization parameters were estimated with the previously reported protocol.[31] In addition, a correction was performed for volume changes (modulation) by modulating each voxel with the Jacobian determinants derived from the spatial normalization, to test for regional differences in the absolute amount of grey matter.[32] All images were subsequently smoothed by convolving with an isotropic Gaussian kernel of 8-mm full-width at half-maximum. Finally, the signal change in rGMV between pre- and post-earthquake images was calculated at each voxel for each participant. Only voxels that showed GMV values >0.10 in both pre- and post-earthquake images were included to avoid possible partial volume effects around the borders between grey matter and white matter, as well as between grey matter and cerebrospinal fluid. The resulting maps representing the rGMV before the earthquake (Pre rGMV) and the rGMV change between before and after the earthquake (Post-Pre rGMV) were then forwarded to the group-level analysis described below.

Statistical analysis

The group-level analysis tested for the relationship between individual severity of PTSD symptoms measured by the CAPS and rGMV. Voxel-by-voxel multiple regression analyses were performed using the CAPS scores for Pre rGMV and Post-Pre rGMV on SPM5. The analysis was performed with age, total brain volume, and periods between pre- and post-earthquake MR imaging data acquisition as additional covariates. Total brain volume was summation of segmented global grey matter and white matter volume in each subject. A significant level was set at $P = 0.05$ corrected for multiple comparisons. Small volume correction[33] was performed to examine each region of interest with a hypothesis (amygdala, hippocampus, insula, ACC and OFC)[6–13] using a lenient threshold of $P = 0.001$, uncorrected as a cluster determined threshold, and a $\kappa = 100$ to suppress the possibility of small clusters arising by chance, as used in previous neuroimaging studies.[7] Small volume correction was applied to each region of interest using anatomical masks (amygdala, hippocampus, insula, ACC and OFC in each hemisphere) from the WFU_PickAtlas (http://fmri.wfubmc.edu/software/PickAtlas)[34,35] and the Anatomical Automatic Labelling Region of Interest package,[36] as used in previous VBM studies of PTSD,[7,8,37,38] as well as functional MR imaging studies.[39,40] Finally, to verify the effect of the structural changes on the CAPS scores, regression analysis was performed using Pre rGMV and Post-Pre rGMV at peak voxels in each cluster as explanatory variables, and total scores of CAPS as independent variables.

RESULTS

Demographic characteristics of the subjects are shown in Table 1. The distribution of the CAPS scores is illustrated in Figure 1. After controlling for age, total brain volume, and periods between pre- and post-earthquake MR imaging data acquisition, the total

Table 1. Demographic characteristics of non-PTSD survivors

Number of subjects (male/female)	42 (33/9)
Age (years)	21.7 ± 1.7
Number of previous lifetime traumas	1.98 ± 0.98
Periods (days)	
From pre- to post-earthquake MR scans	244.4 ± 137.8
From pre-earthquake MR scans *to* the earthquake	142.6 ± 135.4
From the earthquake to post-earthquake MR scans	101.8 ± 8.2
CAPS	
Total	5.7 ± 10.0
Re-experience	1.6 ± 2.5
Avoidance	2.0 ± 4.4
Hyperarousal	2.1 ± 4.5
CESD score	11.6 ± 10.2
STAI scores	
State	41.6 ± 11.4
Trait	43.0 ± 10.0

Abbreviations: CAPS, clinician-administered PTSD scale; CESD, Center for Epidemiologic Studies Depression; MR, magnetic resonance; PTSD, posttraumatic stress disorder; STAI, state-trait anxiety inventory.
Values are shown as mean ± s.d.

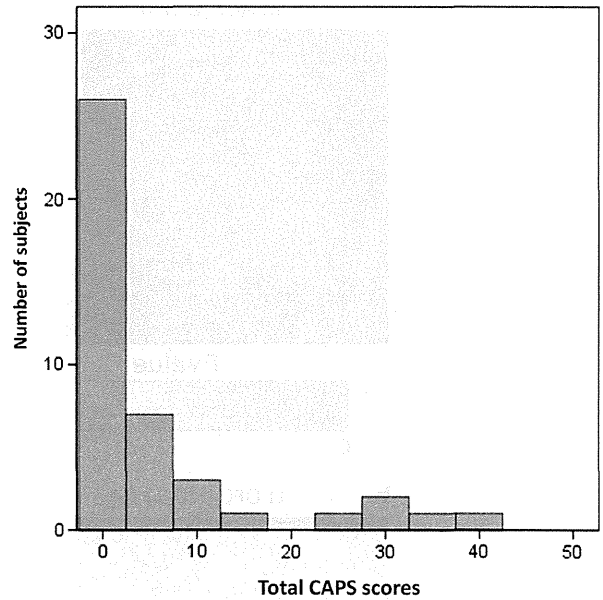

Figure 1. Distribution of total clinician-administered PTSD scale (CAPS) scores. Highest total CAPS score was 39, which is categorized as subthreshold posttraumatic stress disorder (PTSD), according to the following diagnostic categorization of PTSD by CAPS scores; 0–19 (asymptomatic/few symptoms), 20–39 (mild PTSD/subthreshold), 40–59 (moderate PTSD/threshold), 60–79 (severe PTSD symptomatology) and ⩾80 (extreme PTSD symptomatology).[25] All subjects were confirmed as non-PTSD survivors.

scores of CAPS were significantly associated with smaller Pre rGMV in the right ventral ACC ($x = 6$, $y = 32$, $z = 0$; Figure 2a, Table 2), and decreased Post-Pre rGMV in the left OFC ($x = -20$, $y = 52$, $z = -6$; Figure 2b, Table 2), based on region of interest analysis. *Post hoc* regression analysis revealed that Pre rGMV in the right ventral ACC and Post-Pre rGMV in the left OFC accounted for 48% score variance in the CAPS ($F(2, 39) = 18.28$, $R^2 = 0.48$, $P < 0.001$; Figure 3).

