

Longitudinal study of MRS metabolites in Rasmussen encephalitis

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Summary

This study analyses the evolution of metabolite changes in an 8-year-old boy with focal Rasmussen encephalitis. Five MRI examinations, including magnetic resonance spectroscopy (MRS) were performed over 9 months. Following complex partial status, T2-weighted imaging showed transient dramatic signal increase in the left superior temporal gyrus and mesial temporal structures. Subsequent scans showed resolution of the swelling and signal normalization, with development of slight focal atrophy. MRS after status showed a reduction in N-acetylaspartate, total creatine and trimethylamines. Subsequent scans showed complete resolution of these metabolite abnormalities, followed later by development of further abnormal metabolite values. Lactate and

glutamine/glutamate were elevated after status. After surgery, *ex vivo* high-field ¹H and ³¹P MRS confirmed metabolite abnormalities (elevated choline and decreased aspartate, N-acetylaspartate, [¹H]glutamate together with altered [³¹P]phospholipid ratios. These findings suggested active disease process in the anterior region of the excised superior temporal gyrus. We conclude that Rasmussen encephalitis is a combination of progressive encephalitic damage and fluctuating seizure effects, in which neuronal injury and recovery can occur. MRS measurements at a single time point should consider the fluctuating metabolite profile related to seizure activity.

Keywords: Rasmussen; chronic localized encephalitis; epilepsy; magnetic resonance spectroscopy; MRS

Abbreviations: Cho = total choline; CPS = complex partial seizure; Cr = creatine + phosphocreatine; GABA = γ aminobutyric acid; gCOSY = gradient correlated spectroscopy; Glx = glutamine + glutamate; Lac = lactate; ml = myoinositol; MRS = magnetic resonance spectroscopy; NA = N-acetylaspartate + N-acetylaspartyl glutamate; NAA = N-acetylaspartate; PRESS = point resolved spectroscopy; ROI = region of interest; SPECT = single photon emission computed tomography; SPS = simple partial seizure.

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Introduction

Rasmussen encephalitis is a rare, progressive disease beginning in the first decade in previously normal children (Rasmussen *et al.*, 1958). It is characterized by the sudden appearance of refractory partial seizures, often with *epilepsia partialis continua*, or less commonly as complex partial status. The evolution of the disease is typically characterized by progressive neurological and cognitive deterioration with the eventual development of a hemiplegia. Treatment of Rasmussen encephalitis with conventional antiepileptic medication is of limited benefit. Surgical resection or

disconnection of the affected hemisphere may stop the seizures and improve long-term cognitive outcome (Honavar *et al.*, 1992).

Rasmussen encephalitis may have an autoimmune basis (Andrews *et al.*, 1996; Hart, 1994). The pathological course has an initial inflammatory stage, followed by a chronic phase with progressive atrophy of the affected hemisphere (Rasmussen and McCann, 1968; Rasmussen *et al.*, 1958). Histology performed during the acute stages of Rasmussen encephalitis shows signs of chronic encephalitis predom-

antly in the cortex, but also in white matter, with laminar spongiform degeneration, perivascular lymphocytic cuffing, neuronal loss, and proliferation of small round cells and microglia (Gray *et al.*, 1987).

MRI during the initial inflammatory stage shows cortical hyper-intensity on T2-weighted images. The chronic phase is characterized by progressive atrophy of the affected hemisphere and loss of the increased signal in T2-weighted MRI. However, exceptions to this typical course are described (Bien *et al.*, 2002b).

Magnetic resonance spectroscopy (MRS) allows non-invasive assessment of metabolite abnormalities and their response to different treatment methods. Proton MRS can give information on the following metabolites: N-acetyl-aspartate + N-acetylaspartyl glutamate (NA), a marker of neuronal density and function (Petroff *et al.*, 1995); choline-containing compounds (Cho), derived from soluble membrane components and reflecting membrane turnover; creatine (Cr), a component of the cellular energy cycle; myoinositol (mI), an organic osmolyte (Gullans and Verbalis, 1993) and marker of astrocyte density (Petroff *et al.*, 1995). Elevations of glutamine plus glutamate (Glx), measured as a single component because of spectral overlap, may reflect excess levels of the excitatory amino acid glutamate, with the potential for increased levels resulting from the action of autoantibodies on glutamate receptors. The presence of lactate indicates glycolytic metabolism.

Previous proton MRS studies of Rasmussen encephalitis have assessed metabolites *in vivo* at a single time-point (Matthews *et al.*, 1990; Breiter *et al.*, 1994; Geller *et al.*, 1998) or with a second follow up study (Cendes *et al.*, 1995; Turkdogan-Sozuer *et al.*, 2000), and in tissue extracts (Peeling and Sutherland, 1993). No studies of Rasmussen encephalitis have made more than two sequential MRS measurements. The studies (at 1.5 T) focused predominantly on the levels of NA (suggestive of neuronal loss), which were usually decreased in the affected hemisphere, which was always atrophic (Matthews *et al.*, 1990; Cendes *et al.*, 1995; Turkdogan-Sozuer *et al.*, 2000). An abnormally high concentration of soluble membrane precursor (Cho) was reported in one study (Turkdogan-Sozuer *et al.*, 2000), but not in others (Matthews *et al.*, 1990; Cendes *et al.*, 1995) suggestive of variable changes in membrane turnover. Increases in lactate have been observed in Rasmussen encephalitis patients scanned during complex partial status epilepticus, indicative of anaerobic metabolism (Matthews *et al.*, 1990; Cendes *et al.*, 1995). Increased levels of lactate are short-lived and generally induced by an abnormal, anaerobic state such as during or immediately following seizure activity (Breiter *et al.*, 1994; Turkdogan-Sozuer *et al.*, 2000). It is believed that Rasmussen encephalitis may cause changes in membrane composition or turnover but, to date, no ³¹P MRS measurements are reported in studies of resected tissues from patients suffering Rasmussen encephalitis.

The purpose of this study was to describe the longitudinal MRS changes that occur in Rasmussen encephalitis and how

seizures may affect these findings. To do this we have intensively studied a patient with Rasmussen encephalitis using high-field *in vivo* MRI and spectroscopy over a 9-month period. This started 16 months after seizure onset, included an episode of status epilepticus, and continued to the time of surgery with resected tissue being further examined *in vitro* using high-field ¹H and ³¹P spectroscopy.

