Gray-matter volume reduction in the thalamus and frontal lobe in epileptic patients with generalized tonic-clonic seizures

Réduction de volume de la substance grise dans le thalamus et le lobe frontal chez les patients épileptiques avec crises tonicocloniques généralisées

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Available online 26 February 2011

KEYWORDS
Generalized tonic-clonic seizures (GTCS);
Epilepsy;
Voxel-based morphometry (VBM);
Thalamus;
Frontal lobe

Summary
Background and purpose. — Generalized tonic-clonic seizures (GTCS) comprise a common subtype of idiopathic generalized epilepsy (IGE). Previous studies found that patients with GTCS had structural abnormalities in a few specific brain regions. However, the underlying clinical cause leading to these abnormalities remains unclear. The present study aimed to explore the relationship between changes in gray-matter (GM) volume and duration of epilepsy, based on GM volume differences observed between GTCS patients and healthy controls.

Patients and methods. — Voxel-based morphometry (VBM) analysis with DARTEL (diffeomorphic anatomical registration through exponential Lie algebra) was used to investigate GM volume differences in 31 GTCS patients compared with 37 age- and gender-matched healthy controls. Voxel-based correlation analysis was used to explore the relationship between GM volume and duration of epilepsy in GTCS patients.

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doi:10.1016/j.neurad.2010.12.007
Introduction

Idiopathic generalized epilepsy (IGE) refers to a group of genetically determined, age-related epileptic syndromes characterized by bilateral, synchronous and symmetrical generalized spike-and-wave (GSW) or polyspike-wave discharges [1]. IGE is divided mainly into absence epilepsy (AE), juvenile myoclonic epilepsy (JME) and generalized tonic-clonic seizures (GTCS) [1,2]. Each subsyndrome has a specific, distinctive pathophysiological mechanism and clinical presentation [3]. GTCS represents the most common subsyndrome of IGE, and is characterized by the typical seizure symptoms of muscle rigidity, violent muscle contractions throughout the entire body and loss of consciousness.

Although structural magnetic resonance imaging (MRI) analysis with visual inspection usually shows a normal presentation in patients with IGE, an advanced analysis method using quantitative MRI evaluations can increase sensitivity, thereby allowing examination of brain structural abnormalities in detail [4–8]. Several studies using voxel-based morphometry (VBM) analysis have found structural abnormalities in the thalamus and frontal lobe in GTCS patients [9,10]. However, further investigation into the pathology that might result in the structural alterations of GTCS revealed no positive findings between structural abnormalities and clinical variables [9]. In that study, only a small sample of patients was included, and a traditional analytical approach using standard VBM was used to measure gray-matter (GM) volume.

Recently, a new VBM analytical tool dubbed “diffeomorphic anatomical registration through exponential Lie algebra” (DARTEL) has been developed. This set of tools can achieve accurate intersubject registration and improve the anatomical precision of brain images [11–13]. Also, as DARTEL is a more deformable registration method than that used in standard VBM, it enables the detection of brain structural abnormalities with greater sensitivity [11]. For this reason, the present study used this newly developed VBM toolkit to readdress the relationship between changes in GM volume and duration of epilepsy, based on the detection of GM volume differences, in a large study sample of GTCS patients and healthy controls.

Materials and methods

The present study included 31 right-handed patients with GTCS (16 men, 15 women; mean age: 25.89 ± 6.86 years); their demographic and clinical information are presented in Table 1. According to the International League Against Epilepsy (ILAE), these GTCS patients all met the following inclusion criteria:

- typical clinical symptoms of generalized tonic-clonic seizures, such as limb tics, loss of consciousness and no partial seizures, epilepsy duration (time in years from age at seizure onset to time of examination) of at least 2 years and the occurrence of at least three seizures;
- no focal abnormality on conventional anatomical MRI;
- and generalized polyspike-wave discharges on interictal scalp electroencephalography (EEG).

In addition, 37 right-handed, age- and gender-matched volunteers (20 men, 17 women; mean age: 25.51 ± 3.94 years) were recruited as healthy controls (Table 1). None of the controls had a history of either neurological or psychiatric disorder.

All patients and healthy controls gave their written consent to participate. Also, the study protocol was approved by the medical ethics committee of Jining Hospital, Clinical School of Medical College, Nanjing University.

MRI data acquisition

MRI data were collected on a 1.5-Tesla scanner (GE Signa, Milwaukee, WI, USA). The three-dimensional (3D) T1-weighted images for each subject were acquired in the transverse plane with a 0.8-mm slice thickness, using a fast spoiled gradient-echo sequence (FSPGR; TR = 12.1 ms, TE = 4.2 ms, TI = 400 ms, NEX = 1, FOV = 24 × 24 cm², flip angle = 15°, matrix size = 256 × 256).