DISCUSSION

The aim of this study was to investigate the brain structural changes as vulnerability factors and acquired signs of PTSD symptoms in non-PTSD survivors. We found the total scores of CAPS were negatively associated with Pre rGMV in the right ventral ACC, and negatively associated with Post-Pre rGMV in the left OFC.

According to our hypothesis, smaller Pre rGMV in the right ventral ACC and decreased Post-Pre rGMV in the left OFC are structural changes representing vulnerability factor and acquired sign of PTSD symptoms, respectively. Additionally, these structural changes could explain approximately half of the observed PTSD symptoms.

Our longitudinal study provides further evidence of the causal relationships between brain structural changes and posttraumatic responses. Previous longitudinal studies have investigated brain structural changes only after the traumatic events,[11,17,18] but failed to find any brain volume reduction in patients with PTSD. Another longitudinal study revealed that the number of stressful life events is associated with decreased volumes in the ACC and hippocampus/parahippocampus in healthy subjects, but the causal relationship between psychological responses to the stressful events and brain structural changes was not examined.[14] On the other hand, in contrast to our findings, investigations of monozygotic twin pairs with PTSD revealed smaller hippocampal volume as a vulnerability factor,[16] and smaller pregenual ACC as an acquired sign of PTSD.[8] We suppose that these discrepant findings result from fundamental differences in study designs. Monozygotic twin studies cannot distinguish acquired signs of PTSD from acquired signs from birth to trauma, because of the cross-sectional design only after the traumatic events.[8] On the basis of our present findings, smaller ACC volume is not an acquired sign, but an acquired vulnerability of PTSD before exposure to traumatic events, which could have been identified as an acquired sign of PTSD in the monozygotic twin study.[8]

Several lines of evidence support the notion that smaller ventral ACC volume is a vulnerability factor for PTSD symptoms. An essential role of the ventral ACC is the processing of anxiety and fears,[41] which is supposed to be highly related to the manifestation of the clinical symptoms of PTSD.[42] In fact, smaller ACC volume is one of the more robust VBM findings in patients with PTSD,[7–9] also in normal subjects after stressful life events.[14,15] Whether such a smaller volume in the ventral ACC represents an acquired abnormality or a pretrauma vulnerability factor for PTSD is still under discussion.[8] Our present findings provide evidence that smaller ventral ACC volume is a pretrauma vulnerability factor for PTSD.

rGMV in the ACC is associated with personality traits related to PTSD symptoms. Harm avoidance,[43] which is predictive of increased PTSD symptom severity,[44] is positively associated with anatomical variability of the ACC.[45] Individuals with alexithymia, which is positively associated with PTSD symptoms,[46] had smaller ACC volume.[47] Combined with our findings, these observations support the possibility that these psychological characteristics are also vulnerability factors for PTSD.

The cognitive functions of the OFC indicate posttraumatic responses of the survivors soon after the earthquake. A principal function of the OFC is associated with extinction of conditioned fear. Previous lesion studies revealed that OFC lesion caused resistance to the extinction of conditioned fear in both non-human primates[48] and human patients with OFC lesions.[49] Neural responses in the OFC were preferentially enhanced with those in the amygdala during extinction,[50] and this relationship is crucial in the voluntary regulation of emotions.[51,52] In particular, the left lateral part of the OFC is involved in emotional distraction. The left OFC is also involved in suppression of emotional distracters during working memory performance.[53] In fact, patients with PTSD had less activity in the OFC than normal control subjects during extinction to conditioned fear[54] and emotion regulation.[55] Given the previous

