Published in final edited form as:

Sci Transl Med. 2015 October 14; 7(309): 309ra161. doi:10.1126/scitranslmed.aaa7095.

Glutamate Imaging (GluCEST) Lateralizes Epileptic Foci in Non-Lesional Temporal Lobe Epilepsy

Kathryn Adamiak Davis^{1,†,*}, Ravi Prakash Reddy Nanga^{2,†}, Sandhitsu Das³, Stephanie H. Chen¹, Peter N. Hadar¹, John R. Pollard¹, Timothy H. Lucas⁴, Russell T. Shinohara⁵, Brian Litt¹, Hari Hariharan², Mark A. Elliott², John A. Detre², and Ravinder Reddy^{2,*}

¹Penn Epilepsy Center, Department of Neurology, Hospital of the University of Pennsylvania, Philadelphia, PA 19104, USA

²Center for Magnetic Resonance & Optical Imaging, Department of Radiology, University of Pennsylvania, Philadelphia, PA 19104, USA

³Penn Image Computing & Science Lab, University of Pennsylvania, Philadelphia, PA 19104, USA

⁴Department of Neurosurgery, Hospital of the University of Pennsylvania, Philadelphia, PA 19104, USA

⁵Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA 19104, USA

Abstract

When neuroimaging reveals a brain lesion, drug-resistant epilepsy patients show better outcomes after resective surgery than do the one-third of drug resistant epilepsy patients who have normal brain MRIs. We applied a glutamate imaging method, GluCEST (Glutamate Chemical Exchange Saturation Transfer), to patients with non-lesional temporal lobe epilepsy (TLE) based on conventional MRI. GluCEST correctly lateralized the temporal lobe seizure focus on visual and quantitative analysis in all patients. MR spectra, available in a subset of patients and controls, corroborated the GluCEST findings. Hippocampal volumes were not significantly different between hemispheres. GluCEST allowed for high-resolution functional imaging of brain glutamate and has potential to identify the epileptic focus in patients previously deemed non-

*To whom correspondence should be addressed: kathryn.davis@uphs.upenn.edu.

†These authors contributed equally to this work.

Author contributions: K.A.D. and R.P.R.N. contributed equally to this work. K.A.D. developed the clinical protocol, recruited human subjects, and wrote the paper with R.P.R.N. R.P.R.N. assisted with data acquisition and analysis of GluCEST and MRS, in addition to writing the paper with K.A.D. S.D. assisted with hippocampal segmentation. S.C. and P.H. assisted with subject recruitment and clinical data collection. J.P. and B.L. reviewed GluCEST images in addition to contributing to conceptual development. T.L. provided neurosurgical expertise. R.S. provided assistance with statistical analysis. H.H. contributed to development of GluCEST technique. M.E. assisted with image sequence development. J.D. and R.R. contributed to development of GluCEST and conceptual development.

Competing interests: RR and HH hold the patent (US 20120019245 A1) on CEST MRI methods for imaging metabolites and the use of these as biomarkers.

Data and materials availability: All data is available in the supplementary materials.

Supplementary Materials: Detailed patient demographic data, patient and control GluCEST, patient and control GluCEST maps, and patient and control MRS measurements are included in the supplementary materials.

lesional. This method may lead to improved clinical outcomes for temporal lobe epilepsy as well as other localization-related epilepsies.

Introduction

Epilepsy affects ~65 million people worldwide and is a significant source of neurological morbidity. Approximately one-third of epilepsy patients have seizures that are not controlled by medications(1). Ongoing seizures degrade patients' quality of life by limiting driving and employment, and by causing social isolation and psychological harm(2). In addition, uncontrolled seizures are associated with 11 times more mortality than expected on the basis of age(3). The estimated cost of epilepsy in the United States is approximately 10 billion dollars, including medical expenditures and informal care(4).

Localization related epilepsy (LRE), also termed partial onset epilepsy, is the most common type of epilepsy and is present in 80% of drug-resistant patients(5). In adults, temporal lobe epilepsy (TLE) accounts for 65% of LRE(6, 7). Mesial temporal sclerosis can be identified on structural MRI in approximately two-thirds of patients with TLE and is associated with the most favorable outcome from resective epilepsy surgery, with 70-80% of patients seizure-free after temporal lobectomy(8–12). There is a 2-3 times greater chance of a good post-surgical outcome if a MRI or histopathological lesion is identified(13, 14). The distribution of drug resistant (refractory) epilepsy patients is illustrated in Fig. 1.

Currently patients with drug resistant epilepsy undergo multimodal structural and functional imaging for surgical planning. In addition to conventional 3T MRI with fine cuts through the mesial temporal structures, this may include 18-fluoro-deoxyglucose positron emission tomography (FDG-PET), single photon emission computed tomography (SPECT), and magnetoencephalography (MEG). Unfortunately, these methods, even in combination with scalp electroencephalography (EEG), still do not adequately localize the seizure focus in a large percentage of patients. Approximately one-third of patients with TLE show no lesion by conventional MRI(1, 5–10, 12, 13, 15). Nevertheless, in those patients in this group who undergo resective surgery, histopathology is abnormal in 87% (16). This suggests that lesions are present, but that current imaging technology lacks the sensitivity to detect them.

These findings highlight the need for improved tools to map epileptic networks. It is widely postulated that many conventional MRI-negative patients have abnormalities that might be identified by advanced imaging techniques(17). Since it is also well established that patients with lesional epilepsy have better surgical outcomes than non-lesional epilepsy(14), new neuroimaging techniques capable of detecting subtle lesions could potentially improve patient care and increase the chance of seizure freedom after surgery.

