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Neurometabolism in human epilepsy

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Summary

Purpose—Because of the large and continuous energetic requirements of brain function, neurometabolic dysfunction is a key pathophysiologic aspect of the epileptic brain. Additionally, neurometabolic dysfunction has many self-propagating features that are typical of epileptogenic processes, that is, where each occurrence makes the likelihood of further mitochondrial and energetic injury more probable. Thus abnormal neurometabolism may be not only a chronic accompaniment of the epileptic brain, but also a direct contributor to epileptogenesis.

Methods—We examine the evidence for neurometabolic dysfunction in epilepsy, integrating human studies of metabolic imaging, electrophysiology, microdialysis, as well as intracranial EEG and neuropathology.

Results—As an approach of noninvasive functional imaging, quantitative magnetic resonance spectroscopic imaging (MRSI) measured abnormalities of mitochondrial and energetic dysfunction (via ¹H or ³¹P spectroscopy) are related to several pathophysiologic indices of epileptic dysfunction. With patients undergoing hippocampal resection, intraoperative ¹³C-glucose turnover studies show a profound decrease in neurotransmitter (glutamate–glutamine) cycling relative to oxidation in the sclerotic hippocampus. Increased extracellular glutamate (which has long been associated with increased seizure likelihood) is significantly linked with declining energetics as measured by ³¹P MR, as well as with increased EEG measures of Teager energy, further arguing for a direct role of glutamate with hyperexcitability.

Discussion—Given the important contribution that metabolic performance makes toward excitability in brain, it is not surprising that numerous aspects of mitochondrial and energetic state link significantly with electrophysiologic and microdialysis measures in human epilepsy. This may be of particular relevance with the self-propagating nature of mitochondrial injury, but may also help define the conditions for which interventions may be developed.

Keywords

Glutamate; Neurometabolism; Microdialysis; MR spectroscopy; Energetics

The inherent instability of the epileptic brain in many ways can be likened to unstable physical phenomena, such as volcanoes and earthquakes. While on a moment-to-moment basis, the land that gives rise to these events can be described as a static homeostasis, small and unpredictable changes can (repeatedly) set off uncontrolled yet characteristic storms. The epileptic brain resides in a similar “critical state.” What drives the development of a seizure remains heavily debated, but the notion of a homeostatic critical state has led our group to examine the issues of neurometabolic sensitivity and volatility. Brain tissue is well known to be very dependent on available fuel substrate. This, combined with extensive evidence arguing for substantial metabolic dysfunction in epilepsy—significant enough to allow individualized metabolic localization of the seizure focus—has led to investigations regarding how the interdependent metabolism of the elements within the “glialneuronal unit” (GNU) may contribute to the epileptogenic state.

In this review, we consider the basis for the metabolic hypothesis, including perspectives from human and animal work, as well as the brain's metabolic physiology. In doing so, we integrate data from our own group and others to indicate how the various pieces of data may be interrelated. Our tools are geared towards studying the human patient, and bring together several areas, including microdialysis, MR-based metabolic imaging and in vitro studies on resected tissue.

Basis for the Metabolic Hypothesis

The basis for the metabolic hypothesis stems from several avenues of evidence, including imaging data, human physiological measurements and animal model studies.