Patient and methods

The written consent to the study was obtained from the subject and his parents. The ethics committees of Austin Health and Griffith University approved the study.

Patient history

This boy had normal development and was a bright student. At 8 years and 2 months, he had his first complex partial seizure (CPS) where he described difficulties hearing and became vague for 20 min. Seizures escalated in frequency with complex partial status epilepticus (lasting two hours) after 1 month. Simple partial seizures (SPSs) were characterized by a feeling in his throat and peri-umbilical region, nausea, and an auditory agnosia where he could hear voices but could not understand them. The majority of seizures lasted ~10 s. Complex partial seizures occurred with dysphasia, loss of awareness, oral automatisms, deeper and more rapid respiration, and sometimes extension of the right upper limb with right hand automatisms. By 9.5 years, he had simple partial seizures every 5 min and complex partial seizures up to seven times per day. He had marked fluctuation in his receptive speech.

Interictal EEG studies showed interictal left hemispheric slowing most prominent over the left temporal region, and left anterior and mid-temporal epileptiform discharges. Ictal EEG studies showed focal rhythmic sharp activity emanating from the left mid-temporal region. Neuropsychological assessment showed that the patient remained of average intellect at 9.5 years; however, he had made few gains over the last year. He had mild dysphasic features with attention and executive difficulties. Rasmussen encephalitis and focal cortical dysplasia were proposed in the differential diagnosis of his epileptic encephalopathy. Antiepileptic drug treatment included valproic acid, carbamazepine, lamotrigine, gabapentin, clobazam, phenytoin and prednisolone (Table 1). The patient received four pulses of IV methylprednisone, with administration of this drug ending 2 months before the first 3 T MR study.

Structural imaging

In vivo MRI and MRS were recorded on a 3 T MRI scanner (LX2, GE Medical Systems, Milwaukee, WI, USA). Each of the five scanning sessions included MRS acquisition, a T2-weighted sequence in the coronal plane and T1 weighted anatomical imaging. T2-weighted images were acquired

Table 1 Daily medication taken by the patient at the time of each MRS study

First MR	Second MR	Third MR	Fourth MR	Fifth MR
VPA 1500 mg CBZ-CR 800 mg LTG 150 mg	VPA 1500 mg CBZ-CR 800 mg LTG 150 mg CZP 3 mg PHT 300 mg TPM 12.5 mg	CBZ-CR 800 mg LTG 150 mg PHT 125 mg TPM 125 mg	CBZ-CR 800 mg LTG 150 mg PHT 125 mg TPM 125 mg	CBZ-CR 800 mg LTG 150 mg PHT 175 mg TPM 150 mg CLB 10 mg prn
PRED 25 mg alt. day IV MP 500 mg (2.5 months)	Hcort 8 mg, 4 mg alt IV MP 500 mg (5 days)	Hcort 8 mg, 4 mg alt		

VPA = valproate; LTG = lamotrigine; PHT = phenytoin; TPM = topiramate; CZP = clonazepam; CLB = clobazam; CBZ-CR = carbamazepine-controlled release; PRED = prednisolone; IV MP = intravenous methylprednisolone (time prior to MR when last given); Hcort = Hydrocortisone; alt = alternating; prn = as required.

using a T2-weighted fast spin echo (FSE) sequence [TR (relaxation time) = 4500 ms; TE (echo time) = 90 ms; flip angle = 90°; number of acquisitions (NEX) = 2; 512 × 256 matrix; field of view (FOV) = 24 cm; 4 mm slices with a 1.5 mm gap]. Regional image intensities were assessed by side-to-side comparison. A coronal fast spoiled gradient recovery (FSPGR) [TR = 9 ms, TE = 1.9 ms, TI (inversion time) = 500 ms, flip angle = 20; FOV = 25 × 17.5 cm; matrix size = 512 × 512], used for volumetric assessment, was acquired at the first, second and fifth scan. Assessment included bilateral hemispheric, temporal lobe and hippocampal volume measurements. Measurement of hippocampal and hemispheric volumes used previously described methods (Briellmann *et al.*, 2000). Values were not corrected for the intracerebral volume, as changes of the right and left hemispheric volume were considered part of the pathology. To allow comparison, they were expressed as a percentage of the hemispheric volume.

In vivo MRS imaging and data analysis

Three-plane localizing images were acquired to allow prescription of isotropic 2 cm regions of interest (ROI) for MRS. Sagittal plane, 2 cm thick scout images (T1 spin-echo), followed by 2 cm thick coronal images centred in the plane of the ponto-medullary junction were acquired. A ROI in each temporal lobe was selected in the coronal plane with maximal inclusion of the high intensity area in the left superior temporal gyrus seen in the structural imaging and the medial temporal lobe (centred 45 mm from the temporal pole, outlined in Fig. 2) and an equivalent voxel in the right temporal lobe. Volume-localized spectra were recorded using a short-echo point resolved spectroscopy (PRESS) sequence with TE/TR = 30 or 35 ms/3000 ms and either 32 or 64 transients.

In vivo MRS data were analysed to provide information about NA, Cho, Cr, Glx and mI. Metabolite concentrations were determined for each ROI with LCModel software (Provencher, 1993), using a library of reference spectra in a basis set recorded specifically for the scanner and calibrated

with a phantom containing 50 mM NA. The compounds N-acetylaspartate and N-acetylaspartyl glutamate are not differentiated *in vivo*. Therefore, for *in vivo* spectra, NA refers to the two compounds together. For *in vitro* measurements, NAA refers to N-acetylaspartate, which can be differentiated from N-acetylaspartyl glutamate.

The LCModel fitting algorithm uses the multiple peaks contributing to an individual metabolite spectrum to estimate the tissue content of each metabolite (Provencher, 1993). The residual signal corresponds to, and is fitted by, additional broad peaks representing unknown metabolites and other factors such as components with short T1 relaxation times. Results, presented as institutional units (approximating millimolar concentration) based on this process, were compared with temporal lobe spectra of 41 controls (mean age 34 ± 10 years, male/female = 15/26) scanned using a similar acquisition protocol. A normal range of ± 2 SDs from the mean was used. Individual metabolite concentrations were not corrected for incomplete relaxation.