Both human and animal studies suggest that glutamate may serve as a marker of epileptic networks and support the hypothesis that glutamate is elevated in epileptogenic foci (20–23). It has been hypothesized that elevated glutamate within the glial-neuronal unit is a key manifestation of both the mitochondrial and metabolic injury that induces the hyper-excitable state characterizing seizures(19). Microdialysis and pathological studies in human epilepsy reveal increased glutamate ictally, interictally, and post mortem in the epileptic

focus(21, 24). Other imaging markers are concordant with these findings; decreased hippocampal volume on MRI has been associated with increases in extracellular glutamate in drug resistant TLE patients during intracranial EEG evaluation(25).

Magnetic resonance spectroscopy (MRS) studies in patients designed to measure glutamate have been performed mainly with lower field MRI magnets, and the results have not been clear. Unlike the prominent resonances of N-acetyl-aspartate (NAA) and creatine (Cr), which are singlets, glutamate's resonances are triplets or higher order multiplets, resulting in a smaller spectral peaks distributed over a broader range of frequencies. Glutamate also shows spectral overlap with glutamine, which complicates spectral interpretation at low field strength. With data from a 1.5T magnet, the combined resonance of glutamate and glutamine (Glx) has been reported to be decreased in EEG-defined neocortical epileptogenic regions(26). Also with data at 1.5T, it has been reported that the Glx resonance is decreased in sclerotic hippocampi but increased in the epileptic hippocampus in patients with MRI-negative disease(27, 28). In a small study of 5 patients with hippocampal sclerosis on clinical MRI, MRS at 4T showed that glutamate levels were decreased in the sclerotic hippocampi(29). Volume loss in sclerotic hippocampi may have confounded this investigation, as MRS generally obtains data from only one relatively large voxel.

There is evidence for dysfunctional glutamate cycling in TLE with both down regulation of glutamine synthetase (GS) in astrocytes (resulting in slowed glutamate clearance that is most marked in CA1 and CA3 of the hippocampus) and elevated levels of the glutamate synthesizing enzyme phosphate activated glutaminase (PAG) in the epileptogenic hippocampus(30–33). In another study, GS mRNA was increased by ~50% in CA3 in MRI-negative TLE patients compared to patients with hippocampal sclerosis on MRI and controls. In these patients, PAG mRNA was also increased in CA1, CA2, CA3 and the dentate gyrus in MRI negative TLE patients compared to TLE patients with sclerosis on MRI(31).

The chemical exchange saturation transfer (CEST) technique measures proton exchange between the exchangeable protons of the solute with the much larger pool of bulk water protons(34, 35). When the magnetization from the exchangeable protons of the solute is saturated with a frequency-selective RF pulse, there is a proportional decrease of the water signal as a result of accumulation of saturated protons in the bulk water pool. The difference between signals obtained with and without saturation of the solute pool is measured as the CEST effect. For glutamate (Glu), the amine proton resonates at 3 p.p.m down field from water and the exchange rate is within a slow to intermediate exchange regime, making glutamate an ideal neurotransmitter for CEST imaging with MRI scanners at 7T and higher fields (42). GluCEST has at least two orders of magnitude higher sensitivity than traditional ^1H MRS methods for measuring glutamate(42). Indeed, this method images glutamate in-vivo at much higher spatial resolution than can be achieved with MRS or spectroscopic imaging(42). GluCEST has been used to study the brain and spinal cord of healthy subjects and Alzheimer's disease mouse models(44–47). In an Alzheimer's mouse model, glutamate alone contributes >90% of the GluCEST signal with <10% contribution from other metabolites(46, 47).

Here, we applied the magnetic resonance imaging method GluCEST (Glutamate Chemical Exchange Saturation Transfer) at 7 Telsa to patients with non-lesional temporal lobe epilepsy. We hypothesized that the GluCEST method would be able to localize the hemisphere containing the epileptic network by visualizing increased glutamate in the hippocampus of patients with non-lesional temporal lobe epilepsy.

Results

Four non-lesional drug resistant epilepsy patients and 11 healthy controls were included in the analysis. One of the four epilepsy patients subsequently underwent intracranial EEG evaluation and right temporal lobectomy, with pathology consistent with mesial temporal sclerosis. Patient and control hippocampal volume data, GluCEST signal data including clinical data (Table S1), both quantitative values and GluCEST maps, and MRS data (Table S2, Table S3, Figure S1, Figure S2) are available in Supplementary Materials.

In all 4 epilepsy patients, glutamate measured by GluCEST was higher in the epileptogenic (ipsilateral) hippocampus than in the contralateral hippocampus, both qualitatively and quantitatively. Independent visual analysis of bilateral hippocampal images by 2 epileptologists (B.L. and J.P.) blinded to patient information accurately lateralized seizure onset in 4/4 patients. Figure 2 illustrates the lateralized GluCEST signal in two right-sided temporal epilepsy patients and two left-sided temporal epilepsy patients. Seizure onset side was determined by the Penn Epilepsy Center surgical conference consensus blinded to GluCEST results. A t-test comparing the ipsilateral to contralateral hippocampal GluCEST signal in patients was statistically significant, with the higher GluCEST signal in the hippocampus ipsilateral to the location of seizure onset (one-tail, $p=0.011$) (Fig. 3 and Table 1). We also performed a separate analysis on the head and tail subregions of the hippocampus. In the brain slice measured in our experiments, the head of the hippocampus was composed primarily of CA1 region. In patients, the GluCEST signal in the ipsilateral (to seizure onset) head was significantly different from that of the contralateral head (one-tail, $p=0.03$). Similar comparisons in the hippocampal tail, whole hemisphere, and hemisphere not including occipital lobe (consisting largely of temporal lobe and mesial temporal structures) showed no significant differences.

We compared the mean absolute asymmetry in the hippocampus of 11 controls to that of the 4 patients. The confidence interval (GluCEST contrast, %) was -0.42 to 0.73 for the controls, and the mean absolute asymmetry in the patients was 0.16 higher than controls. A t-test comparing the right to left hippocampal GluCEST signal in controls did not reach statistical significance (two-tail, $p=0.268$).

