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Original Article

Functional magnetic resonance and diffusion tensor imaging analysis of verbal working memory in patients with temporal lobe epilepsy

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Abstract: Objective: This study aimed to investigate the relationships between brain functions and verbal working memory (VWM) impairments in patients with temporal lobe epilepsy (TLE). Methods: Functional magnetic resonance imaging was performed on 15 healthy controls and 15 patients with TLE while they performed the N-back task in order to compare the brain areas that were activated during VWM. Diffusion tensor imaging was then performed to examine the integrity of the VWM-related fiber bundles. Results: The patient group exhibited a significant reduction in the activation of the brain areas that were negatively activated in the VWM task in the control group ($P < 0.05$), and no activation was observed in the patient group in the brain areas that were positively activated in the VWM task in the control group. The fractional anisotropy values of both cingulate bundles were significantly reduced in the patient group compared with those in the control group ($P < 0.05$), and these values were positively correlated with the number of activated pixels in the ipsilateral prefrontal areas (left: $r = 0.790$, $P < 0.05$; right: $r = 0.852$, $P < 0.05$). Conclusions: Patients with TLE exhibited impaired VWM functions that were related to subtle alterations in the cingulate bundles.

Keywords: Temporal lobe epilepsy, functional magnetic resonance imaging, diffusion tensor imaging, verbal working memory

Introduction

Patients with temporal lobe epilepsy (TLE) exhibit cognitive impairments, among which memory impairments are the most prominent, and these impairments seriously affect the patients' quality of life. Our brain is a network that consists of spatially distributed but functionally linked regions that continuously share information with each other. Interestingly, recent advances in the acquisition and analysis of functional neuroimaging data have catalyzed the exploration of functional connectivity within the human brain. Functional connectivity is defined as the temporal dependency of neuronal activation patterns of anatomically separated brain regions. In recent years, an increasing body of neuroimaging studies has started to explore functional connectivity by measuring the amount of coactivation among brain regions in resting-state functional magnetic resonance imaging (fMRI) time series investigations. These studies have revealed interesting new findings about the functional connections of specific brain regions and local networks as well as important new insights into the overall organization of functional communication in the brain [1].

Meta-analyses, which examine and analyze the results from a number of different studies, are a powerful tool for integrating the data of functional imaging studies on a broader psychological construct, examining the consistency of the results across various paradigms, and evaluating the effects of different experimental implementations [2]. Cortical and white matter maturation each play unique roles in the development of working memory [3]. The intrinsic resting-state activity has been suggested to facilitate or permit specific brain circuit engagement during the performance of a cognitive

task and to predict subsequent task-evoked brain responses and behavioral performances [4]. These previous studies have also revealed remarkable differences among patients with epilepsy that were consistent with the hypothesis of the reorganization of brain circuitry in epilepsy [5]. Thus, examinations of functional connectivity provide a unique means for identifying abnormalities in brain networks that cannot be discerned at the level of behavioral output through neuropsychological testing. More broadly, the application of functional connectivity methods in task-based fMRI investigations provides the opportunity to define specific task-related networks [6]. Hippocampal activity is progressively suppressed as the working memory load increases, which has been suggested to occur in order to maintain good performance, which has implications on the current understanding of the role of the hippocampus in working memory [7]. These findings suggest that the segregation of the task-positive and task-negative functional connectivity networks that support working memory is disrupted in TLE and that this disruption is associated with the abnormal structural connectivity of the sclerosed hippocampus. The coactivation of parietotemporal regions is associated with poorer working memory might be associated with working memory dysfunction in patients with TLE [8]. These findings provide further evidence that working memory is disrupted in hippocampal sclerosis and that the impaired integrity of both gray and white matter can be seen in the functionally relevant areas. We have suggested that these observations form the structural basis of the impairments of working memory and indicate widespread and functionally significant structural changes in patients with apparently isolated hippocampal sclerosis [9]. Previous reports on the alterations of the visuospatial working memory-related resting-state network in patients with TLE suggest that these abnormalities might underlie the visuospatial working memory impairments in patients with right TLE and that functional compensation might occur by enlarging the functional connectivity within the ipsilateral cerebral network [10]. In this study, blood-oxygen-level dependent (BOLD)-fMRI was performed in order to examine verbal working memory (VWM) activation in patients with TLE and analyze the brain areas that were related to the VWM impairments in these patients. In addition, BOLD and diffusion tensor imaging (DTI) technologies were combined in order to conduct a correlation analysis of the damaged functional regions and the white matter fiber bundles and investigate the relationships between VWM-related brain regions and other brain structures in patients with TLE.

