Magnetic resonance spectroscopy and imaging of the thalamus in idiopathic generalized epilepsy

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Summary
Experimental work in animal models of generalized epilepsy and clinical data in humans with idiopathic generalized epilepsy (IGE) indicate that the thalamo-cortical circuitry is involved in the generation of epileptic activity. The purpose of this study was to evaluate in vivo the chemical and structural integrity of the thalamus in patients with IGE. Thalamic proton magnetic resonance spectroscopic imaging (1H-MRSI), measuring N-acetylaspartate (NAA), choline-containing compounds and creatine (Cr) was performed in 20 IGE patients and in a group of age-matched healthy subjects. Additionally, 1H-MRSI measurements were taken in the insular cortex, the posterior temporal lobe white matter and the splenium of the corpus callosum. MRI volumetric analysis of the thalamus was performed in all patients. At the time of the examination, seizures were well controlled in 10 IGE patients and poorly controlled in nine. One patient was newly diagnosed and had the MRI and MRSI examination prior to starting the antiepileptic medication. In IGE patients, 1H-MRSI showed a reduction of mean thalamic NAA/Cr compared with normal controls; no difference was found in NAA/Cr in the other examined areas. There was no difference in NAA/Cr between patients whose seizures were well controlled and those in whom seizures were not controlled. There was no correlation between thalamic NAA/Cr and mean number of spike and wave complexes. We found a significant negative correlation between thalamic NAA/Cr and duration of epilepsy. The mean thalamic volume in patients with IGE was not different from normal controls. These results show evidence of progressive thalamic neuronal dysfunction in patients with IGE supporting the notion of abnormal thalamo-cortical circuitry as a substrate of seizure generation in this form of epilepsy. The thalamic dysfunction may occur regardless of amount of spike and wave activity.

Keywords: idiopathic generalized epilepsy; thalamus; proton magnetic resonance spectroscopic imaging; MRI

Abbreviations: Cr = creatine; 1H-MRSI = proton magnetic resonance spectroscopic imaging; IGE = idiopathic generalized epilepsy; NAA = N-acetylaspartate; VOI = volume of interest

Introduction
Idiopathic generalized epilepsy (IGE) is characterized by the clinical triad of typical absences, tonic-clonic seizures and myoclonic jerks, with their clinical onset in the first two decades. Patients with different IGE sub-syndromes manifest all or some of these seizure types (Duncan, 1997). The cardinal EEG features are generalized spikes and waves with a normal background activity and, in some patients, photosensitivity. The currently recognized IGE sub-syndromes include: benign neonatal familial convulsions; benign neonatal convulsions; benign myoclonic epilepsy in infancy; childhood absence epilepsy; juvenile absence epilepsy; juvenile myoclonic epilepsy; epilepsy with generalized tonic-clonic seizures on awakening; and epilepsies with seizures precipitated by specific modes of activation (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Prevalence of pharmacoresistant forms account for ~10–20% of all IGE cases (Genton and Gelisse, 2001). In series of 155 consecutive juvenile myoclonic epilepsy cases, it was found that ~15% of them had persisting seizures despite adequate treatment and lifestyle (Gelisse et al., 2001).

The neuroanatomical basis and the neurochemical abnormalities that underlay IGE are not fully defined and this is an area of active research in humans with the condition and in animal models. Accumulating evidence over the years has indicated that the thalamus plays a central role in cortical...
synchronization (Gloor, 1979) (for a review, see Avoli et al., 2001). Recent experimental work on genetic animal models of generalized epilepsy confirmed that the thalamo-cortical circuitry is involved in spike and wave discharge generation (Avanzini et al., 1996). According to these studies, a genetically determined dysfunction in reticular thalamic neurons may alter the electroresponsiveness of the developing thalamo-cortical system and cause a persistent state of abnormal excitation (Avoli et al., 2001).