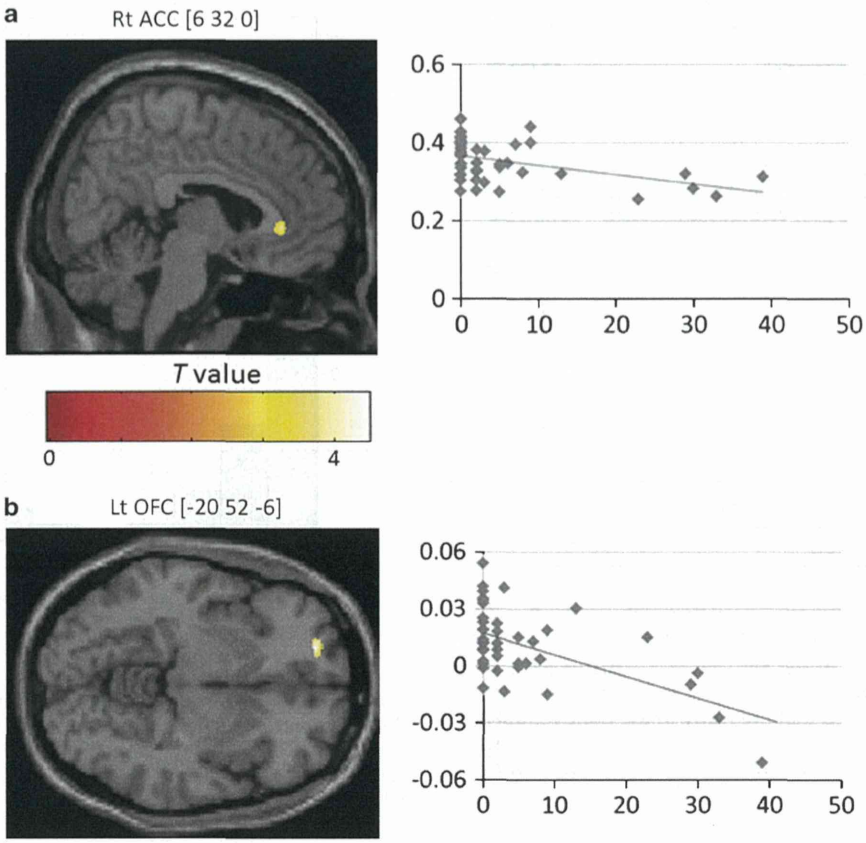

Figure 2. Relationship between total clinician-administered PTSD scale (CAPS) scores and regional grey matter volume (rGMV). Total CAPS scores were negatively associated with Pre rGMV in the right anterior cingulate cortex (ACC; **a**, Spearman's $\rho = -0.42$, $P = 0.005$) and Post-Pre rGMV in the left orbitofrontal cortex (OFC; **b**, Spearman's $\rho = -0.43$, $P = 0.004$), illustrated by the scatter plots on the right side. Vertical axes represent rGMV at peak voxels in each cluster, and horizontal axes indicate total CAPS scores. The predictor brain regions are shown on the left. Rt, right; Lt, left.

Table 2. MNI coordinates, voxel sizes, z-scores and *P*-values for results of the SPM analyses

Brain region	*MNI coordinates*			*κ (voxels)*	*z-scores*	*P-values (SVC)*
	x	y	z			
Pre						
Rt ACC	6	34	0	145	3.76	0.020
Post-Pre						
Lt OFC	−20	52	−6	124	4.03	0.034

Abbreviations: ACC, anterior cingulate cortex; Lt, left; MNI, Montreal Neurological Institute; OFC, orbitofrontal cortex; Rt, right; SVC, small volume correction.

findings, our results indicate that decreased OFC volume might reflect difficulty in distracting emotional memories of experiences related to the earthquake. Therefore, survivors with high CAPS scores are likely to have more difficulty in distracting emotional memories compared with those with low CAPS scores.

Some limitations should be considered when interpreting the present results. First, our study included no subjects with supra-threshold PTSD symptoms and no control group without experience of the earthquake. These were predetermined limitations of this study, because most of the candidates in our pre-earthquake database were supposed to have been affected by the earthquake to some extent, but not exposed to life-threatening experiences. We believe that the investigation of subjects with subclinical PTSD symptoms can provide sufficient evidence, like previous studies.[14,15,56–59] However, a further longitudinal study of patients with supra-threshold PTSD symptoms caused by traumatic events is necessary to examine whether the brain structural changes in our current investigation is applicable to such individuals.

Second, the CAPS scores were not normally distributed across subjects (Figure 1). As this distribution may be consistent with the expected distribution in the normal population, investigating such a population was inevitable. Even if multiple regression analysis on SPM allows independent values of non-normal distribution, the distorted distribution of the CAPS score may reduce the statistical power. Actually, of the 42 participants, 37 scored 0 to 19 and 5 scored 20 to 39 in the CAPS, which are categorized as asymptomatic and subthreshold PTSD, respectively.[25] By applying these two categories to group comparison analysis (that is, two sample *t*-test) as non-PTSD and subclinical PTSD groups, respectively, we found that similar brain regions including the Pre rGMV in the right ventral ACC and the Post-Pre rGMV in the left OFC were negatively associated with the CAPS scores ($P < 0.05$, small volume correction in each region of interest). However, another problem is that the sample size for subjects with subthreshold PTSD was too small to perform group comparison. We could not exclude the possibility that regions other than the ACC and OFC may show significant association with the CAPS scores, if the sample size was improved.

Third, although all subjects were diagnosed as normal, it was suspected that the anxiety level of subjects was relatively high. We

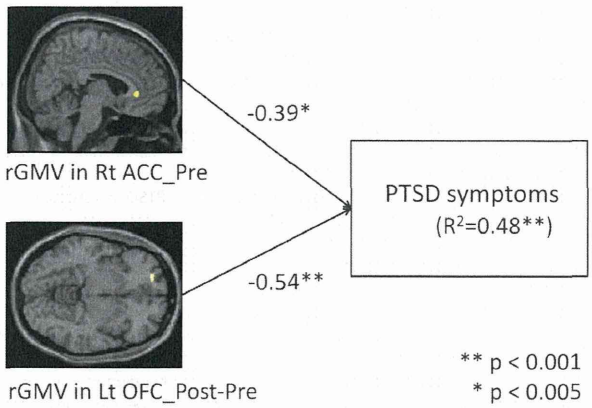

Figure 3. *Post hoc* regression analysis implemented on a path diagram. The relationships between total clinician-administered PTSD scale (CAPS) scores and Pre regional grey matter volume (rGMV) in the right ventral anterior cingulate cortex (ACC; $\beta = -0.39$, $P < 0.005$), and between total CAPS scores and Post-Pre rGMV in the left orbitofrontal cortex (OFC; $\beta = -0.54$, $P < 0.001$) are shown. The predictor brain regions are shown on the left, which predicts posttraumatic stress disorder (PTSD) symptoms evaluated by CAPS. Rt, right; Lt, left.

supposed that such high anxiety level might be caused by the aftermath of the earthquake, such as frequent afterquakes and dispersed radioactive material leaking from the nuclear plants.[60] The high anxiety levels may have some effects on the MR imaging findings. However, even if we adjusted both state and trait anxiety scores as additional covariates, we showed that similar brain regions, including the Pre rGMV in the right ventral ACC and the Post-Pre rGMV in the left OFC, were negatively associated with total CAPS scores ($P < 0.001$, uncorrected). Therefore, our MR imaging findings were robust despite the high anxiety level.