Materials and methods

Subjects

This study was examined, approved, and permitted by the hospital's ethics committee. The diagnostic criteria of TLE were defined according to the Classification Guidelines for seizures (International League Against Epilepsy, 2001). The inclusion criteria for the patients with TLE were as follows: 1) clinical symptoms of epilepsy that suggested that the seizures originated from the temporal lobe; 2) imaging evidence of the presence of temporal lobe lesions, hippocampal atrophy, or sclerosis, while other parts were normal; or 3) electroencephalographic evidence that was recorded during seizure episodes or episode intervals that showed that the epileptic lesions were in the temporal lobe. Patients that met two of the above three conditions were diagnosed with TLE seizures [11]. The exclusion criteria included any of the following: 1) Mini-Mental State Examination scores less than 24 points; 2) comorbid neurological/psychiatric diseases or other progressive systemic diseases that might affect intelligence; or 3) age less than 18 years or over 60 years. Fifteen healthy volunteers who matched the gender, age, educational level, and Mini-Mental State Examination scores of the patients in the TLE group were enrolled in the study. All of the experimental subjects were fully informed of the purpose and methods of the study, and all provided signed informed consent forms (**Table 1**). This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. Written informed consent was obtained from all participants.

BOLD-fMRI experimental program

Our experiments used the classical N-back task model of VWM [12]. This study selected two different tasks (0 back and 2 back, with different levels of difficulty) as the stimuli, and the

Table 1. Basic and clinical information of included patients in the study

Number	Gender/sex	Age	Clinical diagnosis	Seizures	Date of MRI
1	male	26	EP	Tonic-clonic epilepsy	bilateral hippocampus sclerosis
2	female	30	EP	complex partial seizures	right hippocampus sclerosis
3	male	29	EP	Tonic-clonic epilepsy	left hippocampus sclerosis
4	male	27	EP	Sensory Seizures	normal
5	female	30	EP	Tonic-clonic epilepsy	bilateral hippocampus sclerosis
6	male	28	EP	complex partial seizures	bilateral hippocampus sclerosis
7	female	26	EP	complex partial seizures	normal
8	female	23	EP	complex partial seizures	left hippocampus sclerosis
9	female	29	EP	complex partial seizures	left hippocampus sclerosis
10	female	27	EP	complex partial seizures	bilateral hippocampus sclerosis
11	male	28	EP	complex partial seizures	normal
12	male	24	EP	Tonic-clonic epilepsy	bilateral hippocampus sclerosis
13	female	28	EP	complex partial seizures	right hippocampus sclerosis
14	male	28	EP	Tonic-clonic epilepsy	right hippocampus sclerosis
15	male	26	EP	Tonic-clonic epilepsy	right hippocampus sclerosis

0-back task was set as the baseline task. The VWM-activated brain networks were determined by subtracting the brain regions that were activated in the 0-back task from the brain regions that were activated in the 2-back task.