Unlike conventional MRI, which provides structural information based on signals from water protons, proton magnetic resonance spectroscopic imaging (1H-MRSI) provides information about the chemical composition of the brain. Three major peaks characterize long echo-time 1H-MRSI spectra: N-acetylaspartate (NAA), creatine (Cr) and choline. Immunohistochemical studies have suggested that NAA is localized exclusively in neurons and their processes throughout the CNS (Moffett et al., 1991; Simmons et al., 1991; Urenjak et al., 1993). NAA can be used as a neuronal marker (Bjartmar et al., 2002) and a reduction in NAA levels as assessed by 1H-MRSI has been a useful tool for quantifying brain neuronal and axonal integrity in vivo (Matthews et al., 1990; De Stefano et al., 1995; Tsai and Coyle, 1995; Hugg et al., 1996; Nakano et al., 1998; Bjartmar et al., 2002).

Given the evidence for thalamic neuronal involvement in IGE, we conducted a study using 1H-MRSI to test the hypothesis that concentrations of thalamic NAA would be lower in patients with IGE than in healthy control subjects. To assess structural integrity of the thalamus, we used high-resolution volumetric MRI.

**Methods**

**Subjects**

We studied 20 consecutive patients with IGE (14 males; mean age ± SD = 37 ± 9 years; range = 20–52 years). All subjects were right-handed. Demographic and clinical data were obtained through interviews with the patients and their relatives, and by reviewing hospital charts. IGE diagnosis was based on seizure history and semiology, and routine EEG recordings in all patients and video-EEG telemetry in 15 of them. According to the currently recognized IGE sub-syndromes (Commission on Classification and Terminology of the International League Against Epilepsy, 1989), 12 patients had juvenile myoclonic epilepsy and eight had epilepsy with generalized tonic–clonic seizures on awakening. Estimation of the duration and frequency of seizures was based on review of medical records and seizure calendars, and specific questioning of the patient and family members. Age of onset of epilepsy was defined as the age at which the patient developed habitual and recurrent seizures. The duration, or number of years of epilepsy, was defined as the interval between the age of onset and time of the examination. Ten patients had well-controlled seizures (occasional seizures or were seizure free for many years) and nine patients had poorly controlled seizures (1–4 seizures/month). In one patient with newly diagnosed epilepsy, the MRI and 1H-MRSI examinations were performed prior to starting the medication. All had been seizure-free for at least 48 hours prior to the MRI experiments. The Ethics Committee of the Montreal Neurological Institute and Hospital approved the study and informed consent was obtained from all participants.

**Proton MRI/MRSI**

1H-MRSI of the thalamus was obtained in all patients and a group of 11 healthy subjects (seven males; mean age = 34 ± 5 years; range = 27–45 years). Combined proton MRI and MRSI examinations of the brain were obtained in a single session for each examination using a Philips Gyroscan ACS II operating at 1.5 T (Philips Medical Systems, Best, The Netherlands). After scout images in axial and sagittal planes, a multislice transverse spin-echo MRI [TR (repetition time) = 2000 ms, TE (echo time) = 30 ms] was obtained. These conventional MRI images were used to position a spectroscopic volume of interest (VOI) of ~90 mm antero-posteriorly by 90 mm left to right by 18 mm cranio-caudally. The VOI centred on the thalamus was defined for selective excitation prior to phase encoding of the MRSI (Fig. 1). The VOI was oriented parallel to the AC-PC line plus 10° to eliminate susceptibility artefacts generated from the fronto-basal region. MRI spectroscopic images were acquired [32 × 32 phase-encodes, 250 × 250 mm FOV (field of view), 20 mm slab thickness] using a double spin-echo excitation method (TR = 2000 ms, TE = 272 ms) (Ordidge et al., 1987). To suppress the intense water resonance, frequency selective excitation pulses were placed at the beginning of the MRSI sequence (Haase et al., 1985). A quick MRSI without water-suppression was also acquired (TR = 850 ms, TE = 272 ms, 16 × 16 phase encodes, 250 × 250 mm FOV and one signal average) to allow for correction of B0 inhomogeneity during post-processing.