Resected tissue was assessed by histopathology and MR spectroscopy.

Pathology

The resected specimens consisted of cerebral neocortex and white matter, including 40 × 15 × 15 mm of left superior and middle temporal gyrus, 20 × 10 × 7 mm from the depth of the superior temporal sulcus and 10 × 8 × 7 mm from the posterior superior temporal gyrus. Two segments from the resected superior temporal gyrus were used for *in vitro* analysis. These were included in the lateral aspect of the voxel examined by *in vivo* MRS.

High-field MRS studies of excised tissue

Samples of the resected superior temporal gyrus (249 mg middle part and 82 mg posterior section) were collected into liquid nitrogen within 30 s of resection and stored at -70°C until analysis. A double extraction procedure (Lehnhardt *et al.*, 2001) was used to separate the hydrophilic (cytosolic)

components, which were analysed by ^1H MRS (Bruker Avance 14.09 T spectrometer operating at 600 MHz), from the lipophilic (membrane phospholipids) tissue components, which were assessed with ^{31}P MRS (Bruker Avance 7.07 T spectrometer operating at 121 MHz).

^1H NMR measurements were performed on a Bruker Avance DRX 600 MHz (14.09 T) spectrometer at 298 K. Typically, the ^1H spectra were acquired over 32,000 data points, with an acquisition time of 2.3 s and 128 transients. A known quantity of 3-trimethylsilylpropionate (TSP) was added to the ^1H NMR samples to enable estimation of metabolite concentrations. For confirmation of peak assignment, gradient correlated spectroscopy (gCOSY) (Hurd, 1990) was used to acquire spectra over 1024 data points in each dimension. In addition to the metabolites detected *in vivo*, signals from taurine, aspartate, lactate, acetate and gamma aminobutyric acid (GABA) were measured.

^{31}P NMR measurements were performed on a Bruker Avance DRX 300 MHz (7.05 T) spectrometer operating at 121.5 MHz and 298 K. Typically, the ^1H decoupled ^{31}P spectra were acquired over 16,000 data points with an acquisition time of 1.5 s, 6144 transients and a relaxation delay of 2 s. Identification of metabolites was based on published spectra (Arús *et al.*, 1985; Lehnhardt *et al.*, 2001) and normal phospholipid ratios were estimated from published spectra (Lehnhardt *et al.*, 2001).

Results

Timing of the magnetic resonance studies in relation to clinical events

The first 3 T MR study was performed during an elective admission for seizure characterization following two CPS in the previous two weeks, and several simple partial seizures per day. During video-EEG telemetry, several SPS were recorded. The EEG showed localized left temporal interictal and ictal discharges and left temporal slowing. Ictal single photon emission computed tomography (SPECT) [$^{99\text{m}}\text{Tc}$ -HMPAO (hexamethylpropyleneamine oxime monoamine oxidase)] during a CPS showed left lateral temporal lobe hyperperfusion, maximum in the superior temporal gyrus. The first MRI and MRS were performed 6.5 h after this seizure.

Four months after the first MR study or 20 months after the onset of seizures, a second 3 T MR study was performed. After increasingly frequent CPS with lengthening periods of unresponsiveness, emergency admission for complex partial status epilepticus was required. The blood levels of antiepileptic drugs were within or below normal range. This second 3 T MR study was performed 5 days after this complex partial status epilepticus, with no seizures recorded during the days between the status and the MR.

Two months after the second MR study and 22 months after seizure onset, the third 3 T MR examination was performed. During this 2-month period, the patient was relatively stable with infrequent seizures and the oral steroids

were gradually weaned. The third 3 T MR examination was performed a week after a cluster of SPS and CPS.

Two weeks after the third study and one week prior to surgery, the fourth MR was performed. The patient was no longer on steroids and had been free of CPS since the third MR study. However, EEG monitoring confirmed the presence of SPS with focal epileptiform activity over the left anterior and mid temporal region. Slowing over the left temporal region was observed. Pre-operative assessment (video-EEG telemetry, MRI, SPECT, PET and neuropsychological evaluation) suggested the left superior temporal gyrus as the origin of seizure activity. Implanted subdural EEG confirmed focal seizures, confined to the left superior temporal gyrus. Language mapping excluded language in that region. With the differential diagnosis of occult dysplasia or Rasmussen encephalitis, operative resection of the left superior temporal gyrus was performed.

Histological sections from each specimen showed cerebral neocortex and white matter. Definite dyslaminae change was not identified, although single white matter neurons were noted to be frequent. Small numbers of lymphocytes and macrophages were noted in the subarachnoid space and there was focal intraparenchymal perivascular cuffing and scattered foci of neuronophagia (Fig. 1A). Immunohistochemical stains showed both T-lymphocyte subtypes CD68 and CD8 positive cells (macrophage markers) in these foci (Fig. 1B), as reported by Bien *et al.* (2002a). The process was present focally throughout the resected tissue specimens. A diagnosis of a low-grade meningoencephalitis was made, consistent with Rasmussen encephalitis.

Three months after the cortical resection and 25 months after seizure onset, the fifth MR was performed. Seizure frequency was not substantially different following surgery with very frequent SPS per day and 1–2 CPS per week.

Structural MRI

Figure 2 shows the evolution of the structural MRI findings. At the first MRI study, no definite abnormality was seen; in particular, no signal change was evident in the left superior temporal gyrus. The second scan, performed shortly after complex partial status epilepticus, showed a dramatic signal change; the left superior temporal gyrus was swollen, showed high T2-signal intensity and blurred grey–white matter distinction. In the next two MR measurements, recorded after a cluster of CPS (third MRI) and after a relatively seizure-free interval (fourth MRI), there was resolution of the left temporal swelling. Finally, the fifth scan showed the tailored post-operative defect.

Volumetric assessment was performed on the first, second and fifth scans (Table 2). No hemispheric atrophy was apparent. The left temporal lobe was smaller than the right. The hippocampi were initially symmetrical, but the left hippocampus was larger (swollen) at the time of the second MRI scan.

