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Thalamic diffusion and volumetry in temporal lobe epilepsy with and without mesial temporal sclerosis

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Summary
Purpose: As an important connection within the limbic system, considerable attention has been paid to thalamic pathology in temporal lobe epilepsy (TLE). Magnetic resonance imaging (MRI) volumetric studies have yielded variable results and have largely been focused on TLE with mesial temporal sclerosis (TLE+). Diffusion tensor imaging (DTI) provides unique information on microstructure based on the measurement of water diffusion. To date, DTI properties of thalamus have not been well characterized in adult TLE patients with unilateral MTS or without MTS (TLE−). The purpose of this study was to investigate the status of thalamic integrity by using DTI as well as volumetric MRI in adult TLE+ and TLE− patients.
Method: In 17 unilateral TLE+ patients, 10 TLE− patients and 26 controls, the thalamus was segmented by using an automated atlas-based method. Mean diffusivity (MD), fractional anisotropy (FA) and volume were then quantified from DTI and 3D T1-weighted scans.
Results: No significant changes were found in either DTI parameters or volume of thalamus in TLE− patients, as compared to healthy controls. However, both DTI parameters and MRI volumetry showed bilateral thalamic pathology in TLE+ patients, as compared to healthy controls. Also, TLE+ patients showed significant reduction of thalamic volume as compared to TLE− patients. In addition, thalamic FA ipsilateral to seizure focus showed significant correlation with age at onset of epilepsy in TLE+ patients.
Conclusion: Our finding demonstrates bilateral pathology of thalamus in unilateral TLE+ patients. The discrepancy in thalamic pathology between TLE+ and TLE− patients suggests that along with differences in mesial temporal pathology, TLE+ and TLE− have unique extratemporal structural abnormalities.

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Introduction

Temporal lobe epilepsy (TLE) is the most common localization-related epilepsy with mesial temporal sclerosis (MTS) being observed in approximately 70% of cases (Cascino et al., 1991). While TLE without MTS (TLE−) has similar seizure semiology and presumably epileptogenic zones as TLE with MTS (TLE+), its resective surgical success rate is reduced dramatically (Jack et al., 1992; Antel et al., 2002). Advances in quantitative neuroimaging techniques have resulted in increased sensitivity of magnetic resonance imaging (MRI) in detecting MTS. The ability of quantitative MRI techniques such as T2 relaxometry, MR spectroscopy and volumetry to detect MTS in patients with normal conventional MRI scans has lead to the hypothesis that further improvements in MRI techniques will result in the detection of MTS in the majority of nonlesional TLE patients. A recent MRI/histological study, however, suggests that TLE+ and TLE− are two distinct pathological entities (Carne et al., 2004).

As the relay center of the brain, the thalamus has been demonstrated to have well-developed anatomical connections with temporal lobe structures. In TLE, thalamus is thought to play a role as a physiologic synchronizer of seizures (Bertram et al., 1998). Previous histological studies have reported neuronal loss and gliosis in thalamus of both humans with TLE and in animal models (Margerison and Corsellis, 1966; Bertram et al., 2001). Along with these histological observations, various neuroimaging techniques (e.g. positron emission tomography (PET) and single photon emission computed tomography (SPECT)) have also demonstrated thalamic pathology in TLE patients *in vivo* (Henry et al., 1993; Yune et al., 1998; Juhasz et al., 1999; Tae et al., 2005). Recently, volumetric MRI studies further reported atrophy of thalamus in TLE patients (DeCarli et al., 1998; Dreifuss et al., 2001; Natsume et al., 2003; Bonilha et al., 2005). However, many previous studies include only TLE+ patients or TLE patients without specifying the presence of MTS.

Diffusion tensor imaging (DTI) is an MRI technique that can measure the magnitude and directionality of water diffusion in tissue (Basser and Pierpaoli, 1996). This technique can provide quantitative parameters reflecting the integrity of microscopic tissue architecture, indirectly, such as axonal packing and myelination (Beaulieu, 2002). The most popular DTI parameters are mean diffusivity (MD) and fractional anisotropy (FA). MD represents the bulk mobility of water molecules whereas FA is a normalized ratio of diffusion directionality. Notably, the thalamus has anisotropy values higher than other deep gray matter structures due to the presence of numerous myelinated axons, with the axon-rich regions being referred to as thalamocortical striations (Wiegell et al., 2003).

Previous DTI studies in TLE have focused on the hippocampus and white matter tracts including the fornix, cingulum, external capsule, internal capsule, and corpus callosum (Wieshmann et al., 1999; Arfanakis et al., 2002; Yoo et al., 2002; Assaf et al., 2003; Concha et al., 2005; Thivard et al., 2005; Gross et al., 2006). Also, these studies included either only TLE+ or TLE patients without reporting the presence of MTS. Recently, a trend towards elevated MD in the ipsilateral thalamus was reported in TLE children with secondarily generalized seizures, but not in those with only partial seizures (Kimiwada et al., 2006). The diffusion properties of the thalamus have not, however, been well characterized in adult TLE+ or TLE− patients, nor has DTI been applied along with MRI volumetry of the thalamus in the same group of patients. DTI and MRI volumetry provide two related, but distinct, measures of integrity of brain structure. Some abnormalities of microstructure (e.g. axonal loss and demyelination) detectable by DTI may not necessarily lead to macroscopic volume loss detectable by MRI volumetry, and vice versa. The purpose of this study was to investigate potential thalamic abnormalities in TLE+ and TLE− patients using both volumetric MRI and DTI, separately. We hypothesize that TLE+ patients exhibit unique thalamic abnormalities relative to TLE− patients.

Materials and methods

Our protocol was approved by the University of Alberta Health Research Ethics Board, and informed consent was obtained from all participants.