There was no significant difference in hippocampal volume between the ipsilateral and contralateral hippocampal slices in patients ($p=0.478$, assuming equal variances for two-tailed test), or between the volume of the right and left hippocampi in the control groups ($p=0.302$, assuming equal variances for two-tailed test). Data is included in the supplementary materials (Table S3).

We also performed MRS of the bilateral hippocampi in all patients and controls. However, because motion and susceptibility artifacts significantly degrade MRS measurement in this region, interpretable MRS results were only obtainable in the bilateral hippocampi of one patient (patient 4, who underwent intracranial EEG monitoring and right temporal lobectomy). Results were unreliable in one of the hippocampi of the other 3 patients. In controls, bilateral MRS was available in 5 subjects. In the patient with bilateral MRS, glutamate was increased in the hippocampus ipsilateral to seizure onset (Fig. 4) (15.77 millimolar (mM) ipsilateral versus 12.26 mM contralateral). NAA/Cr ratios were also increased in the hippocampus ipsilateral to seizure onset (NAA/Cr=1.39) versus the contralateral hippocampus (NAA/Cr=1.28). In the control subjects, four of the five with available bilateral MRS showed subtle glutamate asymmetries that corresponded to the lateralization of the GluCEST signal. Data is included in supplementary materials (Table S2, Table S3, Fig S1, Fig S2).

One subject included in this study underwent intracranial EEG evaluation to lateralize seizure onset. Symmetric bilateral hippocampal and amygdalae depth electrodes (4) and lateral temporal and subtemporal subdural strip (12) electrodes were placed. A total of four clinical electrographic seizures were captured, all arising from the right hippocampus. On the basis of these intracranial EEG findings, the patient underwent right temporal lobectomy. An example of a seizure arising from the right hippocampal depth electrode is shown in Fig. 5. Coregistration of images was performed using techniques published in prior work and indicate the locations of electrodes (48) (Fig. 5A-G). Intracranial EEG recordings of seizure onset in the right hippocampus were congruent with GluCEST findings, with increased GluCEST in the right hippocampus in this subject. Hippocampal pathology in subject 4 showed dispersion of granule cells, endplate gliosis and mild loss of pyramidal neurons consistent with mesial temporal sclerosis.

Discussion

We present a technique that has the potential to identify seizure foci in epilepsy patients who were previously determined “non-lesional” on the basis of currently available imaging methods. Although our findings were observed in only a small group of 4 patients, we present them now for three reasons: (1) our findings are compelling despite being from an unselected group of patients, (2) the ability to detect seizure foci with GluCEST imaging could potentially greatly impact patient care and quality of life in epilepsy patients, and sharing these results may greatly expedite validation of this technique, and (3) the results are consistent with the presence of inter-ictal increases of glutamate in seizure foci. A larger amount of hippocampal glutamate was measured by GluCEST in the hippocampus in which seizures initiated by visual inspection in all patients with 100% inter-rater reliability. In one patient in whom we could obtain data, glutamate concentration as measured by hippocampal MRS correlated with GluCEST quantification of glutamate in patients and controls, lending further confidence in the GluCEST technique.

Histopathological lesions are present in 87% of non-lesional temporal lobe epilepsy patients (16). Our work indicates that GluCEST is able to identify asymmetric hippocampal glutamate levels in these patients. If validated in a larger population of epilepsy patients,

GluCEST imaging could reduce the need for invasive intracranial monitoring, which is associated with morbidity, mortality and expense. In addition, GluCEST measures may yield prognostic information that can help physicians and patients determine the best form of treatment. A number of new surgical and medical options are now available to epilepsy patients including laser ablation therapy, NeuroPace Responsive Neurostimulator System, and closed loop vagal nerve stimulation(49). Noninvasive methods such as GluCEST could provide an additional tool to help clinicians to stratify patients to the best treatment option and may improve patient outcomes.

The GluCEST technique has multiple advantages over MRS, the only other imaging modality available to measure brain glutamate noninvasively in humans. GluCEST has higher spatial resolution, potentially allowing for more precise visualization of the functional excitatory network, and thus shows promise to further elucidate the etiology and progression of the epilepsy disease state. MRS acquisition is time intensive and only a single voxel at a time is typically acquired (15-20 minutes per voxel in this study with time for shimming). In addition, the rectangular voxels utilized to measure the hippocampal region in MRS likely capture regions outside the hippocampus and vary significantly from subject to subject. The limited MRS data presented here is also representative of the challenges with MRS. MRS requires extensive shimming at 7 Tesla and is exquisitely sensitive to movement artifacts. Finally, the single voxel acquisition limits spatial resolution and results in partial volume effects with contamination of surrounding structures. Furthermore, GluCEST has higher spatial resolution than PET, which has been used to measure glutamate receptors only in healthy controls(50).

These findings are consistent with results from animal models indicating that glutamate synthetase dysfunction leads to accumulation of excessive glutamate in epilepsy(30, 32, 33, 51). In addition, our findings that glutamate is specifically elevated in the hippocampal head, largely composed of the CA1 region of the hippocampus, but not the tail region is supported by the literature(30, 32, 33, 52).

There are multiple limitations to this study, most notably the small sample size, the lack of post-surgical validation of seizure lateralization in all but one patient, and the use of single slice imaging. Substantial variations in GluCEST values observed in control subjects, which appeared to randomly lateralize, may have resulted from differences in slice location in each hemisphere. The single slice method also prohibits measurement of the entire epileptic network. Ongoing work is extending GluCEST to whole-brain imaging.

7T MRI is not uniformly available. However, most epilepsy surgery is performed at academic centers, many of which now have 7T MRI. This should facilitate multisite validation of GluCEST as an imaging biomarker of seizure foci. The ability to accurately localize seizure foci would also provide a strong clinical motivation for the deployment of additional 7T MRI systems or for patients to travel to 7T sites for the procedure. Although this study demonstrated the feasibility of lateralizing seizure foci in TLE, GluCEST could also potentially localize drug resistant neocortical epilepsy, where current epilepsy imaging techniques are even more limited.

