Astrocytes and Epilepsy

Nihal C. de Lanerolle,* Tih-Shih Lee,† and Dennis D. Spencer*

**Department of Neurosurgery, Yale School of Medicine, New Haven, Connecticut 06520; †Department of Psychiatry, Duke University, Durham, North Carolina 27708*

Summary: Astrocytes form a significant constituent of seizure foci in the human brain. For a long time it was believed that astrocytes play a significant role in the causation of seizures. With the increase in our understanding of the unique biology of these cells, their precise role in seizure foci is receiving renewed attention. This article reviews the information now available on the role of astrocytes in the hippocampal seizure focus in patients with temporal lobe epilepsy with hippocampal sclerosis. Our intent is to try to integrate the available data. Astrocytes at seizure foci seem to not be a homogeneous population of cells, and in addition to typical glial fibrillary acidic protein, positive reactive astrocytes also include a population of neuron glia-2-like cells The astrocytes in sclerotic hippocampi differ from those in nonsclerotic hippocampi in their membrane physiology, having elevated Na+ channels and reduced inwardly rectifying potassium ion channels, and some having the capacity to generate action potentials. They also have reduced glutamine synthetase and increased glutamate dehydrogenase activity. The molecular interface between the astrocyte and microvasculature is also changed. The astrocytes are also associated with increased expression of many molecules normally concerned with immune and inflammatory functions. A speculative mechanism postulates that neuron glia-2-like cells may be involved in creating a high glutamate environment, whereas the function of more typical reactive astrocytes contribute to maintain high extracellular K+ levels; both factors contributing to the hyperexcitability of subicular neurons to generate epileptiform activity. The functions of the astrocyte vascular interface may be more critical to the processes involved in epileptogenesis. **Key Words:** Astrocytes, temporal lobe epilepsy, hippocampal sclerosis, NG2 cells, seizures.

INTRODUCTION

It is generally recognized that the immediate cause of epilepsy is an over-excitation of populations of neurons in the brain and the spread of such excitation throughout the brain resulting in behavioral seizures. However, for a considerable period, it was believed that astrocytes play a significant role in the causation of seizure activity. Several brain foci associated with seizure generation are populated by increased numbers of astrocytes. Such foci include hippocampal seizure foci in temporal lobe epilepsy (TLE), several types of mass lesions in the brain (low-grade astrocytomas, oligodendrogliomas, arteriovenous malformations) and tubers in tuberous sclerosis.[1] Gliotic scar formation is a prominent feature of human epilepsy.[2,3] The almost invariable presence of gliotic scars in chronic focal epilepsy has led many to suggest a physiological role for glia in the disease.[4-6] Early studies on the role of astrocytes in epilepsy carried out mostly on gliotic scars induced by topical application of chemicals such as alumina or cobalt to the cortex of animal brains are reviewed by Tiffany-Castiglioni and Castiglioni.[7] In conclusion to their review, the authors write that "astrocytes can hypothetically contribute to epileptogenesis by any of three routes: (1) the initiation of neuronal hyperactivity in previously normal neurons, (2) the promotion of epileptic bursting in abnormal neurons, and (3) the failure to neutralize and arrest neuronal hyperactivity." However, they go on to add that "evidence that astrocytes cause seizures is almost completely lacking, except for the finding that glial scars mechanically distort neuronal morphology." In the years since this review by Tiffany-Castiglioni and Castiglioni,[7] our understanding of the biology and physiology of astrocytes has exploded. As a consequence, so has our understanding of the role of astrocytes in epilepsy.

In this article, we review the information available on the probable contribution of astrocytes to human epilepsy, based on the information gathered on hippocampal seizure foci in patients with TLE, which is a naturally

Address correspondence and reprint requests to: Nihal C. de Lanerolle, D.Phil., D.Sc., Department of Neurosurgery FMB 414, Yale School of Medicine, 333 Cedar St, New Haven, CT 06520-8082. E-mail: Nihal.delanerolle@yale.edu.

occurring seizure disorder. TLE seizures originate from the temporal lobe, particularly the hippocampus.[8] The seizure foci are surgically removed for the control of medically intractable seizures and thus provide for a careful study of their biology.

The examination of hippocampal specimens from patients who had undergone surgery for the control of medically intractable epilepsy has revealed approximately 40% to 65% of cases with hippocampal sclerosis.[9] Hippocampal excision surgery in this type of patient has a better surgical outcome than in those without sclerosis. Analysis of the pathology and electrophysiology of 151 hippocampi removed in the Yale surgical series revealed that after surgery, 84% of patients with sclerotic hippocampi had an excellent (Engel class I) outcome.[9] In these sclerotic hippocampi, in addition to a greater than 50% loss of neurons, the glial density is increased by approximately 80% compared to nonsclerotic hippocampi and neurologically normal autopsy controls[10,11] (FIG. 1). These astrocytes exhibit several unusual properties.