Subjects

TLE+ (left TLE+: $n = 11$; right TLE+: $n = 6$): all MTS patients had ictal semiology of complex partial seizures, and ipsilateral hippocampal T2 greater than 2 S.D. of control values (i.e. left hippocampal T2 > 120 ms; right hippocampal T2 > 119 ms using our methodology listed below). As we have previously demonstrated significantly increased T2 of the contralateral hippocampus in some patients with unilateral mesial TLE (Gross et al., 2006), patients with contralateral T2 greater than 2 S.D. of control values ($n = 2$) were included if the ipsilateral hippocampal T2 was greater than that of the contralateral hippocampus and EEG video telemetry demonstrated ictal EEG onset solely from the ipsilateral temporal region. TLE− ($n = 10$): all TLE patients without MTS had ictal semiology of complex partial seizures, interictal EEG abnormalities in the temporal regions and bilateral hippocampal T2 within 2 S.D. of control values. The mean age of TLE+ and TLE− patients was 38.4 years (range, 20—59 years) and 37.2 years (range, 17—48 years), respectively. These patients were compared to 26 neurologically normal controls with mean age 34.7 (range, 24—58 years). There was no significant age difference between these groups (ANOVA, $F(2, 52) = 0.71$, $p = 0.5$). History and clinical investigation for all patients is summarized in Table 1. The age at onset of epilepsy was different between patient groups (TLE+, 10.5 ± 11.7 years; TLE−, 23.9 ± 11.3 years; t-test, $p < 0.01$). Also, the duration of epilepsy between patient groups was different (TLE+, 27.8 ± 12.4 years; TLE−, 12.3 ± 8.0 years; t-test, $p < 0.01$).

Image acquisition

Images were acquired using a Siemens Sonata 1.5T MRI scanner (Siemens Medical Systems, Erlangen, Germany). T2 relaxometry with coverage of the hippocampus used a high-resolution, multi-echo sequence with 32 echoes, 10 coronal slices, 3 mm slice thickness with 3 mm inter-slice gap, TR = 4430 ms, $TE_1 = 9.1$ ms, TE spacing = 9.1 ms, NEX = 1, acquisition matrix = 192×176 (interpolated to 384×352), FOV = 230 mm × 210 mm, scan time = 8 min 13 s. Diffusion tensor images were acquired by using a twice-refocused single-shot EPI-based sequence: coverage of the whole brain, 2 mm slice thickness with no inter-slice gap, 62 axial slices, TR = 10 s, TE = 88 ms, six diffusion directions with $b = 1000\ s/mm^2$, NEX = 8, acquisition matrix = 128×128 (interpolated to 256×256), FOV = 256 mm × 256 mm, scan time = 9 min 30 s. These acquisition parameters provided good quality DTI data (Figs. 1 and 2). 3D

Table 1 Clinical summary for temporal lobe epilepsy patients

Patients	Sex	Age (year)	Onset of seizures	Febrile seizures	Other history	Neuropsychology	Seizure pattern	EEG (inter)	EEG (ictal)	T2 left (ms)	T2 right (ms)
TLE+ patients ($n = 17$)											
1	M	23	6 months	—	Meningitis at 6 months	L mes T	CPS	LT	LT	141[a]	119
2	F	34	8 months	Prolonged	—	L mes, lateral T	CPS+GTC	LT > RT	LT	127[a]	115
3	F	53	39 years	Prolonged	—	L mes T	CPS + GTC	LT	LT	139[a]	129[a]
4	M	34	29 years	—	—	Unavailable[b]	CPS + GTC	LT	LT	128[a]	115
5	F	59	12 years	—	—	L lateral T	CPS + GTC	LT > RT	Unclear	160[a]	118
6	M	20	3 years	—	Status epilepticus[c]	L lateral T	CPS	LT	LT	131[a]	119
7	F	37	25 years	Yes	—	Bi mes T	CPS	LT	LT	126[a]	116
8	F	20	6 months	Yes	—	L lateral T Bi mes T	CPS	LT	LT	146[a]	119
9	F	57	18 years	—	—	L lateral T	CPS + GTC	LT	LT	132[a]	107
10	F	36	7 years	Yes	—	L mes T	CPS	LT	LT	149[a]	115
11	M	36	6 years	—	—	Bi mes T	CPS + GTC	LT	LT	138[a]	118
12	F	35	18 years	—	Trauma[d] (minor)	No deficit	CPS	RT	RT	127[a]	133[a]
13	F	42	14 years	—	—	R mes T	CPS	RT	RT	114	129[a]
14	F	36	17 months	Prolonged	—	R mes T, lateral F	CPS	RT	RT	117	139[a]
15	F	38	2 months	—	—	R mes T > L mes T	CPS	RT	RT	111	130[a]
16	F	47	6 months	—	Meningitis at 6 months	R mes T	CPS	RT	RT	114	133[a]
17	M	46	5 years	—	Meningitis at 13 months	R T and F	CPS	RT	RT	120	133[a]
TLE— patients ($n = 10$)											
18	F	43	39 years	—	—	R mes T	CPS + GTC	LT > RT	LT	106	109
19	M	34	11 years	—	—	L F, lateral T	CPS + GTC	LT	LT	109	115
20	F	34	26 years	—	—	No deficit	CPS	BiT	unclear	113	115
21	F	42	36 years	—	—	Bi mes T	CPS	LT	LT	113	105
22	M	33	18 years	—	—	Asymmetric mes T	CPS + GTC	BiT	Bi T	118	115
23	F	39	22 years	—	—	Unavailable	CPS	BiT	Bi T	114	114
24	M	46	20 years	—	—	R F and mes T	CPS	BiT	Bi T	117	111
25	M	44	41 years	——	—	Unavailable	CPS	RT	Unavailable	117	118
26	F	30	16 years	—	—	R mes and lateral T	CPS	RT	RT	117	114
27	M	17	10 years	—	—	Unavailable	CPS + GTC	LT	Unavailable	113	116

EEG = electroencephalogram; TLE+ = temporal lobe epilepsy with mesial temporal sclerosis; TLE— = temporal lobe epilepsy without mesial temporal sclerosis; CPS = complex partial seizures; GTC = generalized tonic-clonic seizures; R = right; L = left; mes = mesial; T = temporal; BiT = independent bitemporal; LT > RT = independent bitemporal with left temporal predominance.