Human Imaging Data

There is overwhelming evidence that metabolic dysfunction is common in human epilepsy, and frequently specific enough to identify the seizure onset zone in many patients. As a measure of total glucose consumption, ¹⁸fluorodeoxyglucose (FDG) positron emission tomography (PET) is commonly used to identify hypometabolic areas. The success rate of FDG PET in identifying the region of seizure onset very much depends on the population studied with the best localization rates of ~80–90% in temporal lobe epilepsy (for review, Spencer et al., 1995), although it is less successful in the nonlesional neocortical epilepsy patients. In the mid-1990s, a number of investigators demonstrated the high sensitivity (70–100%) of MRS and MRSI-based methods using decrements in N-acetyl aspartate (NAA) to localize the seizure onset zone in mesial temporal lobe epilepsy (mTLE) patients (Connelly et al., 1994; Hetherington et al., 1995; Cendes et al., 1994). NAA is synthesized only in neuronal mitochondria (Urenjack et al., 1992; Patel & Clark, 1979) and is strongly correlated with oxidative metabolism (Goldstein, 1969; Heales et al., 1995; Bates et al., 1996) and thus provides a complementary approach to PET, which evaluates total glucose consumption. MRS work in patients with cortical malformations has revealed that NAA changes were specific to the epileptogenic zone, and were “normal” in patients whose seizures were well controlled. Furthermore, in patients with on-going seizures the regions which were anatomically abnormal but nonepileptogenic also displayed normal NAA levels (Kuzniecky et al., 1997). An important aspect of the MRSI approach has been its ability to provide more quantitative evaluations, that is, without the use of asymmetry indices and without being biased by tissue loss. As a result, many more of the studies using MRS have shown the frequent occurrence of bilateral involvement in temporal lobe epilepsy (e.g., Fig.

1), which has since also been reported by conventional and diffusion weighted imaging (Concha et al., 2005; Seidemberg et al., 2005; Araújo et al., 2006). Our recent data (Hetherington et al., 2007) has demonstrated that the decrements in NAA are not just localized to the ipsilateral hippocampus, but are also found in a network of involved structures including the contralateral hippocampus and ipsilateral and contralateral thalamus (Fig. 2).

Data from our group has also shown how NAA may provide metabolic information regarding the GNU. Several groups have shown the key reversibility of NAA decrements in the contralateral hippocampus following successful temporal lobectomy (Hugg et al., 1996; Cendes et al., 1997; Serles et al., 2001). The preoperative contralateral NAA decline in a normal appearing hippocampus is unlikely to be measuring neuronal loss, and thus its reversibility suggests that NAA reflects metabolic injury that can recover. Our recent data correlating NAA levels with neuronal and glial (as measured by glial fibrillary acidic protein, GFAP) injury as opposed to neuronal loss (Cohen-Gadol et al., 2004) has also quantitatively indicated that the reductions in NAA are not due to neuronal loss but most likely reflecting injury to the GNU. Further strengthening the link between epilepsy with impaired bioenergetics and mitochondrial function are the abnormal findings of bioenergetics as measured by in vivo spectroscopic imaging of high-energy phosphates (Laxer et al., 1992; Chu et al., 1998; Pan et al., 2005).

A robust measure of bioenergetics has been the ratio of phosphocreatine/adenosine triphosphate (PCr/ATP). As a key regulator and participant in many diverse reactions, the concentration of ATP is important to maintain. By virtue of being in rapid equilibrium with ATP, phosphocreatine allows stability of ATP, with its own concentration varying to reflecting the balance between cellular demand and production. As a result, the ratio of PCr/ATP determined interictally reflects the steady-state ambient energetic condition (and can be thought of as being characteristic of the regional “temperature”). Given the known existing mitochondrial injury it is not surprising that we have found lower PCr/ATP in the epileptogenic region compared to healthy brain (Fig. 3). This view is also consistent with our observations linking in vivo PCr/ATP measurements with electrophysiology from resected tissue studied ex vivo (Williamson et al., 2005). As shown in Fig. 3B, preoperative PCr/ATP values were significantly and positively correlated with the recovery rate of the membrane potential after sustained suprathreshold stimulation (10 s, 10 Hz train). Moreover, there was a similar correlation between PCr/ATP and the ability of dentate granule cells to fire synaptically evoked bursts, a hallmark of epileptogenic hippocampal tissue. We hypothesize that neurons (and possibly astrocytes) in regions with lower PCr/ATP ratios are unable to restore ionic gradients efficiently; this in turn may result in greater likelihood for the occurrence of a seizure, creating a self-propagating injury. In this context, additional loads, such as stress or fatigue that may exacerbate this sensitivity and may in part contribute to the ill-defined but clinically realistic notion of seizure threshold.