Equipment and imaging methods

The Achieva 3.0T superconducting MRI scanner (Philips International B.V., Amsterdam, The Netherlands) was used. The task stimulus was presented to the participants with the SAMRTEC SA-9800 stimulation system (Shenzhen Sinorad Medical Electronics Co., Ltd., Shenzhen, China). For the structural scanning, the settings were the following: spin-echo sequence (T1-weighted), repeated excitation time = 60 ms, echo time = 16 ms, slice thickness = 5 mm, pitch = 1 mm, and scanning field = 220 × 220 mm. For the functional scanning, the settings were the following: gradient echo-echo planar imaging sequence, repeated excitation time = 2,000 ms, echo time = 30 ms, slice thickness = 5 mm, interlayer spacing = 6 mm, scanning field = 220 × 220 mm, and flip angle = 90°, and the scanning pace was the same as the pace used for the structural scanning. The total scanning time was 360 s.

For DTI, a single-excitation echo-planar imaging sequence tensor was used with the following parameters: repeated excitation time = 2,000 ms, echo time = 30 ms, matrix = 112 × 112, slice thickness = 2 mm, interlayer spacing = 2 mm, and b = 1,000, and the diffusion gradients were conducted in 32 directions. The total scanning time was 600 s.

Processing of the fMRI data

The data were analyzed by a MATLAB R2012a workstation and processed with Statistical Parametric Mapping Software (SPM8, http://www.fil.ion ucl.ac.uk/spm/). All of the data underwent space preprocessing, which was followed by intragroup analyses that were performed in order to determine the activated voxel data of the 2-back minus 0-back results for the two groups. The statistical probability threshold was set as $P < 0.05$, and the threshold of the activated voxel range was set to 30 voxels. Subsequently, intergroup analyses were performed with *t*-tests with the same statistical threshold probability and activated voxel range threshold. The brain areas that exhibited significant differences in activation between the two groups were determined, and the xjview software was used to determine the anatomic sites of the differences in activation (Montreal Neurological Institute coordinates), Z values, and number of voxels in the activated areas.

DTI data processing

The DTI studio software (http://cmrm.med.jhmi.edu) was used to process the DTI data, and the image processing sequences were performed

Table 2. Intergroup MRI comparison of VWM activation difference between the 2 groups

Location	Z	MNI			Voxel
		X	Y	Z	
Left parietal lobe	2.87	-60	-41	24	607
Right parietal lobe	2.56	48	-44	33	86
Right temporal lobe	2.59	42	19	-6	337
Left frontal lobe	2.9	-9	-2	-57	110
Right frontal lobe	3.5	48	26	17	835
Left insula	2.5	-42	1	-4	75
Right insula	3.1	48	10	3	180
Left posterior cerebellar lobe	3.03	-21	-65	-45	106
Right posterior cerebellar lobe	3.5	15	-77	-45	152

The voxels represented the numbers of activated brain functional regions, MNI: Montreal template, the *P* values of the above activated regions were all less than 0.05, the Z value was a statistical parameter, and could be converted with t value or *P* value.

in accordance with data processing tools, such as head movement correction, vertex flow correction, tensor computation, and fiber tracking visualization. Finally, the fractional anisotropy (FA) values of the left and right cingulate bundles were calculated, and their fiber tracks were visualized.

Statistical analysis

The average fMRI activation images were analyzed with *t*-tests with the SPM8 software, and the differences were considered statistically significant when the activated areas consisted of 30 or more voxels and the *P* values were less than 0.05. The remaining statistical analyses were performed with SPSS 13.0 software (IBM Corporation, Armonk, NY, USA). The comparisons of the fMRI and DTI data between the two groups were performed with two-sample *t*-tests, and the measurements of the fine structures of the anatomic sites were expressed by the FA values. The comparisons of the correlations between the FA values of the cingulate bundles and the activated areas were performed with Pearson correlation analyses, and *P* values less than 0.05 were considered statistically significant.

Results

General information

Fifteen cases each were enrolled in the TLE patient group and control group. The TLE group consisted of eight males and seven females who had an age of (mean ± standard deviation) 27.30 ± 2.01 years and educational duration of 8.6 ± 2.3 years. The control group consisted of six males and nine females who had an age of 26.5 ± 0.43 years and an educational duration of 9.5 ± 3.9 years.