[a] T2 relaxometry of the hippocampus greater than 2 S.D. of the mean of control subjects (i.e. left: $113 + (2 \times 3.9)$ ms, right: $113 + (2 \times 3.3)$ ms).

[b] Neuropsychology evaluation was not interpretable due to lack of patient cooperation.

[c] At age 3 years the patient presented with nonfebrile status epilepticus (with a negative septic workup, including lumbar puncture).

[d] Seizure onset occurred at age 18 following a minor head injury without loss of consciousness.

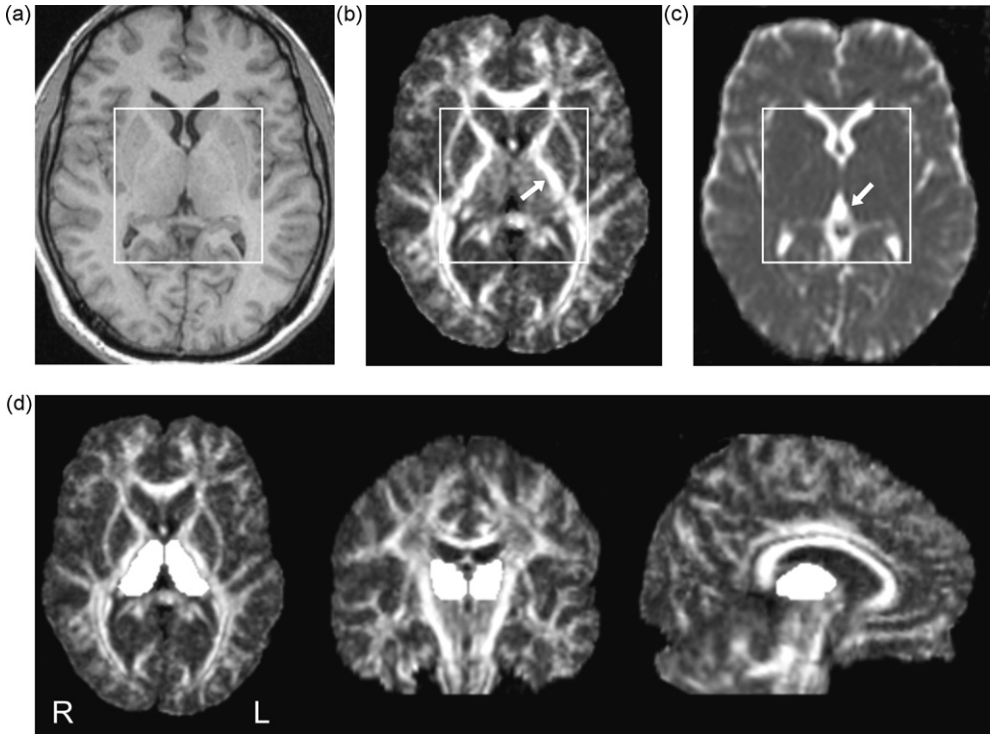

Figure 1 Demonstration of thalamic contrast and segmentation: (a) structural T1-weighted MRI, (b) FA map, (c) MD map and (d) extraction of thalamus. The high FA of the internal capsule was used as the lateral boundary of the thalamus while the high MD of the ventricle was used as a medial boundary (white arrows).

T1-weighted images with high resolution were obtained by a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence with following parameters: 1 mm slice thickness with no inter-slice gap, 144 axial slices, TR = 1890 ms, TE = 4.38 ms, TI = 1100 ms, NEX = 1, acquisition matrix = 256 × 192 (interpolated to 512 × 384), FOV = 256 mm × 192 mm, scan time = 6 min 3 s.

Image preprocessing

For the T2 relaxometry calculation, the signal decay was fitted to a mono-exponential curve on a voxel by voxel basis. Hippocampal T2 values were determined by manually outlining regions of interest (ROIs) around each hippocampus in two consecutive slices.