Materials and Methods

Subjects

All studies were conducted under an approved Institutional Review Board protocol of the University of Pennsylvania. GluCEST MRI was acquired on 7.0T whole body MRI scanner (Siemens Medical Systems) with a 32-channel phased-array head coil (Nova Medical Inc.) in 11 healthy control subjects (3 male, aged 23-54 years; 8 female, aged 24-56 years; mean age 35) and 4 temporal lobe epilepsy subjects (1 male, aged 47 years; 3 female, aged 25-47 years; mean age 40).

The four temporal lobe epilepsy subjects were recruited from the Penn Epilepsy Center and had undergone presurgical evaluation including prior scalp EEG and, in one case, intracranial EEG capturing seizures. As this was initially a pilot study, no predetermined sample size was calculated, and data collection is ongoing. Inclusion criteria included epilepsy classification of mesial temporal epilepsy as determined by the Penn Epilepsy Center conference, at least 18 years of age, and unremarkable clinical MRI as interpreted by a clinical radiologist at University of Pennsylvania. Exclusion criteria were contraindications to 7T MRI scanning (e.g. metallic implant), prior intracranial surgical intervention, claustrophobia prohibiting scanning at 7T MRI without sedative medications, and pregnancy. Imaging for GluCEST was done for each subject at one time point. The investigator (RPRN) analyzing the GluCEST data was blinded to the lateralization of seizure onset in each subject. Table 2 summarizes the clinical data for the 4 epilepsy patients recruited to participate. None of the patients had an identifiable epilepsy risk factor (e.g. no prior history of febrile seizures, no family history of seizures, no history of head trauma). All patients were resistant to multiple antiepileptic medications, having tried and failed between 2-7 prior antiepileptic medications (average 5 prior drug trials). Patient 3 declined evaluation for surgery. Patient 4 underwent a right temporal lobectomy after intracranial EEG monitoring confirmed that seizures arose from right temporal region. Patients 1 and 2 are undergoing presurgical evaluation.

MRI scans and Data Analysis

The MRI protocol consisted of the following steps: a localizer, T1-weighted anatomical 3D magnetization prepared rapid gradient echo (MPRAGE) images of whole brain (176 axial slices, TR/TE/TI = 2800/4.4/1500 ms, $\alpha = 7^\circ$, $0.8 \times 0.8 \times 0.8 \text{ mm}^3$ resolution, iPAT = 2, scan time 4:43), T₂-weighted 3D using the variable flip angle turbo spin-echo (TSE_VFL) sequence (224 coronal slices, TR/TE = 3000/388 ms, $0.4 \times 0.4 \times 1.0 \text{ mm}^3$ resolution, iPAT = 2, scan time 7:42) followed by the acquisition of the GluCEST sequences (42, 46, 47, 53). The T1 images were used to identify the location of the hippocampi and then to manually position the T2 scan such that the direction of the imaged slice was parallel to the long axes of the hippocampi. The TR for the T2 scan was shortened from its optimal setting for T2-contrast in order to bring the scan time to a manageable duration. For GluCEST, anatomical images acquired from T₂-weighted imaging were used to select the axial hippocampal slice. The GluCEST imaging parameters were: slice thickness = 5 mm, field of view (readout) = 200 mm, field of view (phase encoding) = 162.5 mm, matrix size = 256×208 , GRE read out TR = 6.2 ms, TE = 3 ms, number of averages = 2, shot TR = 10000 ms, shots per slice = 2,

with a 800-ms long saturation pulse train consisting of a series of 96-ms Hanning windowed saturation pulses with a 4-msec interpulse delay (100-ms pulse train) at a B_{1rms} of 3.06 μT . Raw CEST images were acquired at varying saturation offset frequencies from ± 1.8 to ± 4.2 ppm (relative to water resonance) with a step size of ± 0.3 ppm. GRE images at two echo times ($TE1 = 4.24$ ms; $TE2 = 5.26$ ms) were collected to compute B_0 map. B_1 map was generated from the two images obtained using square preparation pulses with flip angles 30° and 60° . All of the image processing and data analysis were performed with in-house written programs in MATLAB (MathWorks, version 7.5, R2009b). Overall, acquisition time of CEST images, B_1 and B_0 field maps was approximately 20 minutes. CEST images obtained from ± 1.8 to ± 4.2 ppm were interpolated using the cubic spline method to generate images with a fine step size of 0.01 ppm. B_0 corrected CEST images at ± 3 ppm were generated from the interpolated CEST images by picking signals according to the frequency shift in the B_0 map. The B_0 corrected ± 3.0 ppm images were then used for computing the percentage GluCEST contrast, which is equal to $100 \times (M_{-3ppm} - M_{+3ppm}) / M_{-3ppm}$, where M_{-3ppm} and M_{+3ppm} are B_0 corrected images saturated at -3ppm and +3ppm respectively with respect to water(42). B_1 inhomogeneity artifacts in GluCEST maps were removed using B_1 calibration curves as reported(53). The B_0 and B_1 corrected GluCEST contrasts were then averaged within expertly drawn (performed by Dr. Nanga and confirmed by Dr. Davis) regions-of-interest (ROIs) in the bilateral hippocampi, hippocampal head (largely composed of CA1), hippocampal tail, right hemisphere, left hemisphere, right hemisphere excluding occipital lobe (largely composed of right temporal lobe and mesial temporal structures), left hemisphere excluding occipital lobe (largely composed of left temporal lobe and mesial temporal structures).

Hippocampal volumes were measured from a slice corresponding to the 2D CEST slice. Hippocampal volume of each ROI = $[TP - (NE/2)] \times VP$. (TP = Total number of pixels within the ROI; NE = Number of edge pixels within the ROI; VP = Volume of each pixel in the CEST 2D slice = 3.2 mm^3 ($0.8\text{mm} \times 0.8\text{mm} \times 5\text{mm}$)).