Differences in VWM activation

In the patient group, activation was significantly reduced ($P < 0.05$) in the brain regions that were negatively activated in the VWM task in the healthy control group, and these regions were located in the left frontal lobe, parietal lobe, insula, and posterior cerebellar lobe and the right frontal lobe, temporal lobe, insula, parietal lobe, and posterior cerebellar lobe. No brain regions that were positively activated in the VWM task in the healthy control group were activated in the patient group (**Table 2**; **Figure 1**).

The patient group exhibited significantly less activation in the brain regions that were negatively activated in the control group, and these regions were located in the left frontal lobe, parietal lobe, insula, and posterior cerebellar lobe and in the right frontal lobe, temporal lobe, insula, parietal lobe, and posterior cerebellar lobe. **Figure 1A** displays sagittal, coronal, and transverse views of the brain activation, and the color bar represents the *t* values. **Figure 1B** shows three-dimensional images of the activated brain regions. **Figure 1C** presents cross-sectional images of the activation. The red color represents the brain regions that were positively activated in the normal group more than in the patient group. However, because there were less than 10 voxels, no statistically significant differences were found in the red regions. The blue color represents the brain regions that negatively activated in the normal group more than in the patient group.

DTI results for the cingulate bundles

In the patient group compared with the control group, the FA values for the cingulate bundles, which are associated with VWM function, were decreased. For the cingulate bundles on both sides, the FA values differed significantly [Left: TLE group (0.505 ± 0.235) vs. control group