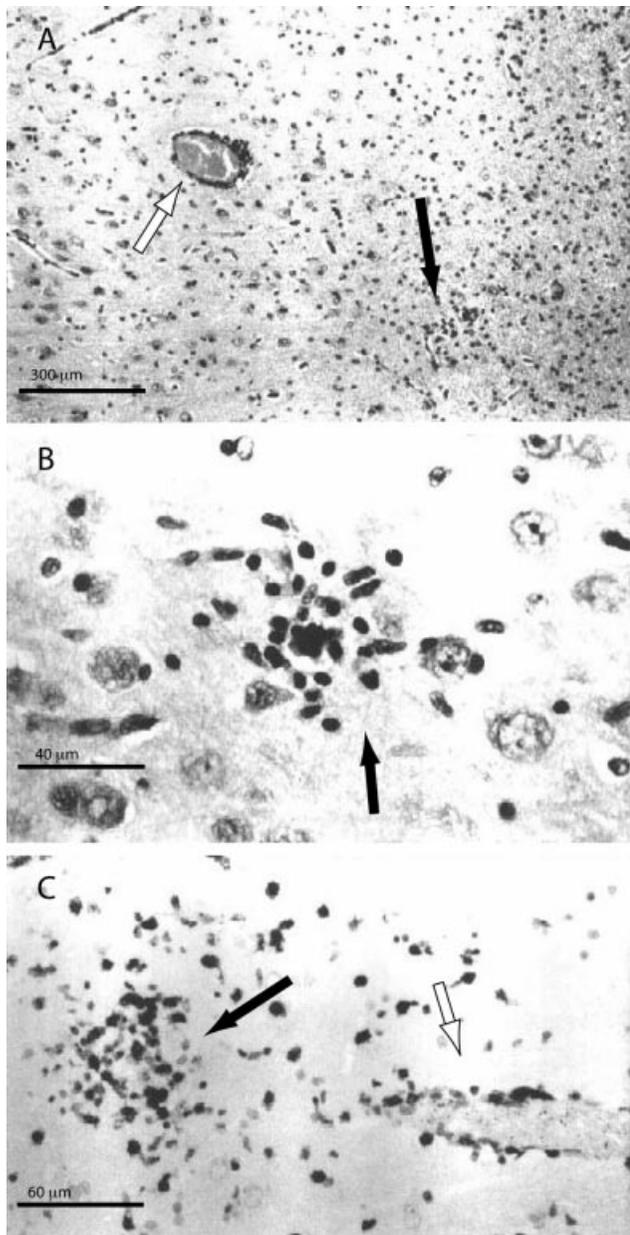


Fig. 1 Histology of surgical samples. (A) Cortex–white matter junction showing microglial cluster suggestive of neuronophagia in superficial white matter (closed arrow) and perivascular lymphocyte cuffing in deep cortex (open arrow). Tissue stained with haematoxylin and eosin, magnification: $\times 100$. (B) Cortical focus of neuronophagia (arrow) in tissue stained with haematoxylin and eosin. Magnification: $\times 400$. (C) Cerebral cortex showing CD8 positive cytotoxic T cells involved in neuronophagia (closed arrow) and perivascular lymphocyte cuffing (open arrow) using labelled streptavidin–biotin technique at a magnification of $\times 250$

MRS findings

The spectra obtained at the four MR sessions prior to surgery are shown in Fig. 3. The concentrations of different metabolites in controls and the longitudinal changes in MRS findings for both temporal lobes in the patient are shown in Table 3 and discussed below.

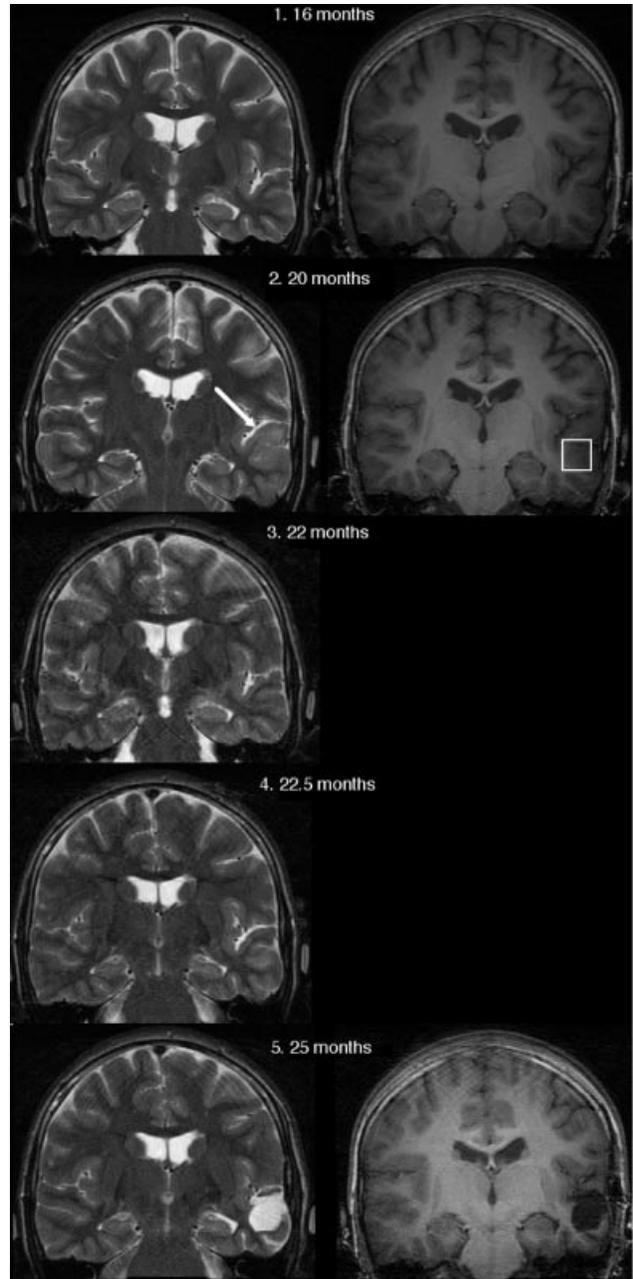


Fig. 2 The coronal MRIs obtained at the five MR sessions. T1-weighted coronal images (right side) were only obtained at the first, second and fifth MR session; T2-weighted scans (left side) were obtained at all five sessions. The MRS voxel position is shown on the first T1 image. Note the signal increase and swelling in the region of the left superior temporal gyrus at the second MR (arrow) and the resolution of this finding over time. There was no apparent hemispheric atrophy.