Aberrant Physiology in in Vivo Epilepsy Patients

Models of neurotransmission and metabolism

There is extensive data from epilepsy patients that are strongly suggestive of a disturbance in metabolic physiology of neurons and glia. As is known from a large body of older data, neurotransmission and metabolism are tightly interwoven linking the neuron and astrocyte (for review, Schousboe et al., 1992). Specifically, glutamate clearance through its conversion into glutamine is accomplished by the glial-specific expression of glutamine synthetase. Glutamine is converted back to glutamate in the neuron through the activity of (phosphate activated) glutaminase. Together, this cycle clearly establishes a directionality of

glutamate neurotransmission flow between the neuron and the astrocyte as shown in Fig. 4A.

The in vitro work of Magistretti and colleagues has further argued strongly for a coupling of neurotransmission with metabolism: with glial glycolysis of glucose enabling synaptic clearance of neurotransmitter glutamate, with the consequent production of lactate being shuttled to the neuron for further oxidation (Magistretti & Pellerin, 1999 and Fig. 4A). Extensive work has also shown that GABA flow stems from the glutamate cycle. GABA is synthesized from glutamate (or glutamine, Fig. 4B) via glutamic acid decarboxylase. It is cleared from the synaptic cleft by either its repackaging into neurotransmitter GABA, or its conversion via GABA transaminase into succinate; in this path it is directly an extension of the TCA cycle. Together, the synthesis and metabolic clearance of GABA is referred to as the “GABA shunt” in order to emphasize its dual nature as a metabolic pathway. GABA flux has been quantitatively estimated in anesthetized (1% halothane) rat brain to be ~20% of the glutamate flux (Patel et al., 2005). Notably, this four-way coupling (neurotransmission and metabolism with neuron and astrocyte) describes a metabolic interdependence with neurotransmission, but intrinsically also permits each cell type to coexist relatively independently. This latter state is likely to dominate under conditions of decreased neurotransmission flow, less glucose consumption, and reduced flux between neurons and glia.

Nonetheless, it is evident that in a glucose-fed brain, the GNU is at the center of a system poised for neurotransmission and metabolism. Disturbances in either cell type, although not necessarily inducing acute cell death, are likely to influence parameters, such as metabolic flow, concentrations of steady-state glutamate, glutamine, GABA, and most importantly excitability, obviously a key issue in epilepsy.

Microdialysis studies

Through microdialysis catheter placement together with intracranial EEG electrodes, we have been able to determine the concentrations of neurotransmitters such as glutamate and GABA in the awake epilepsy patient being studied intracranially for medically intractable seizures. Our early work demonstrated that within the epileptogenic hippocampus, there was a large and rapid increase in extracellular glutamate to neurotoxic levels of 64 μ M with seizure onset. The findings here showed that glutamate went up both in the epileptogenic and nonepileptogenic hippocampi, but also a significantly slow clearance of glutamate in the epileptogenic hippocampus (During & Spencer, 1993). In the context of the GNU, this suggested astrocytic dysfunction, since the glial glutamate transporters (EAAT1, EAAT2) are known to be the primary path for synaptic clearance. More recently, we have studied patients who had probes placed in the hippocampus and cortex, finding that the interictal glutamate levels are significantly elevated in the epileptogenic hippocampus compared to the nonepileptogenic hippocampus (Table 1). Glutamate was also elevated in the epileptogenic cortex although not significantly in comparison to the nonepileptogenic cortex. It is likely that the elevated glutamate is contributing to the local decline in glucose consumption (such as that measured by FDG PET), as regression of glutamate compared with glucose demonstrated significant positive correlations (increasing ecGlu associated with increasing ecGlucose) in the hippocampus ($R = +0.66$, $p < 0.03$). This suggests that in the epileptogenic brain, the local extracellular glucose concentrations are not controlled by transport alone, but biased by local glucose consumption (metabolic clearance, which acts to reduce local glucose levels). Finally, this may be a network distributed process, as preliminary glutamate data from regions classified as seizure onset, propagation or uninvolved regions (as determined from consensus evaluation of intracranial EEG) has also suggested that interictal glutamate is also elevated within the propagated pathway of the network (data not shown).