For single voxel proton magnetic resonance spectroscopy (1H MRS), the voxel of interest (AP 20mm, LR 10mm, HF 5 mm; right/left hippocampus) was positioned on the GluCEST axial hippocampal slice. Automated first order and second order shimming of the B_0 field was performed on voxel of interest in order to obtain a localized water line width of ~ 24 Hz or less using FASTMAP shim method(54, 55) provided by Siemens as work in progress package. Single voxel spectra (SVS) for glutamate were obtained using PRESS (Point RESolved Spectroscopy) sequence with following parameters: number of points = 2048, averages = 8 (water reference spectrum) / 128 (water suppressed spectrum), TR = 3000 ms and TE = 20 ms. The total acquisition time to obtain each spectrum was ~ 7 min 12 s. For post processing of spectroscopy data, we used the raw multi-channel time domain data from the scanner. From the water reference data, channel-wise time-dependent phase shifts due to eddy current and amplitude scale factors were obtained and saved. Both spectra were obtained after channel-wise eddy current correction and adaptive combination(56). Metabolite peaks from water suppressed spectrum were fitted as Lorentzian functions with non-linear least squares fitting (MATLAB “nlinfit” routine) by taking into account the prior knowledge of the 8 macromolecular peaks and 14 metabolite peaks over the frequency range

of 0.5 to 4.3 ppm(57) followed by integration and then normalized by water reference signal for absolute quantification of glutamate.

Statistics

Paired two-sample t-test for means was performed on the control and epilepsy subjects (2 tailed and 1 tailed respectively). GluCEST measurements were calculated for bilateral hippocampi, bilateral hippocampal heads, bilateral hippocampal tails, bilateral hemispheres, and bilateral hemispheres excluding the occipital lobe. N=4 epilepsy patients, N=11 control subjects. Covariates such as age, sex, seizure frequency, concurrent antiepileptic drugs were not included because of low sample size. Hippocampal volume was calculated as described in the methods, and asymmetry was assessed using a two-sample t-test assuming equal variances.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank J. Stein. for assistance with the electrode coregistration (Fig. 5)

Funding: P41 EB015893 (NIH NIBIB), P20 NS12006 (NIH NINDS), McCabe Pilot Award (University of Pennsylvania), Center for Biomedical Image Computing and Analytics Seed Award (University of Pennsylvania)

References

1. Kwan P, Brodie MJ. Early identification of refractory epilepsy. *N Engl J Med.* 2000; 342:314–9. [PubMed: 10660394]
2. Baker GA, Jacoby A, Buck D, Stalgis C, Monnet D. Quality of Life of People with Epilepsy: A European Study. *Epilepsia.* 1997; 38:353–362. [PubMed: 9070599]
3. Fazel S, Wolf A, Långström N, Newton CR, Lichtenstein P. Premature mortality in epilepsy and the role of psychiatric comorbidity: a total population study. *Lancet.* 2013; 382:1646–54. [PubMed: 23883699]
4. Yoon D, Frick KD, Carr DA, Austin JK. Economic impact of epilepsy in the United States. *Epilepsia.* 2009; 50:2186–91. [PubMed: 19508694]
5. French JA. Refractory epilepsy: clinical overview. *Epilepsia.* 2007; 48 Suppl 1:3–7. [PubMed: 17316406]
6. Callaghan BC, Anand K, Hesdorffer D, Hauser WA, French JA. Likelihood of seizure remission in an adult population with refractory epilepsy. *Ann Neurol.* 2007; 62:382–9. [PubMed: 17880009]
7. Wiebe S, Camfield P, Jetté N, Burneo JG. Epidemiology of epilepsy: prevalence, impact, comorbidity and disparities. *Can J Neurol Sci.* 2009; 36 Suppl 2:S7–16. [PubMed: 19760894]
8. Jette N, Wiebe S. Update on the surgical treatment of epilepsy. *Curr Opin Neurol.* 2013; 26:201–7. [PubMed: 23449171]
9. Spencer SS, Berg AT, Vickrey BG, Sperling MR, Bazil CW, Shinnar S, Langfitt JT, Walczak TS, Pacia SV. Predicting long-term seizure outcome after resective epilepsy surgery: the multicenter study. *Neurology.* 2005; 65:912–8. [PubMed: 16186534]
10. Wiebe S. Epilepsy. Outcome patterns in epilepsy surgery--the long-term view. *Nat Rev Neurol.* 2011; 8:123–4. [PubMed: 22290572]
11. Mohammed HS, Kaufman CB, Limbrick DD, Steger-May K, Grubb RL, Rothman SM, Weisenberg JLZ, Munro R, Smyth MD. Impact of epilepsy surgery on seizure control and quality of life: a 26-year follow-up study. *Epilepsia.* 2012; 53:712–20. [PubMed: 22313356]