The NA value on both the left and right temporal lobes was within the normal range at the first measurement but at the second measurement, following the complex partial status epilepticus, it was significantly lower than the normal range indicated by controls (Table 3; second MR, Fig. 3). After seizure control, NA recovered but subsequently had fallen again in the right temporal lobe at the time of the final scan

Table 2 Hemicranial, temporal lobe and hippocampal volumes measured at the first, second and fifth MR examinations

	Hemicranial volume cm ³ (%)		Temporal lobe volume cm ³ (%)		Hippocampal volume mm ³ (%)	
	Left	Right	Left	Right	Left	Right
First MR	750 (50)	753 (50)	57 (3.8)	74 (4.9)	2204 (146)	2201 (143)
Second MR	691 (50)	696 (50)	55 (4)	71 (5.1)	2214 (159)	2065 (148)
Fifth MR	689 (49)	730 (51)	N/A	71 (5)	2031 (143)	2016 (142)

Percentages are relative to the sum of both hemicranial volumes. N/A = not applicable.

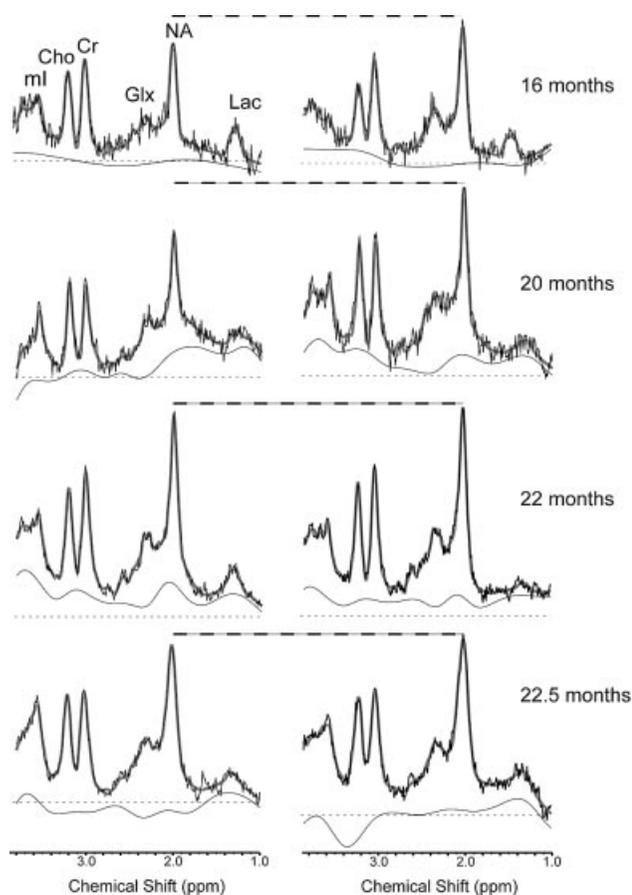


Fig. 3 *In vivo* MRS spectra recorded from left (left spectra) and right temporal lobes. Metabolites are labelled as follows: Lac = lactate; NA = N-acetylaspartate + N-acetylaspartyl glutamate; Glx = glutamine + glutamate; Cr = creatine + phosphocreatine; Cho = total choline; and ml = myo-inositol). The times refer to the sequence of measurements relative to onset of seizures. The dashed lines are drawn to show differences in the NA peak heights.

after surgery, probably reflecting the chronic course of the disease. Creatine was also abnormally low in the left temporal lobe in the second study after the complex partial status (scan 2). The Cr content was within the normal range during the period of good seizure control (scans 1, 3 and 4). Total choline concentration decreased significantly following the complex partial status and returned to normal values thereafter. Total Glx content was in the upper range of normal

control values on both sides. Interestingly, the increased values were more evident on the contralateral side (scans 1–3) with the greatest concentrations observed at the first scan. Lactate, which is not usually detected in spectra recorded from a normal temporal lobe, was markedly increased on the left side at the first MRI scan, which was performed 6.5 h after a seizure. Lactate was also detected at a lower level in subsequent scans 3 and 4, which were performed at least days after a CPS. Myo-inositol was within the normal range for controls during the whole observation period, but was higher in the measurement prior to surgery (scan 4).

High-resolution MRS of tissue extracts

High-resolution 1D and 2D gCOSY ¹H MR spectra of the two tissue samples (predominantly grey matter from the anterior and posterior regions of superior temporal gyrus) were acquired for metabolite identification. Estimated metabolite concentrations in each tissue sample together with published non-encephalitic neocortex metabolite concentrations are shown in Table 4. Compared with the reported values, both samples showed normal creatine content, but an increase in lactate and alanine and a decrease in glutamate.

Notably, the posterior sample showed a large acetate peak (1.9 ppm) that was absent from the anterior sample (Fig. 4). Tissue content of acetate-containing components (including NA) and ml was lower, and the Glx levels were greater in the anterior sample compared with the posterior sample. The acetate signal at 1.9 ppm was not evident in the *in vivo* spectra. Interestingly, only the anterior sample showed the presence of taurine (C-2: 3.27 ppm and C-1: 3.41 ppm). There was a greater intensity of the signal at 2.22 ppm GABA+ than could be accounted for by GABA alone, based on the other GABA signals at 1.83 and 2.94 ppm (confirmed by gCOSY assignment). This additional signal intensity is likely to arise from an unidentified metabolite and could lead to misinterpretation of elevated GABA.

Ex vivo ³¹P MRS spectra, recorded from extracts showed the expected phospholipid components (Fig. 4) of phosphatidylcholine, sphingomyelin, phosphatidylserine and phosphatidylethanolamine.