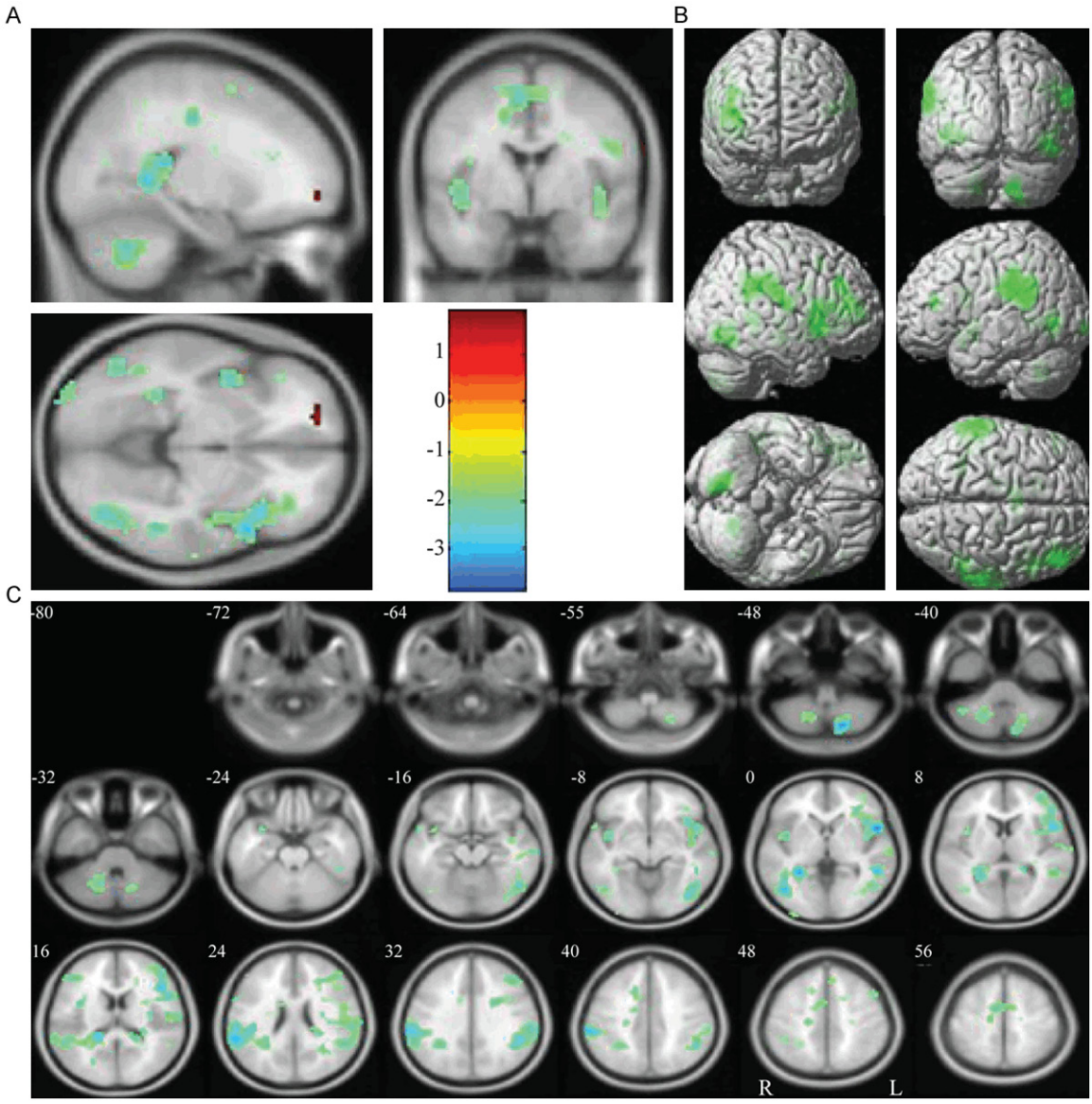

Figure 1. Average brain activation images by intergroup 2-sample t-test.

(0.480 ± 0.326), t = 2.385, P < 0.05; Right: TLE group (0.487 ± 0.020) vs. control group (0.454 ± 0.352), t = 3.105, P < 0.05; **Table 3**; **Figures 2**, **3**].

Correlations of the FA values of the cingulate bundles and the activated voxels in the frontal region in the patients with TLE

The FA values of the left cingulate bundle and the number of activated voxels in the left frontal region in the patients with TLE were positively and significantly correlated (r = 0.790, P < 0.05). Similarly, the FA values of the right cingulate bundle and the number of activated voxels in the right frontal region in the patients with TLE were positively and significantly correlated (r = 0.852, P < 0.05; **Table 4**).

Discussion

Previous findings have shown greater correlations with the seed regions of the default network during working memory tasks compared with at rest. These changes might reflect a decrease in the negative correlations between the default and task-positive networks at rest [13]. A meta-analysis of several experiments that tested the effects of processing on storage corroborated the parameters of the predicted

Table 3. Comparison of FA values of cingulate bundle between the 2 groups ($\bar{X}$ ± sd)

Group	Left cingulate bundle	Right cingulate bundle
Control group	0.505 ± 0.235	0.487 ± 0.020
TLE group	0.480 ± 0.326	0.454 ± 0.352
T	2.385	3.105
P	0.024	0.005

Compared with the control group, the *P* values of left and right cingulate bundles were both less than 0.05.

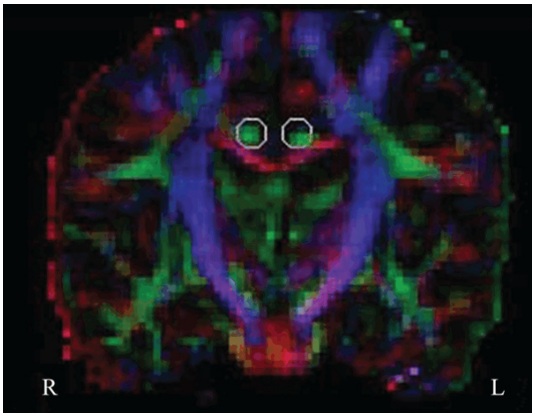

Figure 2. Fiber tracking anatomical sites of bilateral cingulate bundles.