12. de Tisi J, Bell GS, Peacock JL, McEvoy AW, Harkness WFJ, Sander JW, Duncan JS. The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: a cohort study. *Lancet*. 2011; 378:1388–95. [PubMed: 22000136]
13. Siegel AM, Jobst BC, Thadani VM, Rhodes CH, Lewis PJ, Roberts DW, Williamson PD. Medically intractable, localization-related epilepsy with normal MRI: presurgical evaluation and surgical outcome in 43 patients. *Epilepsia*. 2001; 42:883–8. [PubMed: 11488888]
14. Téllez-Zenteno JF, Hernández Ronquillo L, Moien-Afshari F, Wiebe S. Surgical outcomes in lesional and non-lesional epilepsy: a systematic review and meta-analysis. *Epilepsy Res*. 2010; 89:310–318. [PubMed: 20227852]
15. Téllez-Zenteno JF, Hernández Ronquillo L, Moien-Afshari F, Wiebe S. Surgical outcomes in lesional and non-lesional epilepsy: a systematic review and meta-analysis. *Epilepsy Res*. 2010; 89:310–8. [PubMed: 20227852]
16. Cohen-Gadol AA, Wilhelmi BG, Collignon F, White JB, Britton JW, Cambier DM, Christianson TJH, Marsh WR, Meyer FB, Cascino GD. Long-term outcome of epilepsy surgery among 399 patients with nonlesional seizure foci including mesial temporal lobe sclerosis. *J Neurosurg*. 2006; 104:513–24. [PubMed: 16619654]
17. Madan N, Grant PE. New directions in clinical imaging of cortical dysplasias. *Epilepsia*. 2009; 50 Suppl 9:9–18. [PubMed: 19761449]
18. Ozawa S, Kamiya H, Tsuzuki K. Glutamate receptors in the mammalian central nervous system. *Prog Neurobiol*. 1998; 54:581–618. [PubMed: 9550192]
19. Pan JW, Williamson A, Cavus I, Hetherington HP, Zaveri H, Petroff OAC, Spencer DD. Neurometabolism in human epilepsy. *Epilepsia*. 2008; 49 Suppl 3:31–41. [PubMed: 18304254]
20. Lallement G, Carpentier P, Collet A, Pernot-Marino I, Baubichon D, Blanchet G. Effects of soman-induced seizures on different extracellular amino acid levels and on glutamate uptake in rat hippocampus. *Brain Res*. 1991; 563:234–40. [PubMed: 1786536]
21. Millan MH, Chapman AG, Meldrum BS. Extracellular amino acid levels in hippocampus during pilocarpine-induced seizures. *Epilepsy Res*. 1993; 14:139–48. [PubMed: 8095893]
22. Molinari F, Raas-Rothschild A, Rio M, Fiermonte G, Encha-Razavi F, Palmieri L, Palmieri F, Ben-Neriah Z, Kadhon N, Vekemans M, Attie-Bitach T, Munnich A, Rustin P, Colleaux L. Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy. *Am J Hum Genet*. 2005; 76:334–9. [PubMed: 15592994]
23. Petroff OA, Pleban LA, Spencer DD. Symbiosis between in vivo and in vitro NMR spectroscopy: the creatine, N-acetylaspartate, glutamate, and GABA content of the epileptic human brain. *Magn Reson Imaging*. 1995; 13:1197–211. [PubMed: 8750337]
24. Cavus I, Kasoff WS, Cassaday MP, Jacob R, Gueorguieva R, Sherwin RS, Krystal JH, Spencer DD, Abi-Saab WM. Extracellular metabolites in the cortex and hippocampus of epileptic patients. *Ann Neurol*. 2005; 57:226–35. [PubMed: 15668975]
25. Cavus I, Pan JW, Hetherington HP, Abi-Saab W, Zaveri HP, Vives KP, Krystal JH, Spencer SS, Spencer DD. Decreased hippocampal volume on MRI is associated with increased extracellular glutamate in epilepsy patients. *Epilepsia*. 2008; 49:1358–66. [PubMed: 18410365]
26. Pfund Z, Chugani DC, Juhász C, Muzik O, Chugani HT, Wilds IB, Seraji-Bozorgzad N, Moore GJ. Evidence for coupling between glucose metabolism and glutamate cycling using FDG PET and 1H magnetic resonance spectroscopy in patients with epilepsy. *J Cereb Blood Flow Metab*. 2000; 20:871–8. [PubMed: 10826538]
27. Woermann FG, McLean MA, Bartlett PA, Parker GJ, Barker GJ, Duncan JS. Short echo time single-voxel 1H magnetic resonance spectroscopy in magnetic resonance imaging-negative temporal lobe epilepsy: different biochemical profile compared with hippocampal sclerosis. *Ann Neurol*. 1999; 45:369–76. [PubMed: 10072052]
28. Simister RJ, Woermann FG, McLean MA, Bartlett PA, Barker GJ, Duncan JS. A Short-echo-time Proton Magnetic Resonance Spectroscopic Imaging Study of Temporal Lobe Epilepsy. *Epilepsia*. 2002; 43:1021–1031. [PubMed: 12199727]
29. Pan JW, Venkatraman T, Vives K, Spencer DD. Quantitative glutamate spectroscopic imaging of the human hippocampus. *NMR Biomed*. 2006; 19:209–16. [PubMed: 16479532]