Total peak areas for inner and outer membrane phospholipids were the same in both tissue samples. However, of the inner membrane components (phosphatidylserine :

Table 3 Temporal lobe metabolite concentrations measured for the patient in comparison with control values

Metabolite controls (mean \pm SD)	NA		Cr		GPC + PCh		Glu + Gln		Lac		ml	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
Patient	7.81 \pm 0.97	7.82 (6)	4.52 \pm 0.74	5.27 (8)	1.50 \pm 0.21	1.61 (7)	8.04 \pm 1.66	10.87 (7)	2.79 (11)	3.29 \pm 0.69	3.84 (12)	ND
Time after onset												
16 months*	7.18 (7)	7.82 (6)	5.27 (8)	5.18 (9)	1.61 (7)	1.38 (8)	10.87 (7)	11.95 (14)	2.79 (11)	ND	3.84 (12)	ND
20 months	3.67 (9)	4.46 (9)	2.66 (8)	3.63 (8)	1.03 (7)	1.15 (7)	6.40 (16)	9.1 (12)	ND	ND	3.02 (11)	2.78 (13)
22 months	6.37 (7)	7.39 (5)	4.25 (6)	4.77 (6)	1.38 (6)	1.41 (6)	7.49 (13)	10.84 (9)	1.99 (16)	ND	2.99 (10)	3.23 (9)
22.5 months	6.67 (5)	6.90 (6)	4.30 (15)	5.41 (6)	1.68 (6)	1.98 (7)	9.04 (10)	8.54 (12)	1.21 (25)	ND	4.37 (8)	4.00 (12)
25 months	–	4.85 (9)	–	3.94 (6)	–	–	–	8.14 (11)	–	0.49 (19)	–	2.97 (10)

The estimated error in the fit (% SD) is shown in brackets for each metabolite. An error of $\leq 25\%$ was regarded as acceptable. An error of $>25\%$ is reported as not detected (ND). Abnormal values (> 2 SD) are shown in bold. *The first study was made with a TE of 35 ms. The values for 10 control subjects (mean age 33 \pm 7 years, male/female = 3/7) were used to normalize the concentrations to 30 ms. See Fig. 3 legend for abbreviations.

Table 4 Tissue metabolite concentrations estimated using high-field ^1H NMR (14 T)

Metabolite	Anterior $\mu\text{mol/g}$	Posterior $\mu\text{mol/g}$	Non-encephalitic neocortex* $\mu\text{mol/g}$	Temporal lobe** $\mu\text{mol/g}$
Acetate	nd	3.84	nr	0.37 ± 0.06
Aspartate	0.08	1.69	1.05 ± 0.10	1.77 ± 0.15
Alanine	1.47	1.28	0.29 ± 0.02	0.93 ± 0.09
Cholines	1.89	1.38	1.30 ± 0.08	0.49 ± 0.08
Creatine	7.26	7.90	7.22 ± 0.49	9.63 ± 0.28
Glutamine	4.42	2.56	nr	nr
Glutamate	5.05	4.49	8.18 ± 0.39	9.14 ± 0.35
Inositol	5.68	6.41	5.51 ± 0.27	9.02 ± 0.37
Lactate	10.52	14.24	7.66 ± 0.38	nr
NAA	2.52	5.98	5.29 ± 0.28	5.97 ± 0.26
Taurine	0.95	nd	1.33 ± 0.10	1.48 ± 0.07

*From analysis of 32 samples (Peeling and Sutherland, 1993). **From analysis of 10 samples (Petroff *et al.*, 1989). nd = not detected. nr = not reported. Abnormal values (>2 SD) are shown in bold. NAA = N-acetylaspartate.