functions, which suggested that these effects reflected the functions of working memory [14]. Dorsal-"where"/ventral-"what" frameworks that have been applied to working memory maintenance have also been applied to the executive processes of working memory [15]. In this study, we performed fMRI assessments of the performance of patients with TLE on digital-stimulated n-back VWM tests, and two-sample *t*-tests revealed that the patients showed significant reductions in the negative activation of the brain regions that was observed in the healthy control group, while the brain regions that were positively activated in the healthy control group were not activated in the patient group. In addition, we found that the activated regions overlapped to a certain degree between the two groups (frontal lobe and cerebellum), and the two groups exhibited activated sites that were inconsistent with each other. The range of activation on the right was wider than that on the left, which suggested that the differences in VWM activation in the patients with TLE might have exhibited laterality. Such laterality might be related to asymmetries in VWM impairments as the patients with TLE exhibited brain regions with significantly reduced negative activation compared with that in the healthy control group. These findings suggested that the patients with TLE needed to mobilize more brain areas in order to complete the VWM task and that this might be related to the compensatory functions in the brain. One possible explanation is that the activated voxels in the brain increased along with the increased VWM loads, while the VWM-related brain regions that were damaged could not increase their activation relative to the increasing load. Thus, other brain regions had to be mobilized in order to meet the needs of the VWM load.

Based on the above findings, we hypothesized that the deficits in VWM functions in patients with TLE were related to impaired subcortical structures. A meta-analysis has shown that declarative memory retrieval is correlated with activity in the inferior frontal gyrus and anterior cingulate, whereas the updating of working memory corresponds to activation in the inferior parietal lobule, anterior cingulate, and around the inferior frontal gyrus [16]. The cingulate gyrus and the cingulate bundles are important parts of the limbic system. The cingulate bundles connect with the frontal lobe, which is closely associated with VWM. The microstructures of the right cingulum and bilateral cerebellar peduncles appear to be related to VWM cognitive functions, such as sustained attention and working memory, in the human brain [17]. We decided to research the fine structure of the cingulate bundle in patients with TLE after we found that the FA values of the left and right cingulate bundles were significantly decreased in the patients with TLE compared with those in the normal subjects. The frontal lobe occupies an important position in the VWM network, and these findings suggest that the effects of epilepsy on prefrontal network integrity might underlie the memory impairments in patients with epilepsy [18]. We further found that the FA values of the cingulate bundles positively correlated with the activated voxels in the frontal lobe. These results confirmed our hypothesis that the impairments of patients with TLE in VWM functions were related to abnormalities in the structure of subcortical fiber bundles, including the cingulate bundles. These results further suggested that, in