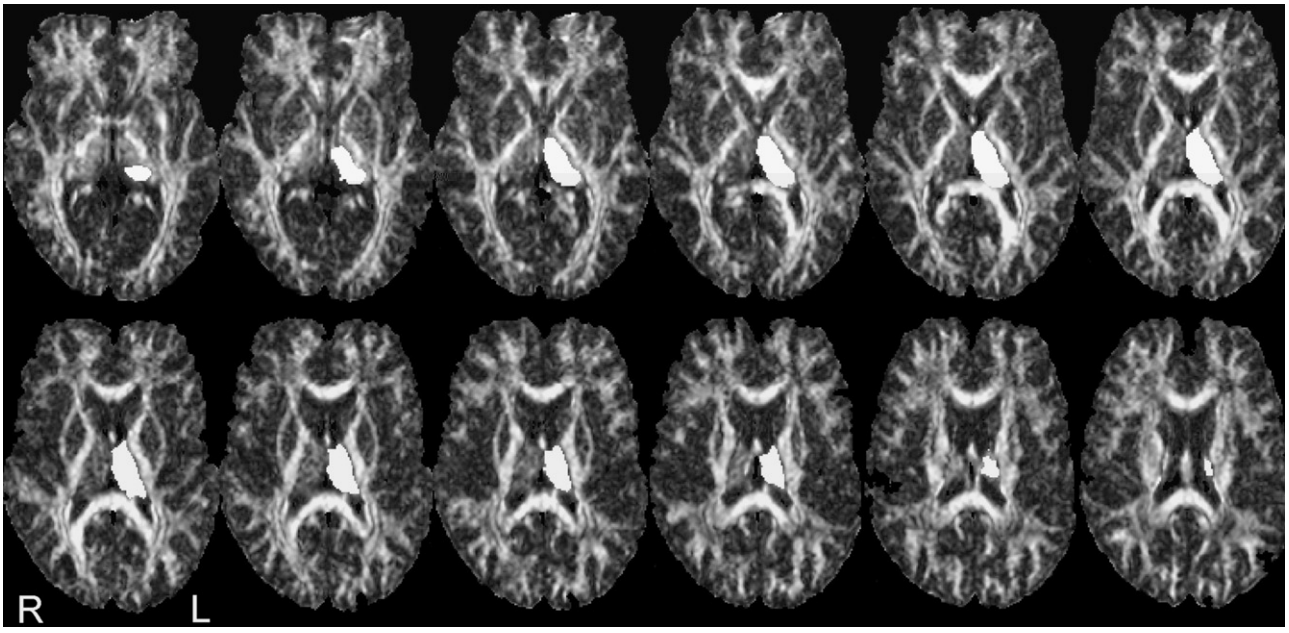

Figure 2 Demonstration of thalamic mask of one hemisphere in native space: all slices (thickness = 2 mm) with left thalamic mask for the same control subject in Fig. 1 were displayed from inferior to superior consecutively. As shown in this figure, the mask covers the thalamus well in the native space.

The diffusion tensor matrix was first calculated voxel-by-voxel from non-diffusion-weighted and diffusion-weighted images (Basser and Pierpaoli, 1996), and diagonalization of the diffusion tensor was performed to yield three eigenvalues and eigenvectors. Mean diffusivity (MD) (i.e. average of three eigenvalues) and fractional anisotropy (FA) were then calculated from the eigenvalues. All these processes were performed in DTIstudio (Johns Hopkins University, Baltimore, MD).

Segmentation of the thalamus

Manual delineation of the thalamus is notoriously difficult on structural T1-weighted MRI because the boundary between the thalamus and adjacent non-thalamic tissues (e.g. the internal capsule) is ambiguous (Fig. 1a). Alternatively, we first mapped images of each subject into a stereotaxic space to realign the thalamus across subjects; then a thalamus-labeled atlas in this space was used to mask out whole thalamus for each subject. Specifically, our processing included several steps as follows.

Co-registration

Each subject's non-diffusion-weighted images (i.e. $b = 0\,s/mm^2$ image) were linearly co-registered and re-sliced to the structural MRI (i.e. T1-weighted image with high resolution). This transformation was implemented using SPM2 (Wellcome Department of Cognitive Neurology, London, UK), and was applied to the diffusion parameter maps (i.e. MD and FA) to match them with the structural MRI.

Mapping images into MNI space

Each subject's images were spatially mapped to the ICBM template (i.e. MNI space) to realign the thalamus across subjects (Evans et al., 1992). Good et al. (2001) have suggested that mapping the segmented gray matter image to the gray matter template can improve the mapping accuracy by removing the contribution of non-brain voxels. Thus, we first segmented the structural MRI to obtain the gray matter image, and then mapped it to the default gray matter template in MNI space. The first step of mapping was optimizing the affine transformation with 12 parameters to match the whole shape of brain to the template. A non-linear deformation ($7 \times 8 \times 9$) was then estimated for the local match of brain. All estimations were implemented in SPM2, and the mapping parameters were then applied to the co-registered diffusion maps above.

Masking out the thalamus

Here, we employed *WFU_PickAtlas* (Wake Forest University, Winston-Salem, NC) to mask out the coarse volume of thalamus (Maldjian et al., 2003). Given the inexact nature of mapping, the coarse thalamus could contain some adjacent tissue, e.g. laterally, the internal capsule and mesially, the lateral ventricle. Although the boundary between thalamus and internal capsule is unclear in structural MRI, the contrast on FA maps is dramatic (Fig. 1b). As well, the MD map offers a good contrast between the thalamus and the lateral ventricle (Fig. 1c). Based on these special properties of diffusion maps, we further polished the coarse volume of thalamus by removing the voxels if $FA > 0.5$ (i.e. to ignore voxels in the internal capsule) or $MD > 1.3 \times 10^{-3}\,mm^2/s$ (i.e. to ignore voxels in the lateral ventricle). Finally, the quality of the extracted thalamus was manually checked in MNI space and was deemed acceptable for all subjects.

Quantification of measures and validation

Jacobian determinant derived from deformation field of mapping can represent the amount of expansion or contraction from stereotaxic space to native space (Ashburner and Friston, 2000). Hence, the Jacobian determinants over all the voxels of each thalamus (left and right separately) in MNI space were summed to calculate the thalamic volume in the native space. For diffusion measures, MD and FA were averaged over all the voxels of each thalamus (left and right separately) in MNI space. The interpolation during registration might have some effect on these diffusion measurements. In order to test this effect, the whole thalamic FA and MD values in the native space were calculated in 10 subjects (randomly selected, five controls and five patients) and compared to their corresponding values in MNI space. The individual thalamic mask in the native space for each subject was obtained by inversely mapping the thalamic mask from MNI space back to native space (Fig. 2). The coverage of the thalamic mask in native space was manually checked across the 10 subjects and was deemed acceptable, which indirectly validates the mask in MNI space. The corresponding thalamic mask of the native space for the same control subject of Fig. 1 is demonstrated in Fig. 2. The results showed no difference in FA values (paired t-test: $p = 0.23$; average difference: 2.3%), but the thalamic MD in MNI space is slightly increased due to the effect of interpolation (paired t-test: $p < 0.01$; average difference: 2.4%) compared to native space. Note that the slight elevation of MD in MNI space is consistent across all subjects, and the MD values in MNI space showed strong correlation with MD values in native space (correlation coefficient: 0.84; $p < 0.001$). Therefore, the slight effect of interpolation on MD has been ignored in the following statistical analysis.