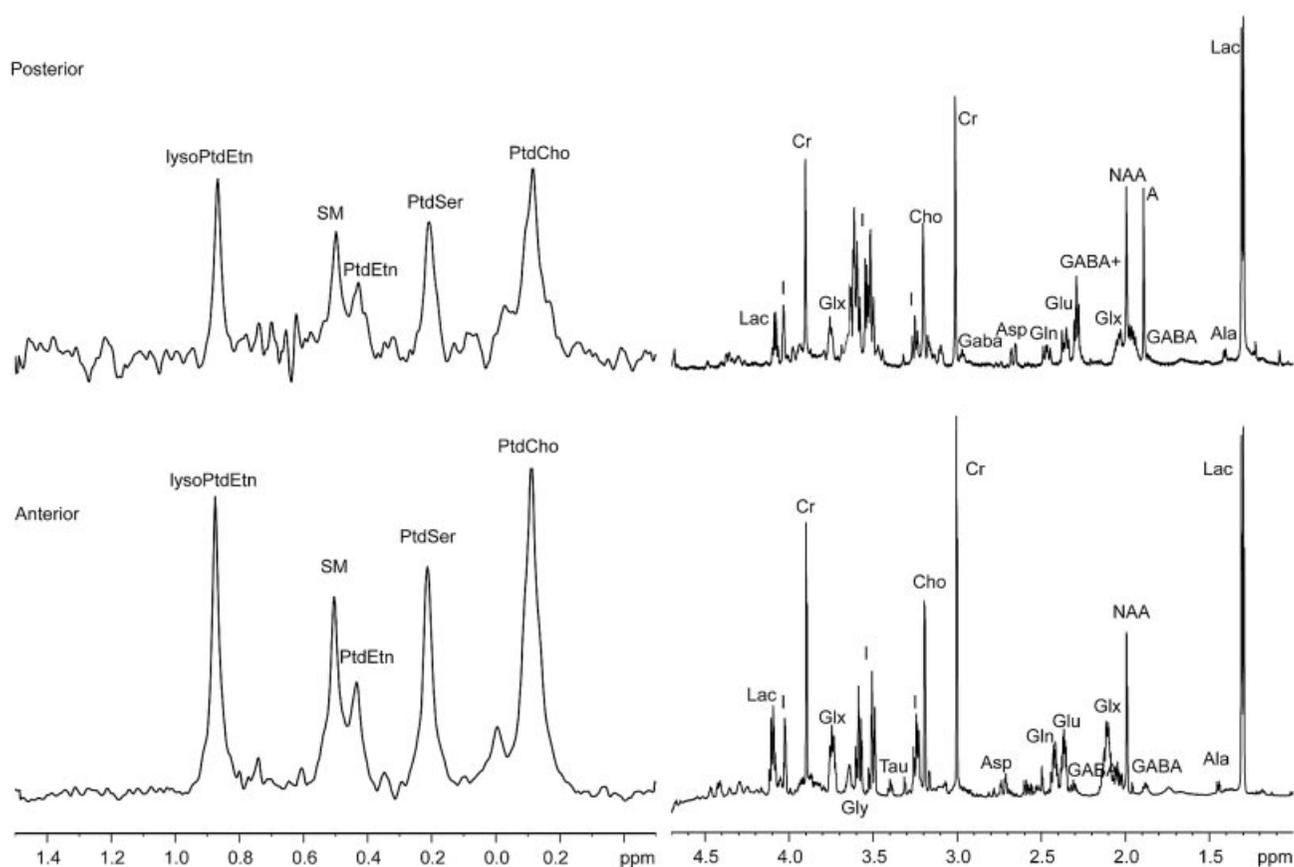


Fig. 4 High-resolution MR spectra recorded from grey matter resected from the anterior and posterior margins of the supratemporal gyrus. The ^1H MR spectra are shown on the right and the ^{31}P MR spectra on the left. ^1H metabolites are labelled as: A = acetate; Ala = alanine; Asp = aspartate; Cr = creatine; I = inositol; Cho = choline; GABA = γ -amino butyric acid; Glu = glutamate; Gln = glutamine; Glx = glutamine + glutamate; Gly = glycine; Lac = lactate; NAA = N-acetylaspartate; Tau = taurine. ^{31}P metabolites are labelled: PtdCho = phosphatidylcholine; PtdEth = phosphatidylethanolamine; PtdSer = phosphatidylserine; SM = sphingomyelin; lyso-PtdEtn = lysophosphatidylethanolamine.

phosphatidylcholine), phosphatidylserine comprised a lower proportion in the posterior sample (0.58) than the anterior sample (0.75) and a published control spectrum (0.88) (Lehnhardt *et al.*, 2001). The outer membrane phospholipid ratio, sphingomyelin : phosphatidylcholine, was higher in both

the anterior and posterior tissue samples (0.48 and 0.46, respectively) than in the control spectrum (0.34) (Lehnhardt *et al.*, 2001). The ratio of total inner to outer membrane phospholipids was 1.14 (anterior) and 1.28 (posterior). This ratio was 1.26 in published control data (Lehnhardt *et al.*, 2001).

Discussion

This high-field MRS study describes serial structural and metabolite changes in an 8-year-old boy with Rasmussen encephalitis. During this 9-month period, an episode of a complex partial status epilepticus occurred. This status was followed by focal swelling and a marked increase in T2-weighted signal intensity in the superior temporal gyrus. Transient brain swelling associated with T2-weighted signal increase following epileptic status has been described previously (VanLandingham *et al.*, 1998). During the observation period, no hemispheric atrophy was detected, reflecting the restricted distribution of the inflammatory disease. Histopathological appearance of the tissue was consistent with an early disease stage in which atrophy is not the main feature, as described by Bien *et al.* (2002b).

Histopathology showed signs of a low-grade meningo-encephalitis, including perivascular cuffing. There was no marked neuronal cell loss or gliosis. These features are consistent with the relatively normal structural and metabolic findings of the resected area on the pre-operative MRI. This suggests that the fluctuations of the MRS metabolites on the earlier scans reflect seizure-associated, transient changes.

The most striking MRS changes were observed following the complex partial status at the second measurement, after which metabolite concentrations returned to normal control values.

N-acetylaspartate (neurons)

There was a bilateral reduction of NA after the complex partial status (second MR), which recovered with treatment of the seizures. To our knowledge, this is the first report describing recovery of NA during the course of Rasmussen encephalitis and suggests that seizures may be important in the measured MR abnormalities. Even after disease duration of 22 months (scans 3 and 4), NA was within the normal range. This contrasts with other studies (Matthews *et al.*, 1990; Cendes *et al.*, 1995; Geller *et al.*, 1998), which report reduced NA in patients with typical Rasmussen encephalitis and brain atrophy. As our patient had no hemispheric atrophy and a very localized seizure focus, the normal NA suggests that despite the focally active disease, the neuronal function was not markedly disturbed once the status was controlled.

This suggests that NA decrease associated with status epilepticus does not reflect irreversible neuron cell death and that adequate control of the status may be followed by recovery of the neuron function. The improvement in tissue NA content was associated with control of the CPS, despite ongoing SPS.

Previous MRS studies of Rasmussen encephalitis have demonstrated consistent findings of reduced NA in small groups of patients (Matthews *et al.*, 1990; Cendes *et al.*, 1995; Geller *et al.*, 1998). One study had sequential data (Cendes *et al.*, 1995) that described multi-voxel long echo time MRS in three patients twice over a period of one year. They found an NA reduction over time and attributed NA reduction to

seizure frequency. However, two of the patients in that study had their second MRS measurement while suffering complex partial status, which may have contributed to the observed NA reduction. Most reports describe MRS profiles recorded after admission to hospital for status epilepticus. These measurements may, therefore, reflect seizure effects and cannot be attributed solely to disease progression. Measurements made in the absence of seizures are necessary to assess Rasmussen encephalitis related metabolite changes.

The reversible nature of the metabolite changes and the inflammation evident on histological examination provide evidence that the observed MRS changes represent the effect of seizures and the disease process rather than permanent changes in tissue composition.

Creatine and choline (cellularity and membrane turnover)

Creatine and choline concentrations of our patient decreased following complex partial status, but subsequently recovered and partly normalized after the operation. The decrease in Cr is consistent with loss of substrates following extended utilization of high-energy phosphates. The Cho decrease in scan 2 after CPS status is consistent with a transient increase in the turnover and loss of membrane components, which was unaffected by progression of the disease and independent of the short-term changes in simple partial seizure frequency.

Metabolites other than NA, Cho and Lac have been assessed by few studies of Rasmussen encephalitis (Geller *et al.*, 1998; Sener, 2000; Turkdogan-Sozuer *et al.*, 2000), making comparison difficult since either the scanning procedures, methods or timing of metabolite measurement with respect to the progression of Rasmussen encephalitis and recent seizure severity were not described or were variable between studies.

Lactate (anaerobic metabolism)

Lactate is typically affected by a seizure occurring immediately before scanning. Lactate increases in patients with Rasmussen encephalitis scanned during status have been described previously (Breiter *et al.*, 1994; Cendes *et al.*, 1995). Interestingly, in our patient lactate increase was observed 6.5 h after a CPS of 90 s duration. This seizure occurred during video-EEG telemetry and was therefore optimally documented. Ictal SPECT obtained during this seizure showed focal hyperperfusion in the left temporal lobe. Video-EEG telemetry excluded ongoing seizure activity beyond the CPS. However, simultaneous EEG was not performed during scanning, and we cannot exclude occurrence of electrographic seizures during the MRI session. The significance of low concentrations of Lac detected in later scans 3 and 4 (see Fig. 3) is unclear, since this spectral region may also contain a signal contribution from macromolecules. However, the high incidence of simple partial seizures may have contributed to lactate production.