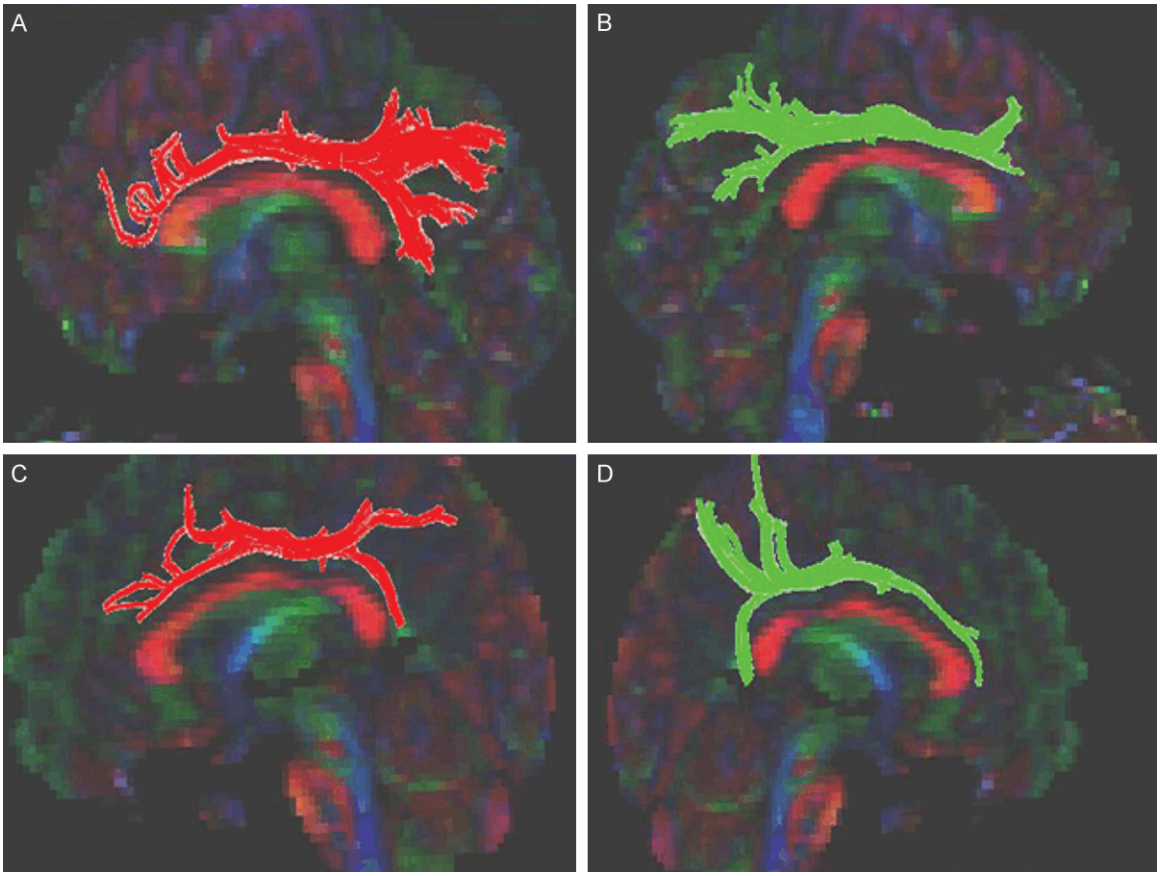

Figure 3. Cingulate bundle fiber tracing images. A and B were fiber tracking images of left and right cingulate bundles of the healthy group, and C and D were fiber tracking images of left and right cingulate bundles of the TLE group.

Table 4. Correlations of FA values of cingulate bundles and frontal activated voxels in TLE patients

	FA value of left cingulate bundle	FA value of right cingulate bundle
R	0.790	0.852
P	0.0004	0.00005

Note: The left and right frontal cingulate bundles were both positively correlated with the ipsilateral activated-voxels, $P < 0.05$.

the context of the expectancy for an imminent cognitive challenge, higher resting-state activity in the posteromedial parietal cortex might be related to increased attentional preparatory resources [19]. The specific involvement of the microstructures of the thalamus and not its volume in modulating working memory performance has been shown, and this might regulate the connections among the cortical areas that are recruited during VWM tasks [11].

A close relationship between resting-state networks and task-evoked activation has been suggested to be functionally relevant for behavior, and spatial multiple regression analyses can be used to examine that relationship [12]. Studies have shown that the combination of the processing of items at the beginning of a list and hippocampal activity patterns have been observed during hippocampal-dependent working memory results in the remembering of these items. However, deactivation of the hippocampus, as has been previously observed during the working memory maintenance of individual items, predicted the failure of long-term memory encoding [20]. This study combined fMRI and DTI technologies, studied the functions and structures of the brains of patients with TLE, detected in vivo nerve functions, and evaluated the integrity of fine white matter structure, and the results are significant for the understanding of a number of clinical diseases. The present study showed that patients with

TLE patients exhibited VWM dysfunction and certain compensatory functions. The FA values of the cingulate bundles in the patients with TLE were decreased, which suggested changes in the fine structure of the white matter fibers in these patients. The activated voxels in the frontal lobe of patients with TLE were positively correlated with the FA values of the cingulate bundles, which indicated that the changes in the fine white matter structure might be related to the changes in the VWM functions. However, this study had a number of limitations. For example, the sample size was small. Therefore, further examinations of the laterality of the abnormalities would be difficult, and an increase in sample size is needed in order to improve the credibility of the results.

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Disclosure of conflict of interest

None.

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