Post-processing of the raw MRSI data included zero filling the non-water-suppressed MRSI to obtain 32 × 32 profiles, followed by a mild gaussian k-space filter and an inverse 2D Fourier transformation to both the water-suppressed and unsuppressed MRSI. Artefacts present in the time domain water-suppressed signal due to static magnetic field inhomogeneities and time-varying gradients were corrected by dividing the water-suppressed MRSI signal by the non-water-suppressed signal (Fu et al., 1998)—a procedure that does not affect relative signal intensities. The residual water signal was then fitted and removed from the water-suppressed data using the Hankel singular-value decomposition procedure (de Beer et al., 1992). To enhance the resolution of the spectral peaks, a lorentzian-to-gaussian transformation was applied before Fourier transformation in the spectral domain. The nominal voxel size in-plane was ~8 × 8 mm, which yielded a spatial resolution of ~12 × 12 mm after filtering.
Metabolite resonance intensities were determined automatically and relative to a spline-corrected baseline using in-house software. Metabolite signals were expressed as ratios of NAA to Cr (a signal arising mainly from both Cr and phosphocreatine). In clinical experiments, it is convenient to quantify NAA in vivo in relation to Cr, which is relatively homogeneously distributed throughout the brain and is not significantly influenced by the epileptic state (Petroff et al., 1995).

A single operator unaware of the subject’s diagnosis selected voxels in the right and the left thalamus for each subject. Each voxel was located entirely within the thalamus to minimize any metabolite contamination from adjacent CSF or grey and white matter outside the thalamus. Artefactually broadened spectra were excluded from the analyses. In the same VOI, additional voxels were selected for each subject in the right and left insular cortex, the right and left posterior temporal lobe white matter and the splenium of the corpus callosum (Fig. 1). The number of spectra averaged for each structure in normal controls and patients was as follows: thalamus 3.8 ± 0.3 versus 3.6 ± 0.5; insular cortex 2.8 ± 0.9 versus 2.6 ± 0.9; posterior temporal lobe white matter 2.4 ± 0.9 versus 2.1 ± 0.6; and splenium 1.8 ± 1.0 versus 2.0 ± 0.9.

**Volumetric MRI analysis**

Thalamic volumes were obtained in all patients and a group of 21 healthy controls (nine males; mean age = 35 ± 14 years; range = 20–65 years). MRI volumetric images were acquired using a T1-fast field echo sequence (TR = 18 ms, TE = 10 ms, one acquisition average pulse sequence, flip angle = 30°, matrix size = 256 × 256, FOV = 256, thickness = 1 mm) giving ~170 isotropic images with a voxel size of 1 × 1 × 1 mm. Visual analysis of the MRI did not reveal any structural abnormality in IGE patients. Volumetric analysis was performed on a Silicon Graphics workstation (Mountain View, California, USA). Images were automatically registered in a standard, stereotaxic space (Talairach and Tournoux, 1988) to adjust for differences in total brain volume and brain orientation, and to facilitate the identification of boundaries by minimizing variability in slice orientation (Collins et al., 1994). Each image underwent automated correction for intensity non-uniformity due to radiofrequency inhomogeneity of the MRI scanner and intensity standardization (Sled et al., 1997). The thalamus was manually segmented using locally developed software, which allows simultaneous viewing of MRI images in coronal, sagittal and horizontal orientations. The anatomical landmarks were determined according to the description by Kretschmann and Weinrich (1992) and the atlas by Schaltenbrand and Wahren (1977). The anterior tip of the thalamus is directed toward the interventricular foramen. Therefore, the anterior margin of the thalamus was defined as the level of the anterior end of the interventricular foramen. The medial margin of the thalamus was defined as the wall of the third ventricle and the interthalamic adhesion, which connects both thalami. The inferior margin was defined as the hypothalamic sulcus, which forms the border in the third ventricle between the inferior thalamus and hypothalamus. Posteriorly, the inferior margin of the thalamus was defined as the superior border of midbrain structures. The lateral margin of the thalamus was defined as the medial border of the posterior limb of the internal capsule. The superior margin of the thalamus was defined as the inferior margin of the central part of the lateral ventricle and the caudate nucleus laterally. The posterior margin of the thalamus was defined as the end of the pulvinar and the medial and lateral geniculate bodies were excluded. The anatomical landmarks are shown in Fig. 2. Intra-rater reliability of thalamic volume measurement was 2.3 ± 2.4% in the left thalamus and 1.9 ± 1.2% in the right thalamus of the 10 normal controls in which measurements were performed twice.