Glutamate and glutamine (Glx – transmitter cycling)

Total Glx levels measured *in vivo* were increased bilaterally at the first scan and were subsequently in the upper range of normal in the contralateral hemisphere. The high Glx concentrations recorded in the initial measurement are consistent with steroid promotion of glutamate and glutamine synthesis (Sun *et al.*, 1999). In the ipsilateral temporal lobe, a lower Glx concentration after the complex partial status is likely to reflect an osmotic response to the associated tissue oedema (Danielsen and Ross, 1999). A short-echo MRS study of two cases with Rasmussen encephalitis (Geller *et al.*, 1998) also found increased Glx, both in the unaffected contralateral hemisphere and in the affected hemisphere.

Tissue extracts

High-resolution ¹H MR studies of the excised tissue were used to compare regional variations between the anterior and posterior parts of the resected tissue with previously reported data (Table 4) (Peeling and Sutherland, 1993). The high lactate concentrations recorded from both tissue samples were consistent with tissue anoxia associated with surgical resection. The lower N-acetylaspartate (NAA) content of the anterior sample suggests substantial reduction in neuronal viability, relative to the posterior sample. Acetate was not detected in the anterior tissue sample, but was elevated in the posterior sample. Aspartate content was reduced in extracts of abnormal tissue (Peeling and Sutherland, 1993), suggesting the anterior sample was more abnormal in this study. While an increased aspartate signal associated with autolysis has been ascribed to breakdown of NAA (Petroff *et al.*, 1988), the normal NAA content in the posterior sample suggests that the high aspartate concentration is not due to NAA degradation accompanying autolysis.

Alanine, which is associated with astrocytes (Danielsen and Ross, 1999), was increased compared with the reported concentration in non-encephalitic cortex (Peeling and Sutherland, 1993), with the greatest concentration in the anterior sample.

The increased concentration of Cho in the anterior sample is consistent with increased membrane breakdown, and with the presurgical *in vivo* MRS increase in Cho. While the anterior glutamine to glutamate ratio was greater than in the posterior sample, both samples had lower glutamate than previously described. Increased glutamate transamination could contribute to elevated glutamine and alanine concentrations.

The osmolyte mI concentration was higher in the posterior tissue extract and within the normal range in the anterior sample. The absence of taurine in the posterior sample may relate to its role as an organic osmolyte (Heilig *et al.*, 1989).

The variable nature of reported metabolite concentrations may reflect regional tissue variation as well as different stages of the disease process. Variations were also observed for

some metabolites in another study using ¹H MRS to assess tissue extracts from patients with Rasmussen encephalitis (Peeling and Sutherland, 1993). Our ¹H MRS data showed more abnormalities in the anterior sample of the resection and demonstrates the presence of metabolite abnormalities despite nearly normal *in vivo* MRS results prior to surgery.

Assessing *in vivo* metabolite changes relative to either Cr or mI showed that the tissue mI concentration was more variable than the other metabolites, particularly in the early measurements. This variability is consistent with the role of mI as an osmolyte and the timing would suggest a change in response to seizure effects.

The differences in concentrations determined from *in vivo* and *in vitro* measurements could be due to a combination of measurement technique and sample differences. *In vivo* spectra were obtained from a larger volume of tissue and were not fully relaxed, whereas *in vitro* spectra were recorded from resected regions of focal abnormality. Changes in metabolite composition during processing can also result in the loss of metabolites or release of bound metabolites that are invisible *in vivo*. The *in vitro* spectra were fully relaxed.

³¹P MRS

There are no previous reports of ³¹P MRS measurements in excised tissue from Rasmussen encephalitis patients. These studies provide information not available from *in vivo* MRS studies, such as the ability to determine the relative proportions of the membrane phospholipid components phosphatidylcholine, sphingomyelin, phosphatidylserine and phosphatidylethanolamine (Bretscher, 1972). Variation in the ratio of individual components may alter the membrane fluidity or function. The major difference compared with the previously reported ³¹P spectrum was a reduced fraction of phosphatidylcholine in the outer membrane layer (suggesting reduced incorporation into membranes or membrane breakdown, consistent with the increased free Cho observed by ¹H MRS). The ratio of inner membrane components phosphatidylserine to phosphatidylethanolamine was reduced compared with the reported control. The significance of this change is unclear. While it does not appear to alter membrane fluidity (Song and Waugh, 1990), the proteins supported in the membrane may be altered. Such changes may be associated with the uncontrolled neuronal activation, responsible for the repetitive seizures in Rasmussen encephalitis. One study (Kwee and Nakada, 1988) gives ranges for grey/white matter showing that sphingomyelin is higher and phosphatidylcholine is lower in white matter compared with grey. Our samples were predominantly grey matter, suggesting that the different phospholipid proportions were disease related.

Medication effects

Although we do not believe that the fluctuations in brain metabolites are due to medication, we cannot exclude an

effect of the various drugs the patient took on the metabolites measured. Carbamazepine, lamotrigine, phenytoin and topiramate were administered throughout most of the study period (Table 1); thus their potential effects would probably have been similar over several MR sessions and not responsible for the observed fluctuations. Steroids may have influenced the results from the first two MR sessions, but steroids could be expected to affect the whole-brain.

Conclusion

This longitudinal high-field MR study of a patient with Rasmussen encephalitis showed a disease process primarily involving the superior temporal gyrus on the left side. This region was identified as the seizure focus on multiple video-EEG telemetry sessions, ictal SPECT and interictal PET examinations. We observed seizure-associated MR abnormalities, consisting of increased T2 signal and reduced NA, Cr and Cho levels in the superior temporal gyrus. These abnormalities resolved completely on subsequent scans performed under better seizure control. This indicates the need for caution in the interpretation of single MRS observations that reflect a single point in a varying state with different combinations of disease and seizure-associated damage.

High-field ^{31}P MRS studies of tissue extracts showed differences in membrane phospholipid content that were consistent with increased Cho measured by *in vivo* ^1H MRS. These findings may inform discussion of the mechanism of the chronic damage to the brain and distinguish seizure related effects from primary progressive disease processes.

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