Fourth, numerous studies revealed smaller rGMV associated with PTSD in the amygdala and hippocampus,[6] and also smaller hippocampal volume represented vulnerability factor for PTSD,[16] but we failed to detect these brain regions either as a vulnerability factor or an acquired sign of PTSD. VBM may not detect such small and localized GMV reduction, because false-positive or false-negative VBM findings may arise from the change of spatial normalization.[7,61] Actually, when applying more lenient thresholds ($P < 0.01$, uncorrected) to this study, we could find negative associations between the CAPS scores and both Pre rGMV and Post-Pre rGMV in the hippocampus.

Despite these limitations, the present longitudinal study could distinguish structural changes representing a vulnerability factor from structural changes representing an acquired sign of PTSD symptoms in the early stage of adaptation to trauma. These findings may be essential to discriminate between survivors with and without PTSD symptoms soon after a traumatic event, and between survivors who will and will not develop PTSD, even in the normal population. These findings provide a better understanding of the posttraumatic responses in the early stage of adaptation to trauma, and may contribute to the development of effective methods to prevent PTSD in the normal population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Causal Relationship Between Psychological Distress After a Severe Earthquake and Brain Structural Changes

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Abstract— **Brain gray and white matter structural alternations in several brain regions including hippocampus, amygdala, the anterior cingulate cortex (ACC) and the prefrontal cortex (PFC) were reported in patients with posttraumatic stress disorder (PTSD) or stressful life events. However, the causal relationship between psychological distress after stressful life events and the structural alteration has remained unclear, because of difficulties with prospective studies. A magnitude 9.0 earthquake hit Japan on March 11, 2011. Many survivors, even those without PTSD, needed psychological support. Since we had much structural MRI data from subjects living around Sendai city area before the quake, this tragedy provided a rare opportunity to investigate longitudinal brain structural changes associated with such a disaster. In this article, we introduce our recent investigations which revealed the brain structural alternations as (a) vulnerability factors and (b) acquired signs of psychological distress after the disaster. Actually, we had collected structural MRI data from a group of healthy subjects before the quake, and we recruited 42 subjects from this group to examine their structural MR images 3 to 4 months after the quake. We demonstrated that smaller regional grey matter volume (rGMV) in the right ventral ACC, and lower fractional anisotropy (FA) in the right cinglum (Cg) before the earthquake were vulnerability factors of psychological distress after the earthquake, and decreased rGMV in the left OFC and increased FA in the left Cg and uncinate fasciculus (Uf) from before to after the earthquake were acquired signs of psychological distress after the earthquake. The findings provide further evidence that the ACC/Cg and the OFC/Uf, which are involved in fear conditioning, and emotional regulation, play an important role in the pathogenesis of PTSD.**

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I. Introduction

The Great East Japan Earthquake, a severe earthquake with a magnitude of 9.0, hit Japan on March 11, 2011. Stress-related disorders such as acute stress disorder and posttraumatic stress disorder (PTSD) are likely to occur among a large number of survivors following a severe disaster [1], and even those without PTSD often require psychological support [2]. In fact, many survivors maintain high anxiety levels due to the earthquake aftermath including frequent aftershocks and dispersed radioactive material leaking from nuclear plants [3]. Distinguishing neurological underpinnings as a vulnerability factor from the acquired signs of psychological distress soon after a disaster among non-PTSD survivors might contribute to a better understanding of posttraumatic responses, early detection of PTSD, and PTSD prevention among survivors.

The neurological underpinnings of patients with PTSD [4–6] as well as those of non-PTSD subjects after stressful life events [7, 8] have been well characterized. Previous neuroimaging studies of patients with PTSD revealed morphological changes in several brain regions, including the hippocampus [4], amygdala [4], anterior cingulate cortex (ACC) [9–11], insula [12] and orbitofrontal cortex (OFC) [13–15], which were also found in healthy adults after stressful life events [7, 8]. Recently, diffusion-tensor imaging (DTI) [16] was used to investigate white matter structural changes in patients with PTSD [17–20], also in health survivor of a disaster [21]. DTI is used to measure the magnitude and direction of water diffusion [i.e., fractional anisotropy (FA)] in brain tissue. FA is modulated by the degree of myelination, axonal membrane thickness and diameter, and/or the amount of axon parallel organization [22, 23] and is thus an indicator of white matter pathway strength or integrity. Previous DTI studies suggest that PTSD patients have altered white matter

integrity in the anterior cingulum (Cg) which is adjacent to the anterior cingulate cortex (ACC) [17–20].

Nevertheless, the causal relationships between the brain structural changes and the psychological response to stressful life events remained unclear, because of difficulties with prospective studies [24]. Although, some longitudinal studies had examined patients with PTSD after traumatic events, they failed to find longitudinal brain structural changes [13, 25, 26]. Longitudinal changes of white matter integrity in subject with PTSD or stressful life events were unexamined. Even a recent longitudinal study revealed that decreased volumes in the ACC and hippocampus/ parahippocampus were associated with the number of stressful life events, the impacts of stress related responses on brain structure were not examined [7]. Evaluation of monozygotic twin pairs with combat-related PTSD has provided evidence that smaller hippocampal volume is a vulnerability factor for PTSD [27], and smaller pregenual ACC represents an acquired sign of PTSD [10]; however, the significance of longitudinal structural changes within individuals as vulnerability factors and acquired signs of psychological distress have remained unclear.