**EEG analysis**

Fourteen patients had generalized spike and slow wave activity at >3 Hz. The remaining six had generalized spike activity <3 Hz or isolated bursts of generalized spikes and slow waves. To examine the relationship between NAA/Cr in the thalamus and the amount of spike and slow wave activity
Because the values of spike and slow wave frequency did not exhibit a normal Gaussian distribution, they were transformed into logarithmic values. To determine the relation between spike and wave activity and thalamic NAA/Cr, we performed Pearson correlation analysis.

Results

Group analysis

Proton MRSI

Patients with IGE and normal controls did not differ in age [F(1,29) = 1.68, P = 0.2] or sex distribution [\( \chi^2 = 0.13 \), degrees of freedom (df) = 1, P = 0.7]. For the 11 normal controls studied, the mean NAA/Cr of the different structures was: left thalamus 2.49 ± 0.13, right thalamus 2.47 ± 0.13 (P = 0.3); left insular cortex 2.56 ± 0.13, right insular cortex 2.54 ± 0.14 (P = 0.4); left posterior temporal lobe white matter 3.56 ± 0.20, right posterior temporal lobe white matter 3.53 ± 0.24 (P = 0.5). The mean NAA/Cr of the splenium was 4.26 ± 0.46. Since there was no difference between the left and right mean NAA/Cr in our normal controls and no lateralized abnormalities were expected to be found in IGE, we used a single thalamic NAA/Cr ratio in controls and patients by obtaining the weighted average of right and left thalamic NAA/Cr (thalamic NAA/Cr). The same was done for the NAA/Cr values in the insular cortex and the posterior temporal lobe white matter.

We found a significant reduction in thalamic NAA/Cr of IGE patients compared with the healthy control group (P = 0.006) (Fig. 3). There was no difference in NAA/Cr between the 10 IGE patients whose seizures were well controlled at the time of the MRSI exam and the nine IGE patients in whom seizures were not controlled (NAA/Cr 2.21 ± 0.26 versus 2.30 ± 0.26; P = 0.4). Power analysis indicated that, based on the mean and SDs obtained from our data, the effect size was 0.39. Given this small effect size and a power of 0.8, we would have required ~120 subjects in each group (well controlled and not controlled) to detect a significant difference.

There was no difference in the mean NAA/Cr of the insular cortex (P = 0.09), posterior temporal lobe white matter (P = 0.07) and splenium (P = 0.12) of patients compared with healthy controls.

We found a correlation between NAA/Cr in the thalamus and the insular cortex (r = 0.54, P = 0.03). There was no correlation between NAA/Cr in the thalamus and NAA/Cr in the posterior temporal white matter (r = 0.38, P = 0.1) and splenium (r = 0.32, P = 0.2).

There was no correlation between the average spike and wave complexes and NAA/Cr in the thalamus (r = 0.06; P = 0.8).

There was no correlation between thalamic NAA/Cr and age in normal controls (r = -0.120, P = 0.7). We found a negative correlation between thalamic NAA/Cr and duration

Statistical analysis

Group differences for age were assessed using one-way analysis of variance (ANOVA). The gender distribution was examined by the \( \chi^2 \) test. In normal controls, the statistical significance of differences in mean NAA/Cr and volumes between right and left sides was assessed using the paired t-test. Group differences for NAA/Cr and volumes between IGE and healthy controls were evaluated using the Student’s t-test. Pearson correlation and partial correlation coefficients were computed between spectroscopic/volumetric measurements of the thalamus and duration of epilepsy, while controlling for age at onset. For analysis of individual patients, we considered values 2 SD below the mean of normal controls to be abnormal.
of epilepsy corrected for age of onset ($r = -0.55$, $P = 0.014$) (Figure 4).