The elevated glutamate also appears to be directly linked to both abnormal in vivo and ex vivo electrophysiology. In Fig. 5A, in vivo hippocampal glutamate measurements from patients with mesial temporal lobe epilepsy are positively correlated with increasing Teager energy ($R = +0.75$, $p < 0.001$), a measure of the high frequency EEG power which by virtue of recent observations on high-frequency oscillations (Staba et al., 2002; Jirsch et al., 2006) may be elevated in epileptogenic tissue. In comparison, ex vivo studies performed on the resected hippocampus have also shown that key measures of hyperexcitability correlate well with extracellular glutamate concentrations. As shown in Fig. 5B several measures of synaptic hyper-excitability are significantly correlated with $[Glu]_{ec}$. These include polysynaptic activity associated with evoked responses and baseline excitatory activity recorded intracellularly in dentate granule cells (Williamson et al., 1995). Both these types of activity are associated with the presence of robust mossy fiber sprouting (de Lanerolle et al., 1997). These data suggest, therefore, that the constellation of anatomic and metabolic changes seen in the MTS hippocampus is associated with an elevated baseline excitability which may prime the tissue for the hypersynchronization that precedes frank seizures involving the entire hippocampus.

The full circle of metabolic abnormalities associated with extracellular glutamate becomes clear when, as the in vivo bioenergetic state couples to ex vivo electrophysiology (Fig. 3), ex vivo and in vivo electrophysiology couples to glutamate (Fig. 5). Thus, we have also observed significant correlations between extracellular glutamate with the bioenergetic state. In this relationship (Fig. 6), lower PCr/ATP is linked with higher concentrations of glutamate as measured in the ipsilateral hippocampus of mTLE patients. Thus, if we view PCr/ATP as a measure of the local energetic state, it is clear that processes that cause energetic perturbation (e.g., changing cerebral demand, fuel availability or stress) may alter the ambient extracellular glutamate concentrations, which in turn contributes towards altered tissue excitability.

Interruptions of metabolic cycling relate to increased excitability

Given the metabolic coupling with neurotransmission as shown in Fig. 4, it is clear that direct interruption of the cycle may have immediate consequences for neurotransmission. This has been specifically tested in the resected rat hippocampus using DON (6-diazo-5-oxo-L-norleucine), an inhibitor of glutaminase (a neuronally dominant enzyme which reconverts glutamate from astrocytically produced glutamine, this would inhibit the glutamate neurotransmission cycle, Newcomb et al., 1997). With DON exposure, a progressive increase in cellular excitability was seen over the course of 2 h in the dentate gyrus (Fig. 7A); this correlated with a significant loss of paired pulse inhibition, implicating loss of GABAergic function. Moreover, following 4 h of incubation in DON, the synaptic response was lost, consistent with the hypothesis that the cycling glutamate is primarily involved in synaptic transmission.

The effect of DON on metabolism was further tested by measuring the total concentrations of glutamate and GABA in hippocampal tissue. As shown in Fig. 7B, there was a progressive loss of both GABA and glutamate content after an hour in DON; it is notable that GABA concentrations fell faster and to a more significant degree than glutamate (Williamson et al, 2004). Moreover, the changes in excitability (shown in Fig. 7A) were seen starting at 60 min exposure, when only 15% of GABA had been depleted. Similar changes were seen in resected human tissue (data not shown). Thus, any interruptions in neurotransmitter metabolism will have complex effects on synaptic network function. These data are consistent with those of Liang et al, 2006 showing that GABAergic neurotransmission is dependent on adequate glutamine transport.

In further support of the hypothesis that interrupting neurotransmitter metabolism is epileptogenic, inhibitors of the neurotransmission cycle have also been demonstrated to result in seizures *in vivo*. For example, Wang et al., 2006 has used chronic exposure to methionine sulfoximine (MSO), which inhibits glutamine synthetase (see Fig. 4), to generate a novel epilepsy model recapitulating much of the neuropathology seen in human epilepsy. Similarly, aminooxyacetic acid, an inhibitor of aspartate-amino transaminase (a key enzyme involved in the recycling of glutamate and the malate aspartate shuttle that transfers redox equivalents from the cytoplasm into the mitochondrion) has also been used to generate seizures (Eid et al., 1999).

