PAPER

Increased thalamus levels of glutamate and glutamine (Glx) in patients with idiopathic generalised epilepsy

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Objective: Abnormal thalamo-cortical oscillations underlie idiopathic generalised epilepsy (IGE). Although thalamic involvement has long been indicated by electrophysiological data, it has only recently become feasible to test this with independent methods. In this magnetic resonance (MR) study, we investigated the metabolic and structural integrity of the thalamus. Possible changes in glutamine and glutamate concentrations and signs of neuronal damage were of particular interest.

Method: Forty three IGE patients and 38 age and sex matched healthy controls were investigated. Quantitative single volume MR spectroscopy (MRS, 1.5 T) was used to measure concentrations of glutamate and glutamine (Glx) and *N*-acetyl aspartate (NAA) in thalamus and occipital cortex. We also measured thalamic volumes on high resolution gradient-echo images and estimated fractions of thalamic grey and white matter with voxel based morphometry (VBM).

Results: IGE patients showed elevated Glx and reduced NAA concentrations in the thalamus compared to controls (12.2 ± 2.6 v 8.9 ± 4.1 mM, $p=0.0022$ for Glx, and 9.9 ± 1.0 v 10.7 ± 0.9 mM, $p=0.017$ for NAA). Thalamic grey matter fraction was reduced in IGE patients, and white matter fraction was increased with the greatest increase in the dorso-medial thalamus. Mean thalamic volume was reduced in patients (6.7 ± 0.7 v 7.2 ± 0.6 ml in controls, $p=0.0001$), as was mean cerebral volume (1163 ± 128 v 1250 ± 102 ml, $p=0.0003$). Patients' thalamus/whole brain ratios were normal.

Conclusion: Quantitative MRS and VBM provide further evidence for involvement of the thalamus in IGE. The observed elevation of Glx levels together with reductions in NAA levels and grey matter fractions are consistent with epilepsy related excitotoxicity as a possible underlying mechanism.

Idiopathic generalised epilepsy (IGE) is a common and complex condition with a predominantly genetic aetiology and variable phenotypes.¹ Our knowledge of the pathophysiology of IGE is based mainly on electrophysiological experiments, the results of which have led to the centrencephalic² and corticoreticular theories.³ The former theory attributes IGE to pathological changes in the thalamic nuclei, whereas the latter postulates that ascending impulses from the thalamus impinge upon a diffusely hyperexcitable cortex. Although this debate has not been settled, it is likely that the cerebral substrate of IGE comprises both the thalamus and cerebral cortex as both structures are involved in thalamo-cortical oscillations,⁴ and destruction of either of them has been reported to modify seizure activity.⁵

Single unit EEG recordings suggest that pacemaker activity of neurons in the reticular nucleus of the thalamus is a precondition for thalamo-cortical oscillations.⁴ These neurons are GABA-ergic and excited via glutamatergic projections from the cerebral cortex. Excitation of neurons in the reticular nucleus leads to inhibitory postsynaptic potentials in thalamic relay cells, causing a de-inactivation of low threshold (T type) Ca^{2+} channels and bursts of action potentials in thalamic principal neurons (relay cells). These bursting relay cells then re-excite cortical neurons via collaterals.^{6,7} Corticothalamic projections ending back on thalamic relay cells, followed by neurons of the reticular nucleus projecting back to the cortex, finally close the circuit. Via this loop, a massive corticofugal excitatory input is conveyed to the thalamus, reinforcing the oscillations and the synchronisation necessary for the spike and wave activity.^{6–8}

This rather convincing notion of thalamic involvement in IGE has recently gained support from magnetic resonance spectroscopy (MRS) studies. In an MRS study of IGE patients, we found significant reductions in the neuronal

marker *N*-acetyl aspartate (NAA) in the thalamus. The loss of NAA was more severe in patients who experienced many generalised tonic-clonic seizures (GTCS) over their lifetime compared with those reporting less than 10 seizures.⁹ Accordingly, Bernasconi *et al* reported NAA reductions in 20 patients with IGE, and a negative correlation between the thalamic NAA levels and duration of epilepsy.¹⁰ Another MRS study of 10 juvenile myoclonic epilepsy (JME) patients showed a significant decrease in thalamic NAA/creatine values.¹¹ Although none of these reports is conclusive with respect to the underlying mechanisms, they clearly indicate a relationship to seizure load. These findings raise the question whether NAA reductions may reflect effects of neuronal excitotoxicity.