**MRI volumetry**

Patients with IGE and normal controls did not differ in age [$F(1,39) = 0.49$, $P = 0.5$] or sex distribution ($\chi^2 = 2.6$, df = 1, $P = 0.1$). For the 21 normal controls studied, the mean volume of the left thalamus was $9670 \pm 744$ mm$^3$ and that of the right thalamus was $9417 \pm 635$ mm$^3$ ($P = 0.08$). Since there was no difference between the left and right mean thalamic volumes in our normal controls, and no lateralized abnormalities were expected to be found in our IGE patients, we used a single thalamic volume in controls and patients by obtaining the average of right and left thalamic volumes (thalamic volume). There was no significant difference between the mean thalamic volume of IGE patients and normal controls ($P = 0.1$).

We found no correlation between thalamic volume and age in normal controls ($r = -0.141$, $P = 0.5$). There was no correlation between thalamic volumes and duration of epilepsy after correcting for age of onset ($r = 0.06$, $P = 0.8$).

**Individual analysis**

The NAA/Cr values were abnormally low in 12 out of 20 (60%) of IGE patients. In particular, NAA/Cr was abnormally low in the patient with newly diagnosed epilepsy in whom the MRI and the IH-MRSI were performed prior to the introduction of antiepileptic medication. Thalamic volumes were abnormally reduced in three out of 20 (15%) of IGE patients.

**Discussion**

Our results showed that there was an abnormally low concentration of thalamic NAA/Cr in IGE patients compared with healthy controls, but there was no difference in the concentration of NAA/Cr in the insular cortex, posterior temporal lobe white matter and splenium of the corpus callosum.

Reductions in NAA are thought to represent either loss of neurons and/or axons, as well as neuronal or axonal injury and metabolic dysfunction (De Stefano et al., 1995; Tsai and Coyle, 1995; Hugg et al., 1996; Bernasconi et al., 1998, 2002). Because NAA is measured in a voxel, e.g. a unit of volume, reduction in NAA could be due to a decreased density of neurons related either to neuronal loss, neuronal shrinkage or increase in water content. In temporal lobe epilepsy, it has been shown that the density of NAA/Cr in the temporal lobes can be reduced even in the absence of significant hippocampal volume loss and that this decrease may be reversible (Cendes et al., 1997b; Connelly et al., 1998). This, along with other evidence from experimental (Dautry et al., 2000; Demougeot et al., 2001) and clinical work (De Stefano et al., 1995; Hugg et al., 1996; Cendes et al., 1997a; Vermathen et al., 1997; Kalra et al., 1998) suggests that NAA reduction can also be due to neuronal metabolic dysfunction in addition to neuronal loss. Neuropathological studies in animal models (Meencke et al., 1981) and in patients with IGE (Meencke et al., 1981; Mouritzen-Dam et al., 1996) did not demonstrate neuronal loss in the thalamus (Meencke and Janz, 1984; Mouritzen-Dam et al., 1996; Sabers et al., 1996). In accordance with
these findings, our patients with IGE did not have significant thalamic volume loss as measured by high-resolution MRI. Although it is conceivable that obtaining an averaged measure from numerous voxels may fail to detect a localized volume reduction within the thalamus (Hazlett et al., 1999), our results are more suggestive of thalamic neuronal/axonal dysfunction in our group of IGE patients.

To the best of our knowledge, this is the first study using a combination of 1H-MRSI and volumetric MRI data to investigate thalamic dysfunction in IGE. One previous study using single voxel MRS (Savic et al., 2000) showed a NAA reduction in the pre-frontal area, but failed to demonstrate a reduction of NAA in the thalamus. In this study (Savic et al., 2000), only the right thalamus was examined and a partial inclusion of areas outside the lateral thalamic border (globus pallidus and internal capsule) could not be avoided using a large single voxel. This might have resulted in a dilution of any thalamic abnormality due to partial volume effects. Studies of regional cerebral blood flow during typical absence seizures using high-resolution H$_2$O PET, have shown a focal increase in thalamic blood flow consistent with thalamic dysfunction in the pathophysiology of IGE (Prevett et al., 1995).

Although we did not find a decrease in cortical NAA/Cr in our patients, there was a correlation between thalamic and insular NAA/Cr. Since thalamo-cortical relay neurons as a group project to all cortical areas (Steriade et al., 1993; Cox et al., 1997), it is conceivable that a primary thalamic dysfunction as shown by a NAA/Cr decrease also affects cortical NAA/Cr.