The metabolic coupling of neurotransmission has been most directly measured by the *in vivo* turnover data from epilepsy patients undergoing medial temporal lobe resection. In these studies, ^{13}C -2-glucose was infused and high-resolution nuclear magnetic resonance (NMR) analysis of the resected and extracted hippocampus was performed, evaluating the distribution of ^{13}C label in glutamate and glutamine. This work, based on a model of ^{13}C utilization *in vivo* (Petroff et al., 2002), demonstrated that the rate of flux through the glutamatergic neurotransmission cycle in comparison to the overall tricarboxylic acid (TCA) cycle flux ($V_{\text{glu}}/V_{\text{tca}}$) was more than five-fold decreased in patients with hippocampal sclerosis (0.08 ± 0.04 , $n = 12$) as compared to the nonsclerotic hippocampus (0.52 ± 0.22 , $n = 5$, significantly different at $p < 0.05$). As a ratio, this value is relatively independent of direct tissue loss, and instead highlights the observation that the normal GNU flow that links neurotransmission and metabolism (Fig. 4) is highly abnormal in the sclerotic hippocampus.

Summary: Physiological evidence for a link between neurotransmission and metabolic cycling with hyperexcitability

Given the extensive neurometabolic literature that links metabolic cycling with neurotransmission within the GNU, it is not surprising that we and other groups have found close relationships between metabolic dysfunction and hyperexcitability. We have discussed the extensive physiological data arguing that in epilepsy patients, there are substantial ictal and interictal abnormalities in glutamate, these being coupled with *in vivo* and *ex vivo* measures of hyperexcitability and metabolic state. Furthermore, it is clear that perturbation of cycling at several levels (glutaminase, glutamine synthetase, and transamination) results in electrophysiological abnormalities and can be used to generate seizure models. Neurotransmission may be especially sensitive, as there are disproportionately depressed cycling rates in comparison to oxidation rates in patients with hippocampal sclerosis. As more data are accumulated, we anticipate that abnormally elevated glutamate will be linked with more widely used measures of injury, including hippocampal volumetry, pathology, and neuropsychological performance (Cavus et al., 2006, data not shown).

In concert with these glutamate data it should be noted that GABA, similar to glutamate, is also tightly coupled to metabolism. In some ways, GABA may be even more metabolically linked because both its synthesis and clearance directly spring from the TCA cycle. This review focuses primarily on glutamate because of its many linkages we and others have observed with hyperexcitability, not thus far clearly seen with GABA. However, it is clear that GABA metabolism may also be a key contributor to excitability, and is a topic of ongoing study.

Mitochondrial Metabolic Injury, and Seizures are Self-Propagating

Thus there is substantial evidence from animal slice and *in vivo* models that metabolic disturbances (e.g., from mitochondrial injury or as generated from specific inhibitors of the neurotransmission/metabolic cycle), can lead to abnormal excitability. Critically, seizures have also been shown to induce mitochondrial and metabolic abnormalities that may evolve

into a pathologic state. It is not surprising that severe mitochondrial injury (e.g., application of cyanide as a strong oxidizing agent or 3-nitropropionic acid which inhibits TCA cycle flow) can cause seizures (Persson et al., 1985; Haberek et al., 2000). More importantly, however, is the observation that even mild exposure to oxidative stress and mitochondrial injury may cause conditions that suggest hyperexcitability. For example, Saransaari and Oja used a mouse hippocampal slice preparation to demonstrate increased spontaneous glutamate and GABA release in the presence of 0.01% H₂O₂ compared to normal conditions (Table 2 compiled from their paper). Addition of 50 mM K⁺ to induce depolarization resulted in further glutamate release, seen in both the normal and H₂O₂ condition. This results in a much higher total glutamate release in the depolarized and oxidatively stressed state. In contrast, GABA release was comparable to glutamate under normal conditions, also elevating with H₂O₂ exposure; however, GABA release failed to further increase with 50 mM K⁺. Together, it is clear that in conditions of depolarization and oxidative stress, the continuing release of disproportionately greater glutamate may contribute to ictogenesis. The etiology of increased neurotransmitter release in conditions of oxidative stress is not wholly determined. However, the major possibilities are decreased oxidative phosphorylation and ATP generation, and/or abnormal intracellular calcium (Ca_i⁺²) regulation. As is well known, the mitochondrion is an important site of Ca_i⁺² sequestration and contributes to the regulation of vesicular glutamate release.