In this article, we introduce our recent investigations which revealed the brain structural alternations as vulnerability factors and acquired signs of psychological distress after the earthquake [28, 29], based on a longitudinal study of structural MRI data obtained before and after the earthquake in normal subjects. In fact, we had collected much structural MRI data from a group of healthy subjects before the quake. Therefore, this tragedy provided a rare opportunity to investigate brain structural changes associated with such a disaster. Forty two subjects were recruited from this group to examine MR images 3–4 months after the earthquake. PTSD symptoms were also assessed using the Japanese version of the clinician-administered PTSD scale (CAPS) structural interview [30]. Anxiety levels were assessed by using the Japanese version of the State–Trait Anxiety Inventory (STAI) [31, 32]. We hypothesized that (a) vulnerability factors for psychological distress after the earthquake could be detected by a significant association with individual differences of bran structures before the earthquake, and (b) the acquired signs could be detected by a significant association with individual difference of brain structural changes from before to after the earthquake around brain regions previously implicated in PTSD.

II. Materials and Methods

A. Recruitment and selection of participants

Eligible participants were recruited from undergraduate and postgraduate students of the Tohoku University community, who met the eligibility criteria of having no history of neuropsychiatric disorders and right-handed. All candidates had participated in past MR imaging experiments conducted in our laboratory undergone structural MR imaging within the 2 years before the earthquake, and declared agreements for re-analyses of MR images scanned before the earthquake in advance. Since all the candidates lived around Sendai city, where was strongly affected by the earthquake, we did not plan to recruit control subjects without experiencing the earthquake. Neuropsychiatric disorders were screened by using the mini international neuropsychiatric interview (M.I.N.I.) [33, 34]. Handedness was assessed by the Edinburgh Handedness Inventory [35]. Among numerous candidates in our database of the past experiments, we could contact with forty two of them. All candidates met above eligible criteria and made written informed consents before participating in the current study to examine possible effect of psychological trauma on brain structure, in accordance with the Declaration of Helsinki [36]. The M.I.N.I. confirmed that no subject had any history of psychiatric illness including PTSD and no subjects were exposed to life-threatening experiences due to the earthquake and tsunami, as well. The current study and all previous studies were approved by the Ethics Committee of Tohoku University.

B. Psychological evaluations

All participants were interviewed by trained psychologists using the CAPS structured interview [30, 37]. In accordance with the M.I.N.I., no subject was diagnosed as having PTSD. Of the 42 participants, 8 subjects filled more than one but not all criteria of three clusters of PTSD symptoms including re-experiencing of the event, avoidance and hyperarousal. In addition, the highest total CAPS score was 39, which is categorized as subthreshold PTSD [38]. Therefore, we could regard all subjects as non-PTSD. Levels of anxiety and depression were evaluated using the state-trait anxiety inventory [31, 32] and the Center for Epidemiologic Studies Depression Scale [39, 40]. The diagnostic structured interview and MR imaging were conducted at 3 to 4 months after the earthquake. All psychological measurements were evaluated only after the earthquake. Demographic characteristics of the subjects are shown in Table 1.

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF NON-PTSD SURVIVORS

Number of subjects (male/female)	42 (33/9)
Age (years)	21.7 ± 1.7
Number of previous lifetime traumas	1.98 ± 0.98
Periods (days) from pre- to post-earthquake MR scans	244.4 ± 137.8
CAPS	
Total	5.7 ± 10.0
Re-experience	1.6 ± 2.5
Avoidance	2.0 ± 4.4
Hyperarousal	2.1 ± 4.5
CESD score	11.6 ± 10.2
STAI scores	
State	41.6 ± 11.4
Trait	43.0 ± 10.0

Values are shown as mean ± standard deviation.

CESD, Center for Epidemiologic Studies Depression; STAI, state-trait anxiety inventory.

C. Image acquisition

All MR imaging data acquisition was conducted with a 3-T Philips Intera Achieva scanner. Using a MPRAGE sequence, high-resolution T1-weighted structural images (240 × 240 matrix, repetition time = 6.5 ms, echo time = 3 ms, field of view = 24 cm, 162 slices, 1.0 mm slice thickness) were collected from all the subjects. Of the 42 subjects, 30 subjects who had undergone DTIs before the earthquake underwent DTIs after the earthquake, as well. The diffusion-weighted data were acquired using a spin-echo EPI sequence (TE = 55 ms, FOV = 22.4 cm, 2 × 2 × 2 mm^3 voxels, 60 slices). The diffusion weighting was isotropically distributed along 32 directions (*b* value = 1,000 s/mm^2). Additionally, a data set with no diffusion weighting (*b* value = 0 s/mm^2; b0 image) was acquired. Detailed information about MR images data acquisition were described in our previous articles [28, 29].

D. Pre-processing of MR imaging data and statistical analysis

(1) MR imaging data analysis for rGMV

Pre-processing and data analysis of rGMV were performed following steps. First, post-earthquake images were coregistered with pre-earthquake images in each subject on SPM2. Preprocessing of the morphological data was performed with VBM2 software [41], an extension of SPM2. The resulting maps representing the rGMV before the earthquake (Pre rGMV) and the rGMV change between before and after the earthquake (Post-Pre rGMV) were then forwarded to the group-level analysis. The group level analysis tested for the relationship between individual severity of PTSD symptoms measured by the CAPS and rGMV. Voxel-by-voxel multiple regression analyses were performed using the CAPS scores for Pre rGMV and Post-Pre rGMV on SPM5. The analysis was performed with age, total brain volume, and periods between pre- and post-earthquake MR imaging data acquisition as additional covariates. A significant level was set at $P = 0.05$ corrected for multiple comparisons. Small volume correction (SVC) [42] was performed to examine each ROI with a hypothesis (amygdala, hippocampus, insula, ACC and OFC) [4, 9–15] using a lenient threshold of $P = 0.001$ uncorrected, and a $\kappa = 100$ to suppress the possibility of small clusters arising by chance. Finally, to verify the effect of the structural changes on the CAPS scores, regression analysis was performed employing Pre rGMV and Post-Pre rGMV at peak voxels in each cluster as explanatory variables, and total scores of CAPS as independent variables.