We addressed this question by analysing the levels of glutamate (Glu) and glutamine (Gln; the sum denoted as Glx = Glu+Gln) in parallel with levels of NAA in patients with IGE. In addition, voxel based morphometry (VBM) of the thalamus was carried out to evaluate whether possible NAA reduction could be attributed to cell loss. Due to time constraints and application of single voxel MRS, only the thalamus and the occipital cortex were investigated with this method. The occipital volume of interest (VOI) served as a reference region to differentiate between specific thalamic changes and general cerebral effects, for example, due to the antiepileptic medication.

Abbreviations: EEG, electroencephalography; FSE, fast spin-echo; Glx, glutamate and glutamine; GTCS, generalised tonic-clonic seizures; IGE, idiopathic generalised epilepsy; JME, juvenile myoclonic epilepsy; MNI, Montreal Neurological Institute; MR, magnetic resonance; MRS, MR spectroscopy; NAA, *N*-acetyl aspartate; SNR, signal to noise ratio; SPGR, spoiled gradient-echo; VBM, voxel based morphometry; VOI, volume of interest

METHODS

Patients

Forty three (20 females) adult patients (age 32 ± 8 years, age at onset 16 ± 5 years with a seizure history of 16 ± 10 years) with IGE according to the International Classification of Epilepsies from 1989 were investigated.¹ Twenty three had JME and the remaining 20 had GTCS.¹ Only those patients whose seizure phenomenology was reliably assessed with the aid of close relatives were included in the study. The diagnosis was based on seizure history, seizure semiology as described by relatives or recorded during hospitalisation, and the results from 129 interictal scalp electroencephalographic (EEG) recordings, all showing bilateral spike and wave or poly spike and wave activity without signs of focal onset. All the patients had a normal routine MR scan of the brain. JME patients had late childhood or teenage onset of awakening myoclonic jerks, most often in the upper but sometimes also in the lower extremities. All had a history of GTCS in addition to myoclonias. Four patients also experienced rare absences. The average age at seizure onset in patients with GTCS only was 15 years. All patients were controlled from the year of their seizure onset and were well known to the neurologist in charge (IS). Their semiology consisted of GTCS with immediate loss of consciousness and without signs of ictal onset or termination. Most GTCS patients reported seizures at random, although some experienced them most frequently on awakening. They had no history of myoclonic seizures and were deemed to fulfil the criteria for the diagnosis epilepsy with GTCS only.¹²

Twenty two patients reported more than 10 seizures during their lifetime, whereas 21 had 10 seizures or fewer. Thirty four patients were medicated with valproate, four with carbamazepine, three with lamotrigine, one with clonazepam, and two were unmedicated. Three patients were on polypharmacy. Except for the seizures, the patients were healthy. None had a progressive condition or a history of status epilepticus, drug intoxication, or drug related encephalopathy. There were no abnormalities on routine CT or MR scans. All patients participated in both MRS and structural MRI. Three patients left the scanner after the MRS but before acquisition of spoiled gradient-echo (SPGR) due to discomfort. Four spectra were excluded because of poor spectral quality.

The control group for structural brain imaging consisted of 38 right handed healthy volunteers (age 29 ± 8 years, 20 females). Ten of the controls were also examined with MRS. All control subjects underwent routine MRI of the brain. None of them was on medication at time of the study, and none had a family history of epilepsy or other neuropsychiatric disorders.

MRI protocol

All subjects were examined with a 1.5 T clinical MR system (Signa Advantage, General Electric Medical Systems, Milwaukee, WI) using a birdcage headcoil. The MRI protocol consisted of axial T2 weighted fast spin-echo images (FSE, effective TE = 85 ms, TR = 2500 ms, FOV = 24 cm, 23 slices of 3 mm thickness) and T1 weighted 3D (TE = 7 ms, TR = 23 ms, FOV = 24 cm, flip angle 25°, 128 slice partitions of 1.2 mm thickness, 2 NEX). The volumetric analyses were carried out on T1 weighted images. VOIs for MRS were positioned on the T2 weighted images as shown by Pouwels and Frahm.¹³ The average VOI size was 4.1 ml in thalamus and 7.2 ml in occipital cortex. A stimulated echo sequence (STEAM) was used for single volume MRS with a short echo time (TE = 30 ms) and a long repetition time (TR = 6 s) to reduce relaxation weighting. In accordance with our previous work,^{9, 14} 64 single acquisitions were corrected for effects of residual eddy currents and patient motion,¹⁵ and analysed by