Data-processing

The 3D T1-weighted images were analyzed with VBM—DARTEL, using SPM8 (http://www.fil.ion.ucl.ac.uk/spm) and MATLAB R2006b software (MathWorks, Natick, MA, USA). VBM preprocessing involved five steps [11,14]:

- checking for scanner artifacts and gross anatomical abnormalities in each subject;
- setting image origin at the anterior commissure (AC) so that the fiducial points were consistently placed [12];
- using the unified standard segmentation [15] option in SPM8 to segment the images into GM and white matter (WM);
Table 1 Characteristics of the patients with generalized tonic-clonic seizures (GTCS) and healthy controls (HC).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GTCS (n = 31)</th>
<th>HC (n = 37)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: male/female (n/n)</td>
<td>16/15</td>
<td>20/17</td>
<td>&gt; 0.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25.89 ± 6.86</td>
<td>25.51 ± 3.94</td>
<td>0.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Handedness: right/left (n/n)</td>
<td>31/0</td>
<td>37/0</td>
<td>&gt; 0.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Duration of epilepsy (years)</td>
<td>8.42 ± 6.96</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD unless otherwise specified.

<sup>a</sup> By Pearson χ² two-tailed test.

<sup>b</sup> By two-sample two-tailed t test.

• using the DARTEL toolbox to obtain a high-dimensional normalization protocol, following John Ashburner’s chapter in the standard version [13];

• and checking for homogeneity across the sample and using standard smoothing (4 mm). Following these steps provided smoothed, modulated and normalized data for statistical analysis.

### Statistical analysis

Statistical analysis of the data was conducted in three steps. First, for each subject, GM, WM and cerebrospinal fluid (CSF) absolute volumes were calculated by estimating the GM, WM and CSF volumes. The total intracranial volume (TIV) was obtained by summing the GM, WM and CSF volumes. Group differences in GM + WM + CSF absolute volume and TIV between GTCS patients and healthy controls were assessed by two-sample two-tailed t test.

In addition, a voxel-wise two-sample t-test was used to compare GM volume differences between the GTCS patients and healthy controls using SPM8. An absolute threshold mask of 0.01 was used to avoid possible edge effects along the border between GM and WM [14]. The results were estimated with a corrected threshold of P < 0.05 (combined height threshold of P < 0.001 and a minimum cluster size of 8 voxels), using the AlphaSim program in the resting-state fMRI data-analysis toolkit (REST), version 1.4 ([http://www.restfmri.net/forum/REST_V1.4](http://www.restfmri.net/forum/REST_V1.4)), which applied Monte Carlo simulation (parameters: individual voxel P = 0.001; 10,000 simulations; FWHM = 4 mm, with mask) [16,17]. Age, GM absolute volume and TIV were regressed out as nuisance covariates.

Following this, a voxel-based correlation analysis was carried out between GM volume and duration of epilepsy. The results were estimated with a corrected threshold of P < 0.05 (combined height threshold of P < 0.001 and a minimum cluster size of eight voxels), using the AlphaSim program as described above. Individual age, GM absolute volume and TIV were regressed out as confounding covariates in this correlation analysis.

### Results

#### Differences in global volume

The GM + WM + CSF absolute volume and TIV in both the GTCS patients and healthy controls are shown in Table 2. The results of group analysis revealed no significant differences in the GM + WM + CSF absolute volume and TIV between the two groups (Table 2).

#### Group comparisons of GM volume using VBM—DARTEL

Compared with healthy controls, the GTCS patients showed significant GM volume reductions in the bilateral thalamus, frontal lobe, insula and cerebellum (Fig. 1, Table 3), whereas there were no regions showing significantly increased GM volume.

#### Correlation analysis between GM volume and clinical manifestations

Voxel-based correlation analysis revealed that GM volume in the bilateral thalami (left thalamus: x = −12, y = −12, z = 0, T value = 4.35; right thalamus: x = 14, y = −12, z = 0, T value = 3.91) and left medial frontal gyrus (x = −8, y = 27, z = 36, T value = 4.09) were significantly negatively correlated with duration of epilepsy in GTCS patients (Fig. 2). At the same threshold, there was no region with a positive correlation with duration of epilepsy.

### Discussion

The present study used a fully automated, voxel-wise analytical method — the VBM—DARTEL — to readdress the relationship between GM volume changes and clinical variables. The results demonstrated a negative correlation between duration of epilepsy and GM volume in the thalamus and frontal lobe, indicating that epilepsy can directly impair these brain regions. Moreover, the association with both the thalamus and frontal lobe suggests an essential role of the thalamocortical network in GTCS patients.

Using VBM—DARTEL, the present study found GM volume reductions in the bilateral thalamus and frontal lobe, thus supporting a central pathophysiological role of the thalamocortical network in GTCS [18]. In addition, not only is the thalamus important, but it is also the most impaired structure in GTCS. Both animal and clinical studies have shown that the thalamocortical circuitry is involved in seizure generalization and maintenance of the GSW discharge [19—22]. In IGE patients, EEG—fMRI studies have found that the activity of generalized spike and slow-wave discharges can increase the blood-oxygen-level-dependent (BOLD) signal, particularly in the thalamocortical network [21]. A study combining positron emission tomography (PET) and EEG
### Table 2
Group comparisons of gray matter + white matter + cerebrospinal fluid absolute volume and total intracranial volume in patients with generalized tonic–clonic seizures (GTCS) and healthy controls (HC).