(2) Diffusion tensor imaging data analysis for FA

Pre-processing and data analysis of diffusion tensor images were performed using statistical Parametric Mapping software (SPM5; Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks, Inc., Natick, MA, USA). The FA map image of each participant was spatially normalized to give images with 2 × 2 × 2 mm voxels and spatially smoothed using a Gaussian kernel of 10 mm FWHM. The resulting maps representing the FA were then forwarded to a group regression analysis. The group-level analysis tested for a relationship between individual state anxiety as measured by the STAI and regional FA. Voxel-by-voxel multiple regression analyses (VBA) were performed using the state anxiety for Pre FA and Post – Pre FA in VBM5 on SPM5. The analysis was performed with sex and period between pre- and post-earthquake MR imaging data acquisition as additional covariates. All tests of FA were performed using an absolute threshold of FA >0.2 [43]. Significant regions were inferred using cluster-level statistics [44]. Only clusters with a *p*-value <0.05 after correction for multiple comparisons at cluster size and an uncorrected voxel-level cluster-determining threshold of $p < 0.0025$ were considered statistically significant in this analysis [45]. Finally, to evaluate the relative association strength between white matter structural changes and state anxiety level, we applied structural equation modeling (SEM) by employing state anxiety scores from STAI, Pre FA, and Post – Pre FA at peak voxels in each cluster as observed variables.

Detailed information about MR imaging data and statistical analysis were described in our previous articles [28, 29].

TABLE 2. MNI COORDINATES, VOXEL SIZES, Z SCORES AND *P* VALUES FOR RESULTS OF THE SPM ANALYSES

Brain region	MNI coordinates			κ (voxels)	z scores	*P* values (SVC)
	x	y	z			
Pre						
Rt ACC	6	34	0	145	3.76	0.020
Post-Pre						
Lt OFC	−24	52	−6	124	4.03	0.034

MNI, Montreal Neurological Institute; Rt, right; Lt, left.

ACC, anterior cingulate cortex; OFC, orbitofrontal cortex; Rt, right; Lt, left

III. RESULTS

A. VBM results for rGMV

After controlling for age, total brain volume, and periods between pre- and post-earthquake MR imaging data acquisition, the total scores of CAPS were significantly associated with smaller Pre rGMV in the right ventral ACC (Montreal Neurological Institute [MNI] coordinates coordinates, x = 6, y = 32, z = 0; Fig. 1a, Table 2), and decreased rGMV from Pre to Post earthquake in the left OFC (MNI coordinates, x = −20, y = 52, z = −6, Fig. 1b, Table 2), based on region of interest (ROI) analysis. Post hoc regression analysis revealed that Pre rGMV in the right ventral ACC and Post-Pre rGMV in the left OFC accounted for 49% score variance in the CAPS ($F(2, 39) = 18.28$, $R^2 = 0.48$, $P < 0.001$, Fig. 1c).

B. VBA results forFA

After controlling for sex and period between pre- and post-earthquake MRI data acquisition, the state anxiety scores were negatively associated with Pre FA in the right Cg (MNI coordinates, x = 20, y = 36, z = 0; Fig. 2a, Table 3) and positively associated with Post – Pre FA in the left

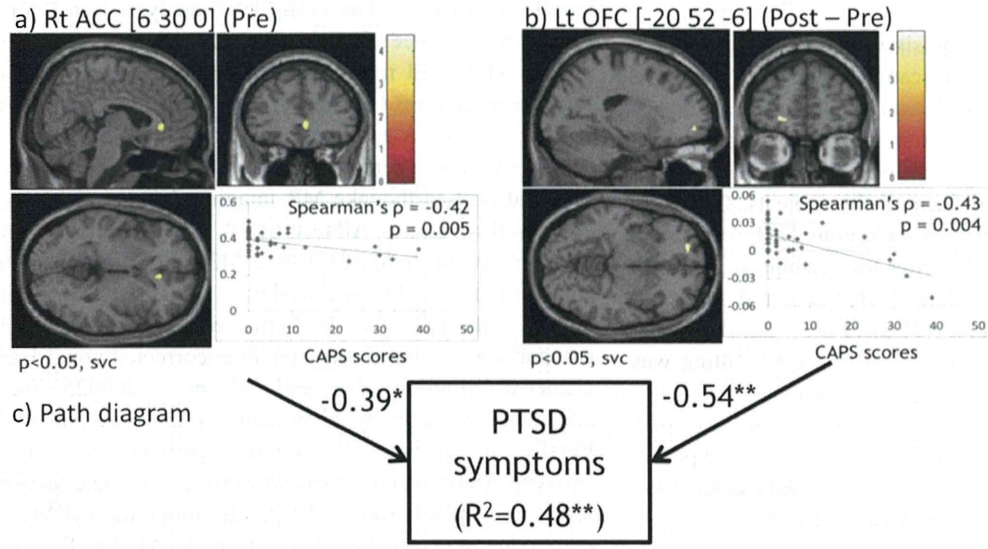

Fig. 1. Relationship between total CAPS scores and rGMV [28]
Total CAPS scores were negatively associated with Pre rGMV in the right ACC (**a**, Spearman's Rho = −0.42, $P = 0.005$) and Post-Pre rGMV in the left OFC (**b**, Spearman's Rho = −0.43, $P = 0.004$), illustrated by the scatter plots on the right side. Vertical axes represent rGMV at peak voxels in each cluster, and horizontal axes indicate total CAPS scores. c) Post hoc regression analysis implemented on a path diagram. The relationships between total CAPS scores and Pre rGMV in the right ventral ACC ($\beta = -0.39$, $P < 0.005$), and between total CAPS scores and Post-Pre rGMV in the left OFC ($\beta = -0.54$, $P < 0.001$) are shown. The predictor brain regions are shown on the left, which predicts PTSD symptoms evaluated by CAPS. Rt, right; Lt, left.