a linear combination of model spectra (LCModel 5.2-2, Provencher, Oakville, Ontario, Canada; see Provencher¹⁶ and <http://www.s-provencher.com>). Concentration estimates in units of mM (mmol/l) were corrected for coil loading, B1 inhomogeneity, and partial volume of cerebrospinal fluid.¹⁷

Image processing and volumetric analysis

Volumetric measurements were performed using MRIcro software.¹⁸ This program displays the volumes simultaneously in the horizontal, sagittal, and coronal planes, thus reducing operator error when outlining the VOIs.

The borders of the thalamus were determined by anatomical landmarks as described by Kretschmann and Weinrich¹⁹ and using the Duvernoy atlas.²⁰ The anterior border was defined as the level of the anterior end of the third ventricle and the interthalamic adhesion. The inferior margin was defined as the superior border of midbrain structures and the medial margin of the wall of the third ventricle and the interthalamic adhesion. The lateral margin of the thalamus was defined as the medial border of the posterior limb of the internal capsule, and the superior margin as the body of the lateral ventricle. The medial and lateral geniculate bodies were excluded. The anatomical landmarks are shown in fig 1. The total brain volume was calculated using MRIcro software.¹⁸ Interrater reliability was tested by delineation of the same thalamus volumes in 10 randomly selected subjects. During the process of delineation the investigators were uninformed about the identity of the subjects.

VBM of the thalamus

Because the thalamus is relatively difficult to delineate, it is best to assess possible thalamic atrophy by a user independent method. This structure was, therefore, also analysed using VBM, as described by Good *et al.*,²¹ using the Statistical Parametrical Mapping software package (SPM99, Wellcome Department of Cognitive Neuroscience, London; <http://www.fil.ion.ucl.ac.uk/spm>).²²⁻²⁴ A template was first created from the T1 weighted images of healthy volunteers by affine spatial normalisation to the standard T1 template of the Montreal Neurological Institute (MNI) provided in SPM99, and spatial smoothing with an isotropic Gaussian kernel (8 mm full width at half maximum). Using this template, each 3D set of high resolution images was segmented into probability maps of grey matter, white matter, and cerebrospinal fluid. These segmented images were derived from matching intensities and an a priori knowledge of the spatial distribution of these tissues in normal subjects.²² Each subject's set of segmented images was then inspected for possible tissue misclassification. One patient showed mis-

Figure 1 Anatomical landmarks for the thalamus on horizontal and coronal slices of a T1 weighted MRI used for manual delineation. The right side is to the left in the image.

Figure 2 Spectra of occipital cortex in a control subject (A) and IGE patient (B). Spectra of thalamus in a control subject (C) and IGE patient (D). Bold lines indicate the LCModel fit to the original data.

classification and was excluded from further analysis. The segmented images were then smoothed using an identical Gaussian kernel and submitted to statistical evaluation. The probability maps reflected fractions of grey matter, white matter, and cerebrospinal fluid. Results from probability maps will, therefore, be discussed in terms of fractions. Data were analysed on a Sun Ultra Blade 150 workstation (Sun Microsystems, Mountain View, CA) using MATLAB 6.5.1 (MathWorks, Natick, MA).