Statistical analysis

Our subjects consisted of 27 TLE patients (17 TLE+ and 10 TLE−) and 26 control subjects. The TLE patients can be further broken down into left TLE+, right TLE+, left TLE−, right TLE− and bilateral TLE− (Table 1). With the addition of right, left and side ipsilateral and contralateral to the seizure focus the number of variables that can be analyzed becomes unmanageable. The primary objective of this study, however, was to look for differences in thalamic volume and DTI parameters between control, TLE+ and TLE− subjects. In preliminary qualitative analysis of the data, no obvious inter or intrasubject differences were observed in volume, MD or FA of TLE− patients and the differences in control and TLE+ subjects was small (only volume shown in Fig. 3). We therefore performed an initial analysis on collapsed data (comparing whole thalamus (left + right)/2 between control, TLE+ and TLE− subjects). In this analysis, one-way analysis of variance (ANOVA) was performed for each parameter (i.e. volume, FA and MD) of thalamus, followed by Dunnet's post hoc test comparing each patient group to the healthy controls. The difference between TLE+ and TLE− patients was separately tested by using a general linear model, in which duration/age at onset of epilepsy were controlled as covariates.

A secondary analysis was subsequently performed to further compare TLE+ patients to controls to test the effect of side (i.e. ipsilateral/contralateral) on the thalamus in TLE+ patients. As four of 10 TLE− patients demonstrated bitemporal seizure onset, this separate ipsilateral and contralateral thalamic analysis was not performed on our TLE− group. Notably, the left and right TLE+ patients were combined as a single patient group here. Given the observed asymmetry of MD and FA of the thalamus in controls, z-score representing relative position of value in a population was calculated for each value of thalamus (Tasch et al., 1999; Natsume et al., 2003). The use of the z-score makes the parameters of both left and right thalamus comparable and therefore allows the mixture of the thalamus of TLE+ patients as two sample groups (i.e. ipsilateral-group: left thalamus in left TLE+ and right thalamus in right TLE+; contralateral-group: right thalamus in left TLE+ and left thalamus in right TLE+). Specifically, each individual value was converted into a standardized z-score by subtracting

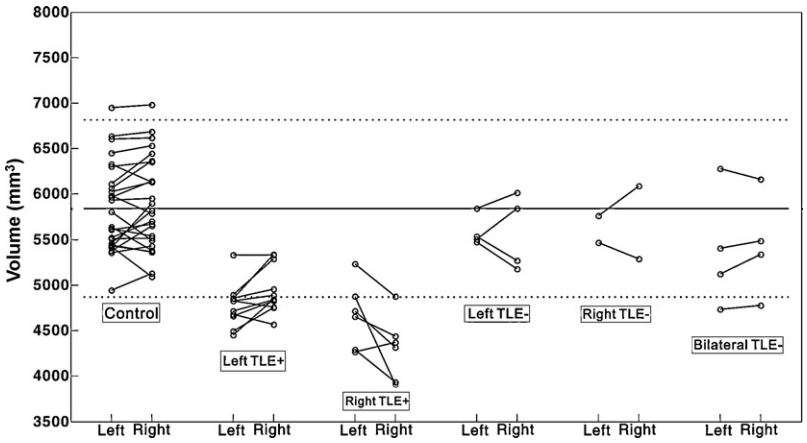

Figure 3 Scatter plot of thalamic volume (left and right separately) across all subjects. The center dark line represents the mean volume of left thalamus in healthy controls, and the upper and lower dash lines represent ±2 S.D. (no significant difference between left and right thalamus in healthy; left $5843 \pm 486\,mm^3$, right $5891 \pm 511\,mm^3$; $p = 0.25$). Based on the absence of inter or intrasubject differences in TLE− patients and the very small side to side differences in control and TLE+ subjects, TLE− subjects were collapsed into a single group and mean whole thalamus values were used for the initial statistical analysis.

the mean of healthy controls and then dividing the difference by the standard deviation of healthy controls for each thalamus (left or right). As well, individual T2 of hippocampus was converted into a *z*-score relative to the healthy control. For any individual, a *z*-score of −1 on any parameter indicates a raw value that is 1SD below the mean of normal controls on that parameter. By using *z*-scores, two samples *t*-test was performed to test the difference between controls and TLE+ patients with thalamus being classified as ipsilateral or contralateral to the seizure focus. In TLE+ patients, the degree of difference between ipsilateral and contralateral thalamus was examined by using paired *t*-test if bilateral thalamus both show significant difference, as compared to controls.

Pearson correlation was performed in both TLE+ and TLE− patient groups between thalamic parameters and either age at onset or duration of epilepsy. Further, partial correlation was calculated between thalamic parameters and age at onset while controlling for duration of epilepsy, as well as between thalamic parameters and duration of epilepsy while controlling for age at onset. In addition, Pearson correlation was performed between thalamic parameters and hippocampal T2 in TLE+ patients in our secondary analysis.