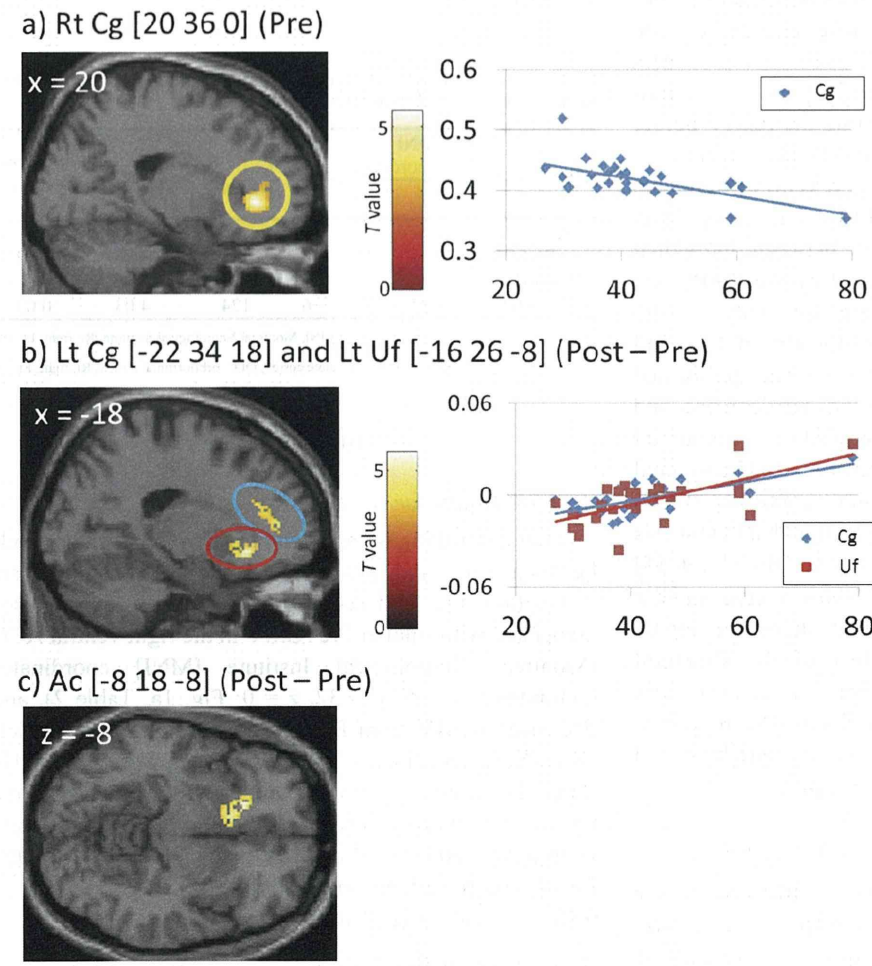

Fig. 2. Relationship between state anxiety and FA[29]
State anxiety scores were negatively associated with Pre FA in the right anterior Cg (**a**, $r = -0.61$, $p = 0.0004$) and Post – Pre FA in the left anterior Cg (**b**, $r = 0.70$, $p = 0.00002$) and the left Uf (**b**, $r = 0.65$, $p = 0.0001$), as illustrated by the scatter plots on the right. Vertical axes represent FA values at peak voxels in each cluster and horizontal axes indicate total state anxiety scores. The left Uf and the Ac were included in the same cluster.
FA, fractional anisotropy; Rt, right; Lt, left; Cg, cingulum; Uf, uncinate fasciculus.

anterior Cg (MNI coordinates, x = −22, y = 34, z = 18; Fig. 2b; Table 3), and a cluster including the left uncinate fasciculus (Uf; MNI coordinates, x = −18, y = 26, z = −8; Fig. 2b, Table 3) and the anterior commissure (Ac; MNI coordinates, x = −10, y = 18, z = −8; Fig. 2c, Table 3). Furthermore, SEM data showed that Pre FA in the right anterior Cg and Post – Pre FA in the left anterior Cg and the left Uf accounted for 60% of the score variance in state anxiety ($R^2 = 0.60$; Fig. 3).

TABLE 3. MNI COORDINATES, VOXEL SIZES, Z-SCORES AND *P*-VALUES FOR RESULTS OF THE SPM ANALYSES

Brain region	MNI coordinates			κ (voxels)	z scores	*p*-values (cluster level)
	x	y	z			
Pre						
Rt Cg	20	36	0	310	4.62	0.010
Post-Pre						
Lt Cg	−22	34	18	128	4.34	0.026
Lt Uf	−18	26	−s8	161	4.12	0.013
Ac*	−10	18	−8		3.83	

MNI, Montreal Neurological Institute; Rt, right; Lt, left.

Cg, cingulum; Uf, uncinate fasciculus; Ac, anterior commissure

* The Ac is included in the same cluster as the Lt Uf.