Another important avenue by which mitochondrial dysfunction may be contributing to seizures and epilepsy may be considered from the work on uncoupling proteins. As has been discussed by Kim-Han and Dugan, 2005, the mitochondrial uncoupling proteins provide an important influence on mitochondrial function, by physiologically separating electron transport from oxidative phosphorylation. These proteins (UCP1–4) effect a proton leak in the inner mitochondrial membrane. This is important since otherwise much free radical generation and consequent oxidative stress could result from excessive electron transport flow without appropriately coupled ATP synthesis. In brain, the expression of uncoupler protein UCP2 in particular has been shown to increase in conditions of acute trauma, ischemia as well as the ketogenic diet (Mattiasson et al., 2003; Sullivan et al., 2004). It is therefore particularly of interest that a transgenic mouse model that over-expresses UCP2 (which is an otherwise healthy mouse without apparent CNS abnormality, equivalent hippocampal volume compared to wild type) demonstrated greater than five-fold less neuronal loss with acute pilocarpine induced seizures in comparison to control mice. This was seen, in spite of the presence of equivalent seizure severity between the two groups (Diano et al., 2003). While this work does not argue that depressed UCP2 is itself epileptogenic, its physiological role may be important. Increased UCP2 expression results in decreased free radical concentrations (Mattiasson et al., 2003) and increased mitochondrial numbers (Wu et al., 1999). These in turn can result in less oxidative stress and potentially improved Ca⁺² regulation (Andrews et al., 2005).

Finally, extensive data has shown that injury from recurrent seizures can in turn induce significant metabolic and mitochondrial injury. We have used a pilocarpine rat model to show that even well before the onset of spontaneous seizures (2 days after status), magnetic resonance measurements of hippocampal NAA show significant declines of NAA/Cr, these changes being less in those rats who did not proceed into stage 4 or 5 seizures. This study was performed in the latent period and demonstrates that mitochondrial injury happens early, recovers somewhat a few days later but does not return to normal levels. These events happen before there is substantial neuronal loss, which typically starts at approximately 10–20 days after status (Turski et al., 1989; Peredery et al., 2000). The injury may be resulting from several sources, including oxidative stress, which has been shown by several groups. Using a kainate model, Bruce and Baudry (1995) demonstrated increased lipid peroxidation and protein injury soon after (8–16 h) prolonged (5–6 h) seizure activity. Liang & Patel

(2006) also used the kainate model to find both increased concentrations of reactive oxygen species and decreased mitochondrial redox state (CoASH/CoASSG, reduced CoA and CoA-disulfide glutathione), the latter seen acutely and chronically (7 days after status). Several studies from human tissue and animal models have shown the presence of severely abnormal respiratory chain function, these data from TLE and the pilocarpine model of epilepsy (Brines et al., 1995; Kudin et al., 2002; Nasseh et al., 2006). Kudin and colleagues (2002) have argued that the reduction of oxidative enzymes is due specifically to loss of mitochondrial DNA, which has been shown to be especially vulnerable to seizures. Finally, the injury may in part be led by calcium accumulation, which has been shown to increase dramatically with status epilepticus (for review, DeLorenzo & Sun, 2006; Holmes, 2002). As is well known, increased Ca_i^{+2} can influence neuronal plasticity, apoptosis as well influence seizure occurrence.

Conclusions

In summary, there is an increasing body of evidence from human and animal studies that finds (1) significant mitochondrial and metabolic injury can induce a hyper-excitabile state and seizures; and (2) that seizures very commonly induce mitochondrial and metabolic injury. We would hypothesize that the elevation in glutamate is a key manifestation of this two step process which points in the direction of a dysfunctional GNU. Like several other possible contributing factors in the pathogenesis of epilepsy, such as aberrant axonal sprouting or GABA dysfunction (among many possibilities), these two steps establish a self-propagating state, which once initiated, cascades from being a physiological response to an initial insult into a pathologic state. In such a view, multiple other factors, such as genetic background, nature of the initial insult, medication effect, etc., can clearly modify the velocity in which the development of chronic epilepsy may occur. Nonetheless, if the GNU and mitochondrial dysfunction with glutamate hyperexcitability is to be established as an independent contributor to the pathogenesis of epilepsy, additional work is needed in order to demonstrate that the data that have supported this two step model are not coincidental or bystander effects, but are fundamentally contributing to chronic epilepsy.