Results

Initial analysis

The results of group comparisons by using left—right collapsed value of thalamus are shown in Fig. 4. All parameters of thalamus showed significant group difference by ANOVA (volume, $p < 0.001$; MD, $p = 0.01$; FA, $p = 0.026$). As compared to healthy controls (volume $5867 \pm 487\,mm^3$; MD

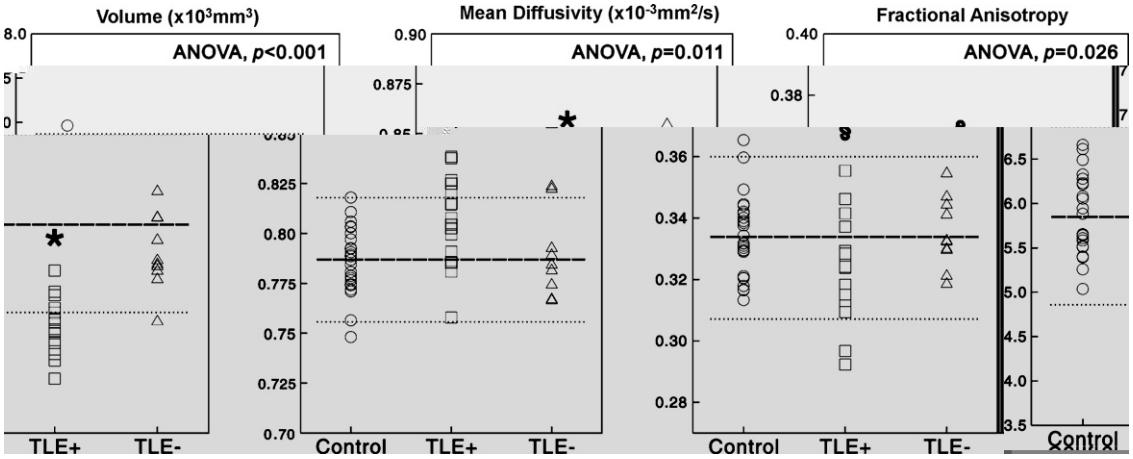

Figure 4 Scatter plots of overall thalamic volume (left), MD (middle) and FA (right) in healthy controls, TLE+ patients and TLE− patients. The center dark line represents the mean of healthy controls, and the upper and lower dash lines represent ±2 S.D. of healthy controls. TLE+ patients showed significant difference in Volume, MD and FA, as compared to controls. However, no significant difference was observed in TLE− as compared to controls ($^{\S}p < 0.05$ and $^{*}p < 0.01$, as compared to healthy controls by using Dunnet's post hoc test).

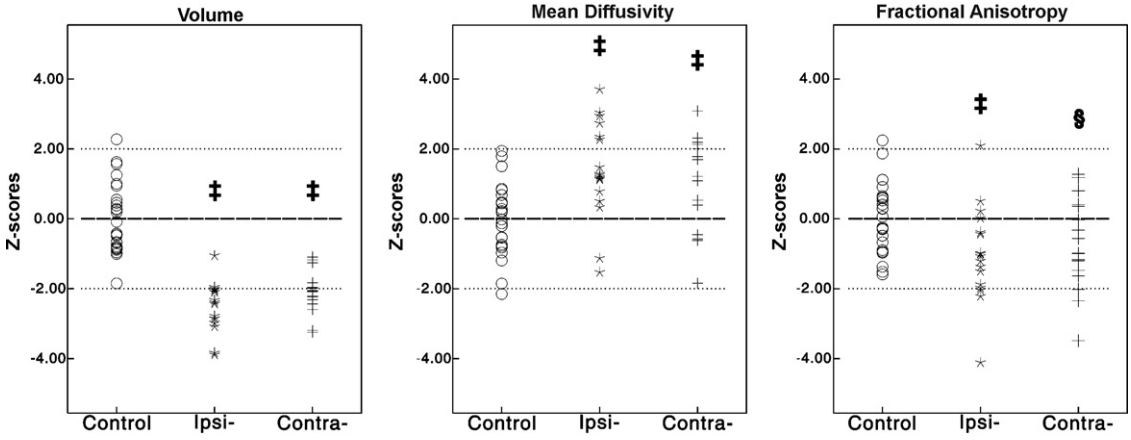

Figure 5 Scatter plots of *z*-score of thalamic volume (left), MD (middle) and FA (right) in healthy controls and TLE+ patients with thalamus being classified as ipsilateral or contralateral to seizure focus. TLE+ patients showed bilateral significant differences of reduced volume, increased MD and reduced FA, as compared to controls ($^{\S}p < 0.05$ and $^{\ddagger}p < 0.01$, as compared to healthy controls).

$(0.79 \pm 0.02) \times 10^{-3}$ mm^2/s; FA 0.33 ± 0.01), TLE+ patients showed significant changes in all parameters with a sizeable drop in volume (4731 ± 311 mm^3, $p < 0.001$) and small increases of MD ($(0.81 \pm 0.02) \times 10^{-3}$ mm^2/s, $p = 0.006$) and reduction of FA (0.32 ± 0.02, $p = 0.03$). In contrast, no significant difference was found between TLE− patients (volume 5528 ± 420 mm^3, $p = 0.07$; MD $(0.80 \pm 0.03) \times 10^{-3}$ mm^2/s, $p = 0.44$; FA 0.34 ± 0.01, $p = 0.95$) and healthy controls. After controlling for the duration and age at onset of epilepsy, only the volume of thalamus showed significant difference between TLE+ and TLE− patients (volume, $p = 0.001$; MD, $p = 0.23$; FA, $p = 0.23$).