<table>
<thead>
<tr>
<th>Absolute volume (cm³)</th>
<th>GTCS (n = 31)</th>
<th>HC (n = 37)</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gray matter</td>
<td>797.1 ± 51.3</td>
<td>807.3 ± 56.5</td>
<td>0.6027</td>
</tr>
<tr>
<td>White matter</td>
<td>452.3 ± 38.3</td>
<td>469.7 ± 45.2</td>
<td>0.2534</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>445.4 ± 97.0</td>
<td>459.7 ± 75.0</td>
<td>0.6647</td>
</tr>
<tr>
<td>Total intracranial volume</td>
<td>1694.9 ± 132.6</td>
<td>1736.8 ± 104.9</td>
<td>0.3584</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD.

* By two-sample two-tailed t test.

### Table 3
Regions of decreased gray-matter volume in patients with generalized tonic-clonic seizures (GTCS) compared with healthy controls (HC) according to VBM—DARTEL.

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Voxels</th>
<th>T value</th>
<th>MNI coordinate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Right cerebellum</td>
<td>1003</td>
<td>4.82</td>
<td>42</td>
</tr>
<tr>
<td>Left cerebellum</td>
<td>769</td>
<td>4.54</td>
<td>−40</td>
</tr>
<tr>
<td>Left inferior frontal gyrus</td>
<td>197</td>
<td>4.38</td>
<td>−48</td>
</tr>
<tr>
<td>Right precentral gyrus</td>
<td>83</td>
<td>4.16</td>
<td>20</td>
</tr>
<tr>
<td>Left thalamus</td>
<td>230</td>
<td>4.13</td>
<td>−14</td>
</tr>
<tr>
<td>Left precentral gyrus</td>
<td>91</td>
<td>3.72</td>
<td>−32</td>
</tr>
<tr>
<td>Medial frontal gyrus</td>
<td>182</td>
<td>3.54</td>
<td>−2</td>
</tr>
<tr>
<td>Right insula</td>
<td>165</td>
<td>3.46</td>
<td>40</td>
</tr>
<tr>
<td>Left insula</td>
<td>165</td>
<td>3.36</td>
<td>−46</td>
</tr>
<tr>
<td>Right inferior frontal gyrus</td>
<td>30</td>
<td>3.33</td>
<td>48</td>
</tr>
<tr>
<td>Left middle frontal gyrus</td>
<td>98</td>
<td>3.20</td>
<td>−42</td>
</tr>
<tr>
<td>Right thalamus</td>
<td>37</td>
<td>2.88</td>
<td>10</td>
</tr>
</tbody>
</table>

VBM—DARTEL: voxel-based morphometry—diffeomorphic anatomical registration through exponential Lie algebra; MNI: Montreal Neurological Institute.

### Figure 1
Results of voxel-based morphometry analysis show gray-matter (GM) volume reductions in the bilateral thalami, frontal lobe, insula and cerebellum in patients with generalized tonic-clonic seizures (GTCS) compared with healthy controls (HC). There is no region showing increased GM volume in the GTCS patients. Voxels with P < 0.001 and a minimum cluster size of 8 voxels were used to identify significant clusters. The criterion was set at a corrected threshold of P < 0.05. The colored bar represents T-values.
The insula is a region of convergence of multisensory inputs, with strong connections to the thalamus and several other cortical areas. The present results are consistent with previous findings of insula impairment in both focal and generalized epilepsy [21]. In addition, it is generally accepted that the cerebellum is involved in epilepsy and has epileptogenic potential. The cerebellum also has well-known reciprocal network connections with other regions, including the cerebral cortex via the thalamus and pons [26]. Cerebellar ictal hyperperfusion has been observed in GTCS patients using single-photon emission computed tomography (SPECT), and positive correlations have been found in cerebral blood flow between the cerebellum and thalamus [26,27]. In fact, as the cerebellum has widespread connections with a vast number of afferent and efferent tracts implicated in many neurological diseases [28], it is not surprising that GTCS patients have also shown subtle structural abnormalities of the cerebellum.

**Conclusion**

In the present study using VBM—DARTEL analysis, GM volume reductions in the thalamus and frontal lobe were associated with progressive epileptic seizures, indicating that epilepsy may directly impair the thalamus and frontal lobe. The present study also provides further insights into the underlying pathophysiological mechanism of GTCS.

**Conflict of interest statement**

The authors declare that they have no conflict of interest.

**Acknowledgements**

The present research was supported by grants from the National Natural Science Foundation of China (Grant nos. 30470510 to Guangming Lu, 30800264 and 81020108022 to Zhiqiang Zhang, 30971019 to Qing Jiao, 60628101 to Yijun Liu, 90820006 and 30707090), and by local key medical research projects (Grant nos. 07z030 to Guangming Lu and Q2008063 to Zhiqiang Zhang).

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