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Figure 1. MRSI statistical map showing the bilateral but dominant left hippocampal abnormalities in NAA/Cr. The data are corrected for tissue content variability and are independent of cerebrospinal fluid (CSF) and parenchymal tissue volume. The color bar indicates level of statistical significance, starting at $p < 0.05$ (red). *Epilepsia* © ILAE

Figure 2.

A limbic network of involved loci is seen in MTLE that heavily links the ipsilateral epileptogenic hippocampus with the ipsilateral anterior thalamus, and therein links with numerous other areas including contralateral limbic structures. For each pair of linked structures is shown its R value and significance. *Epilepsia* © ILAE

Figure 3.

(A) ^{31}P spectra from an epilepsy patient showing the ipsilateral depression of PCr/ATP in the epileptogenic hippocampus. The voxel size of the ^{31}P measurement is ~ 12 cc, for example, from a small sphere with a radius of ~ 1.4 cm. (B) Preoperative PCr/ATP values determined from the ipsilateral mesial temporal lobe correlates significantly with electrophysiology from the resected tissue, that is, recovery of the membrane polarization after a sustained train of suprathreshold stimulation. *Epilepsia* © ILAE

Figure 4. Glutamate (**A**) and GABA (**B**) neurotransmission is directional involving multiple cellular compartments. *Epilepsia* © ILAE

Figure 5.

(A) In vivo measures of extracellular glutamate increases with increasing teager energy, $R = +0.75$, $p < 0.001$. 95% confidence intervals and prediction bands are shown. (B) Ex vivo electrophysiology measures link hyperexcitability with in vivo extracellular glutamate concentrations. *Epilepsia* © ILAE

Figure 6. Relationship between preoperative PCr/ATP and microdialysis measurements of log extracellular Glutamate, $R = -0.03$. 95% confidence intervals and prediction bands are shown. *Epilepsia* © ILAE

Figure 7.

(**A**) Electrophysiology of resected rat hippocampus with exposure to DON, an inhibitor of glutaminase. With progressive time of exposure, increasing hyperexcitability is seen. (**B**). Concentration of GABA and glutamate both fall with exposure to DON, with glutamate falling less rapidly than GABA. Data are from resected rat hippocampi. *Epilepsia* © ILAE

Table 1
Interictal ECF levels of glutamate, glutamine, glucose, and lactate from epilepsy patients (n = 35) (From Cavus et al., 2005)

Probe location	Glutamate	Glutamine	Glucose	Lactate
Hippocampus				
Epileptogenic (n = 14)	12.1 ± 3.4**	601.4 ± 131.3	2.9 ± 0.7	6.8 ± 0.7*
Nonepileptogenic (n = 21)	2.6 ± 0.3	702.7 ± 131.7	2.2 ± 0.2	4.6 ± 0.4
Cortex				
Epileptogenic (n = 8)	4.9 ± 1.2	798.2 ± 181.3	1.4 ± 0.2	5.2 ± 0.8
Nonepileptogenic (n = 18)	2.4 ± 0.3	617.8 ± 81.0	1.6 ± 0.3	4.7 ± 0.5

** Significantly different between epileptogenic and nonepileptogenic groups, p < 0.0001.

* Significantly different between epileptogenic and nonepileptogenic groups, p < 0.05.

Table 2
Neurotransmitter release from mouse hippocampal slices (Adapted from Saransaari & Oja, 1997)

	Normal		H2O2	
	Spontaneous	K+ induced	Spontaneous	K+ induced
Glutamate	0.03	0.2	0.7	1.25
GABA	0.03	0.25	0.25	0.35