Statistics

Differences in NAA and Glx concentrations between patients and controls were tested with one sided *t* tests at a significance level of $p < 0.05$. An unpaired *t* test was also used for comparisons between patients with high (>10 GTCS yearly) and low seizure frequency (<10 GTCS yearly) ($p < 0.05$). Pearson's coefficient of correlation was calculated to test whether seizure frequency, duration of seizures, age at onset, and seizure recency (that is, days between the last seizure and the MR investigation) were related to thalamic concentrations of Glx and NAA ($p < 0.05$). Pearson's coefficient of correlation was also used to test the possible correlation between thalamus concentration of NAA and Glx, and the interrater reliability of the estimated thalamus volumes. Finally, differences in the estimated local grey and white matter were tested with SPM99, restricting the search

volume to the thalamus by use of a rectangular mask (Talairach coordinates $x = -27$ to $+27$, $y = -35$ to $+4$, $z = -6$ to $+16$). This mask included both hemispheres, a minor part of the third ventricle, and the surrounding white matter. Statistically significant differences between patients and controls were calculated with random effect analysis (using the height *T* threshold of 0.001, and a *p* threshold of 0.05). Separate contrasts were used for grey and white matter fractions contrasting patients with controls (patients-controls to evaluate an increase in patients; and controls-patients to assess a decrease in patients). The local maxima for significant differences between the two groups of subjects are given in Talairach's coordinates.

RESULTS

The thalamic concentrations of Glx were significantly higher in IGE patients than in controls (12.2 ± 2.6 v 8.9 ± 4.1 mM, $p = 0.0022$), while the thalamic concentrations of NAA were lower than in controls (9.9 ± 1.0 v 10.7 ± 0.9 mM, $p = 0.017$). Typical spectra are shown in fig 2. In contrast, no significant differences were found in the occipital cortex (table 1, fig 3). Although only NAA and Glx levels were of interest for the present study, the spectral acquisition automatically provided the levels of creatine, choline, and myo-inositol. Whereas the thalamus creatine did not differ (6.1 ± 1.1 mM in controls and 6.2 ± 0.7 mM in patients), both the choline and

Table 1 Metabolite concentrations and values from selected parameters

	NAA (mM)	Glx (mM)	Tissue water content (%)	Water T2 (ms)
Patients, right thalamus	9.9 ± 1.0*	12.2 ± 2.6**	65.8 ± 2.3*	72.3 ± 2.5
Controls, right thalamus	10.7 ± 0.9	8.9 ± 4.1	67.5 ± 3.2	74.2 ± 3.6
CR bound†, right thalamus	0.84 ± 0.17	2.66 ± 0.63	–	–
Patients, occipital cortex	11.0 ± 1.1	11.9 ± 2.9	69.6 ± 2.3	73.4 ± 2.5
Controls, occipital cortex	11.3 ± 0.7	11.1 ± 2.7	68.0 ± 2.2	74.3 ± 3.0
CR bound, occipital cortex	0.65 ± 0.56	2.46 ± 0.69	–	–

Mean values and standard deviations.

* $p < 0.05$; ** $p < 0.01$, significant differences between IGE and controls.

†Cramér-Rao lower bounds of fitting error: %SD values provided by LCModel converted to absolute concentration units.

myo-inositol were lower in patients (2.0 ± 0.4 v 1.6 ± 0.3 mM, $p = 0.0005$ for choline, and 4.8 ± 1.5 v 3.3 ± 1.4 mM, $p = 0.006$ for myo-inositol).

No correlation was observed between concentrations of NAA and Glx in the thalamus or in the occipital cortex. Neither did we find a correlation between individual concentrations of Glx or NAA, and the measured clinical parameters (age, age at seizure onset, duration of epilepsy, or time between MR scan and the preceding seizures). Patients with more than 10 GTC seizures during their lifetime (22 patients) had lower thalamic NAA concentrations than those with less than 10 seizures (9.5 ± 1.0 v 10.4 ± 1.1 mM, $p = 0.014$). No corresponding difference was observed with respect to Glx (12.6 ± 2.8 v 11.7 ± 2.3 mM). Neither the MRS results nor the volumetric or VBM analysis showed any significant difference between patients with JME and GTCS (two sided unpaired t test with unequal variances, $p < 0.05$).

Although the populations were matched with respect to gender distribution, the mean thalamic volume was significantly reduced in patients when compared to controls (6.7 ± 0.7 v 7.2 ± 0.6 ml, $p = 0.0001$), as was the mean cerebral volume (1163 ± 128 v 1250 ± 102 ml, $p = 0.0003$) (table 2). The thalamic volumes in relation to the whole brain

Figure 3 Absolute concentrations of NAA and Glx in controls (blank) and IGE (shaded). The boxes indicate the inner quartiles, the vertical lines the outer quartiles. Outliers are shown as circles.**Table 2** Volumetry

Subjects	Thalamus (ml)	Total brain (ml)	Ratio thalamus/total brain
Controls	7.2 ± 0.6	1250 ± 102	57.4 × 10 ⁻⁴
Patients	6.7 ± 0.7***	1163 ± 128***	57.6 × 10 ⁻⁴

*** $p < 0.001$.

volume did not differ between patients and controls. The interrater correlation in the measurements of the thalamic volumes was 0.96.