30. Eid T, Thomas MJ, Spencer DD, Rundén-Pran E, Lai JCK, Malthankar GV, Kim JH, Danbolt NC, Ottersen OP, de Lanerolle NC. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet*. 2004; 363:28–37. [PubMed: 14723991]
31. Eid T, Lee TSW, Wang Y, Perez E, Pérez E, Drummond J, Lauritzen F, Bergersen LH, Meador-Woodruff JH, Spencer DD, de Lanerolle NC, McCullumsmith RE. Gene expression of glutamate metabolizing enzymes in the hippocampal formation in human temporal lobe epilepsy. *Epilepsia*. 2013; 54 available at <http://cpnp.org/resource/reference/129134>.
32. Petroff OAC, Errante LD, Rothman DL, Kim JH, Spencer DD. Glutamate-glutamine cycling in the epileptic human hippocampus. *Epilepsia*. 2002; 43:703–10. [PubMed: 12102672]
33. Eid T, Hammer J, Rundén-Pran E, Roberg B, Thomas MJ, Osen K, Davanger S, Laake P, Torgner IA, Lee TSW, Kim JH, Spencer DD, Ottersen OP, de Lanerolle NC. Increased expression of phosphate-activated glutaminase in hippocampal neurons in human mesial temporal lobe epilepsy. *Acta Neuropathol*. 2007; 113:137–52. [PubMed: 17115168]
34. Guivel-Scharen V, Sinnwell T, Wolff SD, Balaban RS. Detection of proton chemical exchange between metabolites and water in biological tissues. *J Magn Reson*. 1998; 133:36–45. [PubMed: 9654466]
35. Wolff SD, Balaban RS. NMR imaging of labile proton exchange. *J Magn Reson*. 1990; 86:164–169.
36. Ward KM, Aletras AH, Balaban RS. A new class of contrast agents for MRI based on proton chemical exchange dependent saturation transfer (CEST). *J Magn Reson*. 2000; 143:79–87. [PubMed: 10698648]
37. Dagher AP, Aletras A, Choyke P, Balaban RS. Imaging of urea using chemical exchange-dependent saturation transfer at 1.5T. *J Magn Reson Imaging*. 2000; 12:745–8. [PubMed: 11050645]
38. van Zijl PCM, Jones CK, Ren J, Malloy CR, Sherry AD. MRI detection of glycogen in vivo by using chemical exchange saturation transfer imaging (glycoCEST). *Proc Natl Acad Sci U S A*. 2007; 104:4359–64. [PubMed: 17360529]
39. Saar G, Zhang B, Ling W, Regatte RR, Navon G, Jerschow A. Assessment of glycosaminoglycan concentration changes in the intervertebral disc via chemical exchange saturation transfer. *NMR Biomed*. 2012; 25:255–61. [PubMed: 22253087]
40. Zhou J, Tryggstad E, Wen Z, Lal B, Zhou T, Grossman R, Wang S, Yan K, Fu DX, Ford E, Tyler B, Blakeley J, Larterra J, van Zijl PCM. Differentiation between glioma and radiation necrosis using molecular magnetic resonance imaging of endogenous proteins and peptides. *Nat Med*. 2011; 17:130–4. [PubMed: 21170048]
41. Haris M, Cai K, Singh A, Hariharan H, Reddy R. In vivo mapping of brain myo-inositol. *Neuroimage*. 2011; 54:2079–85. [PubMed: 20951217]
42. Cai K, Haris M, Singh A, Kogan F, Greenberg JH, Hariharan H, Detre JA, Reddy R. Magnetic resonance imaging of glutamate. *Nat Med*. 2012; 18:302–6. [PubMed: 22270722]
43. Haris M, Singh A, Cai K, Kogan F, McGarvey J, DeBrosse C, Zsido GA, Witschey WRT, Koomalsingh K, Pilla JJ, Chirinos JA, Ferrari VA, Gorman JH, Hariharan H, Gorman RC, Reddy R. A technique for in vivo mapping of myocardial creatine kinase metabolism. *Nat Med*. 2014; 20:209–214. [PubMed: 24412924]
44. Kogan F, Singh A, DeBrosse C, Haris M, Cai K, Nanga RP, Elliott M, Hariharan H, Reddy R. Imaging of glutamate in the spinal cord using GluCEST. *Neuroimage*. 2013; 77:262–7. [PubMed: 23583425]
45. Cai K, Singh A, Roalf DR, Nanga RPR, Haris M, Hariharan H, Gur R, Reddy R. Mapping glutamate in subcortical brain structures using high-resolution GluCEST MRI. *NMR Biomed*. 2013; 26:1002/nbm.2949
46. Haris M, Nath K, Cai K, Singh A, Crescenzi R, Kogan F, Verma G, Reddy S, Hariharan H, Melhem ER, Reddy R. Imaging of glutamate neurotransmitter alterations in Alzheimer's disease. *NMR Biomed*. 2012; 25:1002/nbm.2875

47. Crescenzi R, DeBrosse C, Nanga RPR, Reddy S, Haris M, Hariharan H, Iba M, Lee VMY, Detre JA, Borthakur A, Reddy R. In vivo measurement of glutamate loss is associated with synapse loss in a mouse model of tauopathy. *Neuroimage*. 2014; 101:185–92. [PubMed: 25003815]
48. Wu J, Azarion A, Pearce A, Krish V, Wagennar J, Chen W, Zheng Y, Wang H, Lucas T, Gee J, Litt B, Davis K. An open-source, automated platform for visualizing subdural electrodes using 3D CT-MRI coregistration. *Epilepsia*. 2014; 55:2028–37. [PubMed: 25377267]
49. Morrell MJ. Responsive cortical stimulation for the treatment of medically intractable partial epilepsy. *Neurology*. 2011; 77:1295–304. [PubMed: 21917777]
50. Wong DF, Waterhouse R, Kuwabara H, Kim J, Braši JR, Chamroonrat W, Stabins M, Holt DP, Dannals RF, Hamill TG, Mozley PD. 18F-FPEB, a PET radiopharmaceutical for quantifying metabotropic glutamate 5 receptors: a first-in-human study of radiochemical safety, biokinetics, and radiation dosimetry. *J Nucl Med*. 2013; 54:388–96. [PubMed: 23404089]
51. Eid T, Lee TSW, Wang Y, Pérez E, Drummond J, Lauritzen F, Bergersen LH, Meador-Woodruff JH, Spencer DD, de Lanerolle NC, McCullumsmith RE. Gene expression of glutamate metabolizing enzymes in the hippocampal formation in human temporal lobe epilepsy. *Epilepsia*. 2013; 54:228–38. [PubMed: 23384343]
52. Eid T, Lee TSW, Wang Y, Perez E, Pérez E, Drummond J, Lauritzen F, Bergersen LH, Meador-Woodruff JH, Spencer DD, de Lanerolle NC, McCullumsmith RE. Gene expression of glutamate metabolizing enzymes in the hippocampal formation in human temporal lobe epilepsy. *Epilepsia*. 2013; 54:228–38. [PubMed: 23384343]
53. Singh A, Cai K, Haris M, Hariharan H, Reddy R. On B1 inhomogeneity correction of in vivo human brain glutamate chemical exchange saturation transfer contrast at 7T. *Magn Reson Med*. 2013; 69:818–24. [PubMed: 22511396]
54. Gruetter R. Automatic, localized in vivo adjustment of all first- and second-order shim coils. *Magn Reson Med*. 1993; 29:804–11. [PubMed: 8350724]
55. Gruetter R, Tkác I. Field mapping without reference scan using asymmetric echo-planar techniques. *Magn Reson Med*. 2000; 43:319–23. [PubMed: 10680699]
56. Natt O, Bezkorovayny V, Michaelis T, Frahm J. Use of phased array coils for a determination of absolute metabolite concentrations. *Magn Reson Med*. 2005; 53:3–8. [PubMed: 15690495]
57. Pfeuffer J, Tkác I, Provencher SW, Gruetter R. Toward an in vivo neurochemical profile: quantification of 18 metabolites in short-echo-time (1)H NMR spectra of the rat brain. *J Magn Reson*. 1999; 141:104–20. [PubMed: 10527748]
58. Yushkevich PA, Pluta JB, Wang H, Xie L, Ding SL, Gertje EC, Mancuso L, Kliot D, Das SR, Wolk DA. Automated volumetry and regional thickness analysis of hippocampal subfields and medial temporal cortical structures in mild cognitive impairment. *Hum Brain Mapp*. 2014; 36:258–87. [PubMed: 25181316]