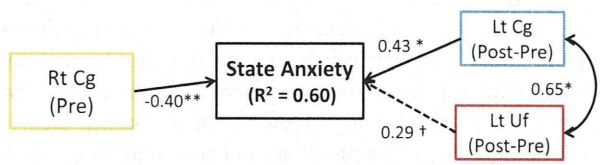

Fig. 3. SEM implemented on a path diagram [29]
The strength of the path coefficients between state anxiety scores and Pre FA in the right anterior Cg (−0.40, $p < 0.001$) and between state anxiety scores and Post – Pre FA in the left anterior Cg (0.43, $p < 0.01$) and the left Uf (0.29, $p = 0.06$) are shown. The path coefficient strength between Post – Pre FA in the left anterior Cg and the left Uf (0.65, $p < 0.005$) are shown as well. The brain regions that predict state anxiety level evaluated by STAI are shown on the left.
FA, fractional anisotropy; Rt, right; Lt, left; Cg, cingulum; Uf, uncinate fasciculus; STAI, State–Trait Anxiety Inventory

IV. DISCUSSION

This article introduces the first evidence of the causal relationship between psychological distress after a severe earthquake and brain structural changes. The longitudinal MRI studies demonstrated that smaller rGMV in the right ventral ACC, and lower FA in the right anterior Cg before the earthquake were vulnerability factors of psychological distress after the earthquake, and decreased rGMV in the left OFC and increased FA in the left anterior Cg and Uf from before to after the earthquake were acquired signs of psychological distress after the earthquake.

Our longitudinal studies provide further evidence of the causal relationships between brain structural changes and psychological responses to the disaster. Previous longitudinal studies investigating brain structural changes only after the traumatic events [13, 25, 26], even a prospective study of combat related PTSD [46], have failed to find the longitudinal brain structural changes in patient with PTSD. Another longitudinal study have revealed that the number of stressful life events are associated with the decreased volumes in the ACC, hippocampus/parahippocampus in healthy subjects, the causal relationship between psychological responses to the stressful events and brain structural changes were not examined [7]. On the other hand, in contrast to our findings, smaller hippocampal volume as a vulnerability factor [27], and smaller pregenual ACC as an acquired sign of PTSD [10] were unveiled by investigations of same monozygotic twin pairs with PTSD. Apparent discrepancy existed; however, the reason of the discrepancy came from fundamental differences in study designs. Monozygotic twin studies could not distinguish an acquired sign of PTSD from an acquired one from birth to trauma, because of the cross-sectional design after the traumatic events [10]. Taken our present findings into account, smaller ACC volume is not an acquired sign, but an acquired vulnerability of PTSD before exposed to traumatic events.

Several evidence support the notion that smaller ventral ACC volume and lower white matter integrity in the right anterior Cg are vulnerability factors for psychological responses to a stressful life event. The anterior Cg bundle is a part of the principal white matter tract in the Papez circuit, which includes the ACC and the amygdala [47]. An essential role of the ventral ACC is the processing of anxiety and fears [48], which is supposed to be highly related to the manifestation of the clinical symptoms of PTSD [5]. In fact, smaller ACC volume and lower white matter integrity in Cg are robust findings in patients with PTSD [9–11, 18–20], also in normal subjects after stressful life events [7, 8, 21]. Our present findings provide evidence that the smaller ventral ACC volume and the lower white matter integrity in the right anterior Cg are the pretrauma vulnerability factors for psychological responses to the disaster.

The cognitive functions of the OFC and the Uf indicate psychological responses of the survivors soon after the earthquake. A principal function of the OFC is associated with regulation of emotions [49, 50]. Then, the Uf is a principal white matter tract that connects the OFC and limbic regions including the amygdala and the anterior temporal cortices [51, 52]. We supposed that the decreased OFC volume might reflect difficulty in cognitive control of emotion, and the increased FA in the Uf reflect frequent access of emotional regulation related to post-earthquake stress. The frequent access strengthens white matter integrity, based on the notion of previous cognitive training studies suggesting the white matter integrity related to trained cognitive functions in question increased [53, 54]. This interpretation is consistent with the interpretation of FA elevation caused by temporary activation of the Cg in PTSD [17]. Therefore, those who have difficulty in emotional regulation were likely to have higher psychological distress, and needed to regulate emotions more frequently than did those with lower psychological distress soon after the earthquake.

V. Conclusion

The longitudinal investigations distinguished structural brain changes that distinguish a vulnerability factor rather than an acquired sign of psychological distress after the disaster. These findings might be essential for discriminating between survivors with and without emotional distress soon after a disaster and between survivors who will and will not experience elevated levels of psychological distress, even in the normal population. These kinds of brain structural studies allow us to infer cognitive functions, dysfunctions, and personalities associated with the brain structures in question. Therefore, the findings improve our understanding of the psychophysiological responses to disasters and might contribute to the development of effective methods to prevent stress-related disorders in the normal population, specifically PTSD. In particular, the findings let us know what kind of cognitive functions, dysfunctions, and personalities we should screen for in a disaster area. In addition, the cognitive functions and personalities would be a target of psychotherapy for stress-related disorders. We also believe that investigations of normal populations without PTSD make a significant social contribution in that a large population was resilient to the disaster.

Finally, we present the future directions of our on-going investigations. First, we plan to follow the subjects for least 2 years after the earthquake. These follow-up investigations will allow us to observe whether the brain structural changes recovered or continued. In addition, we will be able to detect late-onset brain structural changes, as the present study detected brain structural changes soon after the disaster only. Second, we will attempt to recruit a control group that did not experience the earthquake. The lack of a control group was a predetermined limitation of the present study, because most of the candidates in our pre-earthquake database were believed to be living near Sendai, which was affected by the earthquake. To overcome this issue, we plan to make a longitudinal magnetic resonance imaging (MRI) dataset of healthy subjects who did not experience the disaster. We have started to obtain brain MR images and psychological data from such healthy subjects. Finally, we propose combining the brain MRI data, psychological data, and genomic information to explore genomic vulnerability factors for brain structural changes and psychological distress after a disaster.

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