Secondary analysis

In healthy controls, paired *t*-test demonstrated a significant right-greater-than-left asymmetric pattern in absolute MD (left $(0.78 \pm 0.02) \times 10^{-3}$ mm^2/s, right $(0.80 \pm 0.02) \times 10^{-3}$ mm^2/s; $p < 0.01$) and a significant left-greater-than-right asymmetric pattern in absolute FA (left 0.34 ± 0.01, right 0.33 ± 0.02; $p < 0.01$) of the thalamus, which is consistent with prior findings (Fabiano et al., 2005). There was no significant absolute volumetric asymmetry observed of the thalamus (left 5843 ± 486 mm^3, right 5891 ± 511 mm^3; $p = 0.25$). Given the diffusion asymmetry of the thalamus in controls, *z*-score was used for comparison in our secondary analysis, which aims to clarify the contribution of ipsilateral and contralateral thalamus to the overall difference between controls and TLE+ patients as detected in initial analysis. The results of group comparisons using *z*-scores are demonstrated in Fig. 5. Significant volumetric reduction was observed in both ipsilateral (mean ± S.D. of *z*-score: -2.51 ± 0.71; $p < 0.001$) and contralateral thalamus (-2.05 ± 0.64; $p < 0.001$) in TLE+ patients, as compared to healthy controls. As well, bilateral thalamus both showed significant elevation in MD (ipsi: 1.38 ± 1.4, $p < 0.001$; contra: 1.01 ± 1.31, $p = 0.005$) and reduction in FA (ipsi: -1.02 ± 1.35, $p = 0.006$; contra: -0.72 ± 1.3, $p = 0.043$) in TLE+ patients. Paired *t*-test showed no significant difference in MD ($p = 0.12$) and FA ($p = 0.17$) but significant difference (ipsi lower than contra) in volume ($p = 0.003$) between ipsilateral and contralateral thalamus, which indicated more severe atrophy of thalamus ipsilateral to seizure focus in TLE+ patients.

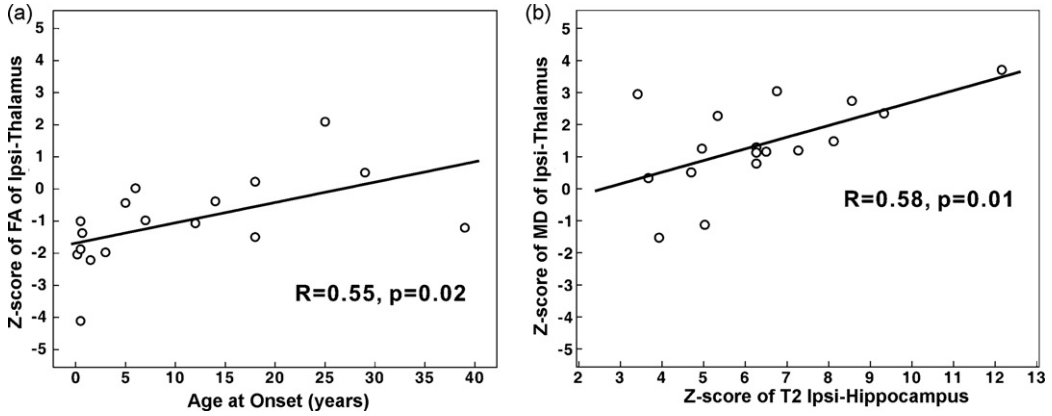

Figure 6 Scatter plots of significant positive correlations (a) between age at onset and *z*-score of ipsilateral thalamic FA and (b) between *z*-score of ipsilateral hippocampal T2 and *z*-score of ipsilateral thalamic MD in TLE+ patients.

Correlations

In our initial analysis for whole thalamus, neither Pearson correlation nor partial correlation was observed between any thalamic parameters (i.e. volume, MD and FA) and age at onset or duration of epilepsy in TLE+ or TLE− patients. Only thalamic FA showed tendency of correlation with age at onset (Pearson correlation, $r = 0.47$, $p = 0.06$; partial correlation while controlling for duration, $r = 0.46$, $p = 0.07$) in TLE+ patients. In our secondary analysis with ipsi- and contra-thalamus analyzed separately with z-scores, we found that only ipsilateral thalamic FA showed significant positive correlation ($r = 0.55$, $p = 0.02$) with age at onset in TLE+ patients (Fig. 6a). Partial correlation of ipsilateral FA with age at onset while controlling for duration of epilepsy remains significant ($r = 0.54$, $p = 0.032$). No other significant correlation was observed between ipsilateral/contralateral thalamic parameters and age at onset or duration of epilepsy. In addition, significant Pearson correlation was observed between ipsilateral hippocampal T2 and ipsilateral thalamic MD ($r = 0.58$, $p = 0.014$) (Fig. 6b) but not for thalamic FA ($r = -0.427$, $p = 0.088$).

Discussion

By using a manual-outlining method, previous studies have reported atrophy of ipsilateral thalamus in TLE+ patients (Natsume et al., 2003) and ipsilateral (DeCarli et al., 1998) and bilateral thalamus (Dreifuss et al., 2001) in TLE patients where the presence or absence of MTS is not specified. The discrepancy across these studies might be related to difference in criterion for outlining thalamus and patient selection. In contrast, voxel-based morphometry (VBM) has consistently demonstrated gray matter atrophy in bilateral thalamic regions of TLE+ patients (Bernasconi et al., 2004; Keller et al., 2004; McMillan et al., 2004; Bonilha et al., 2004; Mueller et al., 2006). Moreover, Bonilha et al. (2005) demonstrated intense gray matter concentration reduction in the anterior thalamic portion relative to the posterior portion bilaterally in TLE+ patients. In agreement with these previous studies, we found bilateral thalamic atrophy in TLE+ patients (Fig. 5), which indicated an important but nonspecific loss of structural components of bilateral thalamus in unilateral TLE+ patients. Notably, thalamic atrophy ipsilateral to the seizure focus was more severe than contralateral, suggesting the underlying mechanisms responsible for volumetric loss may differ ipsilateral and contralateral to seizure focus.