Operator independent VBM revealed a significantly lower thalamic fraction of grey matter and a significantly elevated fraction of white matter in patients (table 3, fig 4).

The peak significance for the reduction in grey matter was located in the right ventro-medial thalamus (at Talairach's coordinates $x = 6$, $y = -10$, $z = 2$; fig 4) as was the peak significance for the increase in white matter ($x = 4$, $y = -10$, $z = 6$). The grey or white matter fractions did not differ between patients who experienced more than 10 GTCS over their lifetime compared to those having less than 10 GTCS during their lifetime.

DISCUSSION

Using established MR methodology we investigated whether thalamic alterations can be detected in patients with IGE. Our major finding, reduction in NAA accompanied by elevation of Glx and reduced grey matter fractions, supports this assumption. Prior to discussing the possible underlying mechanisms, some methodological aspects need to be scrutinised.

Methodological error of the Glx concentration estimate is mainly due to low signal to noise ratios (SNR) and strong coupling effects. These errors could have masked putative correlations to NAA and clinical parameters. LCModel is an automated, user independent curve fitting routine for the quantification of metabolite concentrations from in vivo MR spectra.¹⁶ The program uses a library of spectra at calibrated concentrations of each individual metabolite. This allows discrimination between metabolites with partly overlapping

Figure 4 VBM showing decreased thalamus grey matter fraction in patients compared to controls. The right side is to the right in the image. The white areas in the centre of each image indicate z scores of ≥ 4 .

Table 3 VBM of white and grey matter in the thalamus

	Peak z value	Cluster size (ml)	Talairach's co-ordinates (x, y, z)	
Grey matter	5.3	1.5	6, -10, 2	Right thalamus
Controls-patients	4.4	0.4	-4, -12, 6	Left thalamus
White matter	4.2	1.44	4, -10, 6	Right thalamus
Patients-controls	NS	-/-	-/-	Left thalamus

signals by exploiting spectroscopic information from resonances at different frequencies. However, it is difficult to differentiate between glutamine and glutamate at 1.5 T due to the similarity of these compounds. Even in spectra of standard locations in parietal grey and white matter the concentration of Glx = Glu+Gln is commonly determined, because the fitting error of the sum is much smaller than those of the single compounds. The flexible base line estimated by LCModel includes resonances with broad line widths, thus minimising macromolecular contribution to the metabolite peaks. Thus, LCModel has successfully been applied in several MRS studies of epilepsy patients at 1.5 T, even when analysing small voxels.^{25, 26} The Glx concentrations in our control subjects were higher in cortex than in thalamus. This reproduces findings obtained at 2 T using similar methodology (9.8 mM in thalamus, 12.5 mM in occipital cortex¹³) albeit at slightly lower levels. Systematic deviations for single metabolites when comparing different field strengths and echo times may be due to a systematic influence of the SNR dependent baseline.²⁷ The Cramér-Rao bounds for the fitting error are an estimation of the precision of estimates. For comparison with the standard deviations (SD) and group differences, the relative values provided by LCModel ("'%SD'") were converted to absolute concentrations. These were consistent in patients and controls, and mean values are given in table 1. They accounted for a large fraction of the observed SDs but were considerably smaller than the differences of mean NAA and Glx concentration.

A potential bias with the single voxel method is the difficulty of avoiding partial inclusions of areas outside the lateral border of the thalamus (globus pallidus and internal capsule). As discussed previously, the influence of systematic partial volume changes would lead to "dilution" of any thalamic abnormality. Consequently, elimination of partial volume effects is expected to enhance the present finding of increased Glx and reduced NAA concentrations.