The neurochemical abnormality underlying IGE is not fully defined, and this is an area of active research in both affected humans and in animal models. There is evidence that hypersynchrony within the thalamo-cortical circuitry is maintained through GABA-mediated mechanisms (Danober et al., 1998; Huntsman et al., 1999). It has been proposed that recurrent inhibition within the thalamic reticular nucleus serves to reduce synchrony and thus prevent seizures. Low GABA and high glutamate CSF concentration have been described in canine generalized epilepsy (Podell and Hadjiconstantinou, 1997). An increase in GABA and glutamate + glutamine in the occipital lobe of patients with IGE has been reported (Simister et al., 2002). Recently, Petroff et al. (2002) showed an increase in cellular glutamate content and a tight metabolic coupling between NAA and glutamate concentration in the human epileptogenic hippocampus. Thalamic abnormal electrical activity in IGE could be explained by hypereexcitability due to an increase in cellular glutamate. Additional thalamic MRS studies in IGE are needed in order to further assess the metabolite levels, in particular GABA and glutamate.

In a previous 1H-MRSI study of children with newly diagnosed temporal lobe epilepsy (Miller et al., 2000), we showed that decreased NAA/Cr in the temporal lobes was present from the onset of disease and was not entirely the result of chronic recurrent seizures, although repeated generalized tonico-clonic seizures can exacerbate this abnormality (Bernasconi et al., 2002). The lack of difference in NAA/Cr between IGE patients with adequate seizure control and those with persistent seizures, and the lack of association between thalamic NAA/Cr and the amount of spikes and waves in our patients supports the idea that neuronal dysfunction in IGE could be primarily related to the underlying epileptogenic process rather than to the effect of the seizures themselves or interictal activity. However, our study was not powered to detect a difference as small as our effect size when comparing patients whose seizures were well controlled at the time of the MRSI exam and those in whom seizures were not controlled.

We found a negative correlation between NAA/Cr and the duration of epilepsy, suggesting that progressive thalamic neuronal dysfunction may evolve in patients with IGE, even when seizures are clinically controlled. Because duration is a composite variable, consisting of both age of onset and the age of the patient, one might suspect that any change in that variable might be related to the age of onset or increasing age. The relationship we found with duration was present after controlling for age of onset. Given the cross-sectional design of this study, we could not statistically control for the effects of aging in our patient population. However, there was no such relationship in our healthy control subjects.

The effects of medication on NAA have not been thoroughly investigated. In schizophrenia, 1H-MRSI studies of patients treated with neuroleptics (Bertolino et al., 1996) and unmedicated patients (Bertolino et al., 1998) revealed no significant differences in thalamic NAA measures between these two groups. Furthermore, a post-mortem study (Tsai et al., 1995) failed to find differences in NAA in normal subjects compared with neuroleptic-treated subjects. Abnormally low NAA/Cr found in the temporal lobes of an unmedicated child with newly diagnosed temporal lobe epilepsy (Li et al., 2000) and in our patient with newly diagnosed IGE also argues against a significant effect of medication on regional NAA measures in the brain. Thus, it appears unlikely that antiepileptic medication is responsible for the lower concentrations of thalamic NAA/Cr in our group of IGE patients.

In conclusion, we have found evidence of thalamic neuronal metabolic dysfunction in patients with IGE supporting the notion of an abnormal thalamo-cortical circuitry as the underlying substrate of seizure generation in this form of epilepsy. However, it is not known whether thalamic dysfunction is specific to IGE. Future work should address the functional integrity of the thalamus in other forms of epilepsies. The thalamic dysfunction appears worsened with increasing duration of epilepsy and possibly unrelated to the clinical control of the seizures or the amount of spike and wave activity. Similar thalamic dysfunction in patients with juvenile myoclonic epilepsy and epilepsy with generalized tonic–clonic seizures on awakening support the clinical notion of a common pathophysiological abnormality and a
neurobiological continuum of IGE syndromes (Andermann and Berkovic, 2001).

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References


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