While most studies have focused on TLE+ patients, Mueller et al. (2006) and Natsume et al. (2003) have reported contradictory findings on thalamic volume in TLE− patients. As in our study Mueller et al. reported no difference in thalamic volume of TLE− patients as compared to controls. In contrast Natsume et al. reported significant reductions in thalamic volume in TLE− patients ipsilateral to the seizure focus. It is possible that the difference in criterion for defining MTS may be responsible for the contrasting findings (our study used hippocampal T2 relaxometry to define MTS while Natsume et al. used hippocampal volumetry). As Bernasconi et al. (2000) have demonstrated T2 relaxometry is more sensitive in the detection of MTS than hippocampal volumetry, a direct comparison between our results and those of Natsume et al. is likely not valid. In addition, previous [^{11}C]flumazenil (FMZ) binding PET findings in TLE− patients showing no FMZ binding changes in thalamus provide evidence supporting our negative finding (Hammers et al., 2002).

Consistent with our volumetric findings, DTI also showed bilateral pathology of thalamus in TLE+ patients but no diffusion changes of the thalamus in TLE− patients, as compared to healthy controls (Fig. 5). Notably, despite the significance of the diffusion changes, the difference in DTI parameters between TLE+ patients and healthy controls is small especially as compared to the dramatic difference of volume. Given the plethora of myelinated axons in the thalamus, the slight elevation of MD and reduction of FA likely reflects damage of the remaining axons (e.g. less myelin or less densely packed axons) in TLE+ patients. While we demonstrated bilateral thalamic diffusion abnormalities, albeit small, in TLE+ patients (Fig. 5), neither Thivard et al. (2005) nor Kimiwada et al. (2006) observed significant thalamic diffusion abnormalities in TLE patients. Kimiwada et al. reported a trend towards elevated MD in children with TLE who experienced generalized convulsions ($p = 0.09$) using 2D region-of-interest analysis of the thalamus whereas Thivard et al. (2005) used voxel-based analysis of the whole brain and reported no diffusion differences of the thalamus in TLE+ adult patients. It is possible that the absence of thalamic abnormality in this report could relate to differences in image analysis and patient selection. In the case of the report from Kimiwada et al., differences in patient selection could account for the contrasting results. While we analyzed adult patients with MTS and without MTS separately, the report from Kimiwada et al. (2006) combined children with and without MTS, e.g. only 5 of 14 had hippocampal sclerosis.

Several possible explanations for thalamic abnormalities in TLE+ patients include the chronic effects of excitotoxic damage from repeated epileptic discharge and/or transneuronal degeneration secondary to deafferentation (Torch et al., 1977; Kodama et al., 2003). While the observation of DTI and volumetric abnormalities ipsilateral to the seizure focus in TLE+ patients is consistent with downstream changes secondary to ongoing seizure activity, the contralateral thalamic abnormalities are more difficult to explain. While contralateral abnormalities could reflect secondary deafferentation along interhemispheric pathways, the presence of bilateral abnormalities in patients with unilateral seizure onset could suggest the possibility that the observed abnormalities may be present prior to the onset of seizures in TLE+ patients, which is also partly supported by the absence of Pearson correlation and partial correlation between any of thalamic parameters and duration of epilepsy in our TLE+ patients. Although speculative, these pre-existing abnormalities in thalamus could be a required state for the development of the epileptic circuit in TLE+ patients.

Furthermore, a significant correlation between age at onset and ipsilateral thalamic FA, while controlling for duration of epilepsy, was present in the TLE+ patients (Fig. 6a). Previously, Hermann et al. (2002) reported that early onset TLE patients rather than late-onset are associated with reduced brain tissue volume, particularly total white matter

volume, which suggests a global adverse neurodevelopmental impact of early onset of TLE on brain structure. Our observed positive correlation of age at onset with ipsilateral thalamic FA z-score implies that early onset TLE+ patients have more severe thalamic pathology, although volume of thalamus z-score was not correlated with age at onset. This is not surprising as MRI volumetry and DTI are measuring aspects of two related but distinct pathological phenomena. Volumetric reduction is suggestive of an overall and nonspecific loss of structural components of thalamus, whereas DTI abnormalities are believed to mainly reflect the damage of axonal integrity and/or myelin loss in the thalamus. While Keller et al. (2002) reported relations between gray matter concentration of thalamus and duration/age at onset of epilepsy in TLE patients, patient selection (e.g. TLE+ and TLE− patients were combined in the study of Keller et al.) and methodological difference may be responsible for our contrasting findings. In addition, ipsilateral hippocampal T2 showed significant correlation with thalamic MD ipsilateral to the seizure focus in our TLE+ patients (Fig. 6b), which suggests a thalamohippocampal network pathology in TLE+. This network pathology is likely to be associated with the downstream damage from recurrent discharge along the ''Papez Circuit'' during the seizure in TLE+. Further studies are needed to clarify this issue.

As compared to healthy controls, our TLE+ and TLE− patients exhibited different patterns of thalamic properties in both MRI volumetry and DTI. The direct comparison with controlling for age at onset and duration of epilepsy further showed significant difference in the thalamic volume between TLE+ and TLE− patients, but no significant difference in FA and MD. Given our small sample size, we cannot rule out the possibility that TLE+ and TLE− actually have similar pathology of thalamus and the absence of thalamic abnormalities in our TLE− patients is associated with their shorter duration/later age at onset (as compared to our TLE+) leading to fewer seizures experienced and potentially less damage of thalamus. However, Carne et al. (2004) recently confirmed the high consistency between hippocampal atrophy from current high-resolution MRI and histopathological confirmation of hippocampal sclerosis (HS) (one of 10 MRI-negative TLE patients versus 23 of 23 MRI-positive TLE patients was confirmed to have histopathological evidence of HS), suggesting that TLE+ and TLE− are two unique clinicopathologic entities. Besides the hippocampal differences, our findings suggest the presence of differences in extra-temporal (i.e. thalamic) pathology between TLE+ and TLE− patients.

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