Although VBM suggests a stronger involvement of the right thalamus, no statements about lateralisation can be made, as changes were also observed on the left side. No asymmetries were found between the right and left thalamus in the volumetric analysis. Because of time constraints, only the right thalamus was investigated with MRS in the present study. All examined subjects were right handed. A difference between patients and controls due to systematic lateralisation was, therefore, not expected, which is in agreement with several published studies reporting no asymmetries in thalamic NAA levels.^{10, 11}

Antiepileptic drug therapy may, theoretically, have affected the observed concentrations of Glx. Most of our patients were treated with valproate and three were medicated with lamotrigine. Lamotrigine is expected to reduce rather than elevate glutamine and glutamate concentrations.²⁸ Changes in glutamine levels have been reported in patients treated with valproate in some studies^{29, 30} but not in others.³¹ Our patients showed, however, normal Glx levels in the occipital

cortex, suggesting that the observed thalamic changes in this study were not an overall effect of antiepileptic medication. Furthermore, no differences or trends to differences were observed in thalamic Glx values between patients treated with valproate, lamotrigine, and carbamazepine.

Because of rather poor grey/white matter differentiation, manual volumetry of the thalamus is cumbersome and prone to operator bias. Like others, we obtained an SD of about 10 % in healthy controls.³² In view of previous negative results,³² volumetry may not be sufficiently sensitive to detect changes in the thalamus associated with IGE. In contrast, VBM showed a reduction in thalamic grey matter in IGE. This finding is consistent with the slight decrease in mean water content in the MRS volumes ($p = 0.019$, table 1).

Glutamate and aspartate are thought to play an important role in the modulation of thalamic activity initiated by corticothalamic projections.³³ Since neurons and glia are tightly coupled with respect to glutamate and glutamine metabolism, an elevation in Glx may result from several processes. Tentative explanations involving altered neuronal or glial numbers, intercellular concentration due to flux rates, or activity of the tricarboxylic acid cycle remain purely speculative as altered Glx levels reflect changes of both glutamate and glutamine in both neurons and glia. Nevertheless, Glx has proven valuable in evaluations of several pathological conditions, including IGE, provided that comparisons are carried out between matching populations.²⁶ Increases in Glx in parallel with decreases in NAA have also been found in the epileptogenic regions of patients with partial epilepsy.^{25, 34} Reduced thalamic cholines and myo-inositols in IGE are consistent with our previous findings.^{9, 14} Though the origin is not clear, these findings argue against the presence of reactive gliosis. One possible explanation, compatible with the reduced grey matter fractions, is thalamic atrophy. Glutamine and glutamate (and thus Glx) are highly involved in excitatory neuronal activity. After transcranial magnetic stimulation, a persisting increase in Glx has been observed, and glutamate level is shown to increase after epileptic seizures.³⁵ Furthermore, studies with microdialysis showed increased ictal and postictal extracellular glutamate.^{36, 37}

One suggested mechanism for the elevated extracellular glutamate concentration in epilepsy is the reduction in glutamate reuptake. The levels of glutamate transporters are decreased prior to seizure occurrence in the cortex and the thalamus,³⁸ which underlines the possible involvement of glutamine and glutamate transmission in this region.

The finding of altered grey and white matter fraction in the thalamus in our patients questions the current definition of IGE as a condition without regional morphological changes. The present data also add a new factor in understanding the underlying pathophysiology of IGE, by suggesting elevations in glutamine and/or glutamate levels, which is a common characteristic of epileptogenic tissue. The present observations may reflect seizure associated changes in the thalamus even though no direct relationship to seizure load (total number, frequency, duration) was found, possibly due to the low spread of these parameters within our patient population. The results also provide a possible rationale for the recent observation of an increased interictal BOLD signal in the thalamus and cerebral cortex of patients with absence epilepsy,³⁹ and increased interictal blood oxygenation level dependent signal in thalamus during the spike and wave discharges.⁴⁰ Additional prospective studies of larger numbers of patients (including comparisons with patients having secondarily generalised seizures) are, however, necessary to further elucidate the cause-effect relationship between epileptic seizures and their subsequent effect on the thalamus in IGE.

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ELECTRONIC-DATABASE INFORMATION

Information on LCModel 5.2-2 can be found at <http://www.s-provencher.com> and on SPM99 at <http://www.fil.ion.ucl.ac.uk/spm>.

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