Figure 1.
The distribution of epilepsy patients worldwide, with details for those with drug-resistant epilepsy.

Figure 2. Coronal sections from four patients with drug-resistant temporal lobe epilepsy, showing the GluCEST signal

(A) 40 year-old female with non-lesional right TLE, with a visible increase in the GluCEST signal in the right hippocampus. (B) 47 year-old female with non-lesional right TLE, with a visible increase in the GluCEST signal in the right hippocampus. (C) 25 year-old female with non-lesional left TLE, with a visible increase in the GluCEST signal in the left hippocampus. (D) 47 year old male with non-lesional left TLE, with a visible increase in the GluCEST signal in the left hippocampus.

Figure 3. Increased glutamate in the hippocampus ipsilateral to seizure onset, as measured by GluCEST

GluCEST contrast in the hippocampi ipsilateral and contralateral to seizure onset, as measured by percentage displaced water protons. Green and grey portions of each box represent the second and third quartile values, respectively; the upper and lower range of values is indicated by whiskers. $p=0.011$, one-tailed t-test

Figure 4. Increased glutamate in the hippocampus ipsilateral to seizure onset in patient with right TLE

Single voxel proton magnetic resonance spectroscopy (^1H MRS) was performed individually on left and right hippocampus of non-lesional right TLE subject (NAA: N-Acetyl Aspartate; Glu: Glutamate; Cr: Creatine; Cho: Choline; mI: myo-Inositol; Glx: Glutamate+Glutamine). Heights of the peaks were measured in arbitrary units (A.U.).

Figure 5. Intracranial EEG data from patient 4, with non-lesional TLE arising from right hippocampus

Background shows EEG data from the individual contacts of subdural and depth electrodes in patient 4, indicating the localization of seizure onset to the CA1 region of the hippocampus (red arrow). (A, C and E) Three MRI sections through the hippocampus and amygdala showing the multi-contact right mesial temporal depth electrode (each contact of the electrode is shown as a yellow square; seizure onset electrode is the electrode most distal from scalp (first electrode from left)). (B, D, and F) Duplicate of the sections shown in (A, C, and E) with segmentation of hippocampal subfields superimposed on the image. Red, CA1; green: dentate gyrus; dark blue, Brodmann Area 36; yellow, CA3; light blue Brodmann Area 35; violet, subiculum. Segmentation was performed as described (48). (G) Right lateral view of the reconstructed MRI brain image of patient 4. The position of the right hemisphere electrodes is shown in green, coregistered with the MRI image. The electrodes used for seizure localization are labeled (Depth electrode, right hippocampal and amygdalar), RPT=right posterior temporal, RMT=right mid-temporal, RAT= right anterior temporal, RAF=right anterior frontal). Unlabeled electrodes were not used in the analysis described in this article.

Table 1
Summary of GluCEST findings in non-lesional patients with temporal lobe epilepsy

Comparisons are made between regions ipsilateral and contralateral to seizure onset.

Bilateral Regions tested	Two sample paired t-test for means, comparing ipsilateral to contralateral	GluCEST values ipsilateral (%), range of values	GluCEST values contralateral (%), range of values	Ipsilateral - contralateral (%), range of values
Hippocampi	p=0.011 * (one-tailed)	8.69-11.16	7.41-10.32	0.46-1.28
Hippocampal Heads	p=0.03 *	9.06-12.36	7.59-10.87	-0.02-1.53
Hippocampal Tails	p=0.11	8.41-10.15	7.24-10.53	-0.38-1.17
Hemispheres	p=0.19	7.47-7.82	6.49-7.97	-0.50-0.98
Hemispheres excluding occipital lobe	p=0.11	8.28-9.49	7.90-9.24	-0.09-0.73

* =significant with $p < 0.05$, N=4

Table 2

Epilepsy patient clinical information

Patient Number	Age	Gender	Clinical MRI	FDG-PET	Duration of seizures	Seizure frequency	Seizure Localization
1	40	Female	Normal	Mild right anterior temporal hypometabolism	6 years	1 CPS/month, occasional CPS clusters	Right temporal (Scalp EEG)
2	25	Female	Normal	Pending	2.5 years	3 CPS/week	Left temporal (Scalp EEG)
3	47	Male	Normal	None	40 years	1-2 CPS/year	Left temporal (Scalp EEG)
4	47	Female	Normal	Right temporal hypometabolism	27 years	2-3 CPS/week 1-2 GTC/month	Right Temporal (Intracranial EEG) *

SPS, simple partial seizure, CPS, complex partial seizures, GTC, generalized tonic clonic seizure,

* underwent right temporal lobectomy