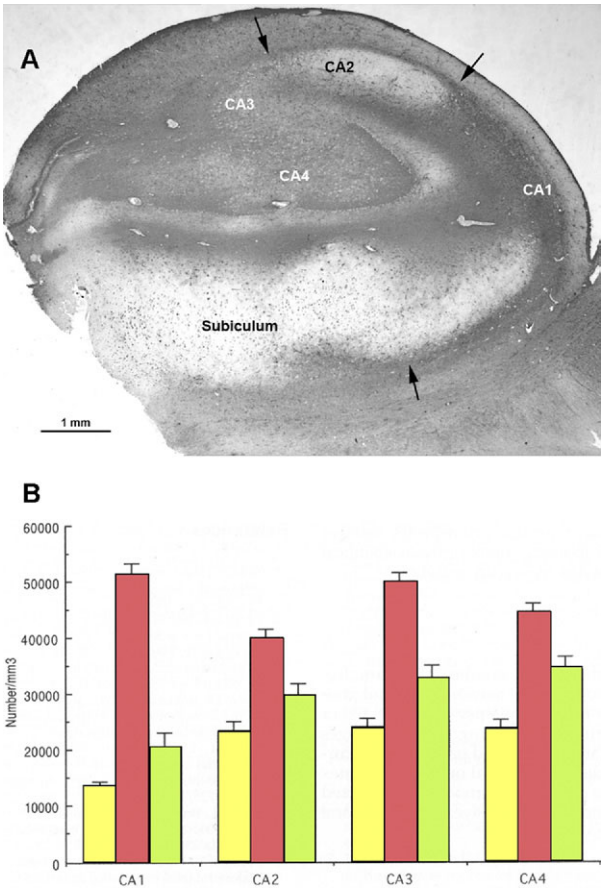

FIG. 1. A: Photomicrograph of a section of sclerotic human hippocampus immunostained for glial fibrillary acidic protein (GFAP). The clear regions (dentate granule cell layer, CA2, and subiculum) are those with little or no neuronal loss and thus weak GFAP immunoreactivity. The hilus, CA3, and CA1 regions have considerable neuronal loss strong GFAP positivity. CA = Cornu Ammonis or Ammon's horn. (Courtesy of Dr. Jung H. Kim, Department of Pathology, Yale University). B: Glial density as expressed by mean glial number per mm^3. Error bar = standard error of the mean. Yellow bar = autopsy control (n = 12); red bar = sclerotic hippocampi (n = 83); green bar = temporal lobe epilepsy with extrahippocampal pathology (nonsclerotic). (Courtesy of Dr. Jung H. Kim, Dept. of Pathology, Yale University.)

ASTROCYTE RECEPTORS

Astrocytes express similar sets of receptors as do neurons, but with varying relative strength of expression. These receptors can be activated by synaptically released neurotransmitters, by "glio" transmitters, or by molecules diffusing in the brain extracellular space.[12] Among the receptors expressed on astrocytes are glutamate receptors, and both ionotropic and metabotropic receptors. Ionotropic glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype made up of subunits GluR1 to GluR4 are found on astrocytes. An elevated flip-to-flop mRNA ratio of the GluR1 splice variant is reported in reactive astrocytes from sclerotic hippocampi, suggesting an increase in responsiveness of these astrocytes to glutamate.[13,14] Immunoelectron microscopic examination of sclerotic hippocampi has revealed the expression of mGluR2/3, mGluR4, and mGluR8 in reactive astrocytes. In the same manner, mGluR3, mGluR5, and mGluR8 are reported to be up-regulated in the hippocampus in experimental animal models of TLE.[15] Activation of these receptors leads to an increase in intracellular $Ca2^+$ and $Ca2^+$ wave propagation, leading to the release of glutamate from astrocytes according to some investigators.[16] However, whether Ca^{2+} waves or oscillations lead to glutamate release remains controversial.[17]

ASTROCYTE TRANSPORTER MOLECULES

On their cell membrane, astrocytes have a variety of transporter molecules through which they exchange a variety of molecules with the extracellular space. In the normal activity of the brain, astrocytes play a major role in the clearance of glutamate released by synapses into the extracellular space. Astrocytes achieve this through the activity of two glutamate transporter molecules excitatory amino acid transporter (EAAT)1 and EAAT2. Increased levels of extracellular glutamate are detected by in vivo dialysis in sclerotic seizure foci.[18,19] Some authors have reported a down regulation of EAAT1 and EAAT2 glutamate transporters[20,21] in sclerotic hippocampi and propose that this accounts for the observed increase in extracellular glutamate. However, others have been unable to confirm such an observation.[22] A careful review of these discrepant data lead to the conclusion that astrocyte glutamate transporter differences in sclerotic versus nonsclerotic hippocampi are an inad-

equate explanation for high extracellular glutamate levels observed by *in vivo* dialysis in sclerotic seizure foci.[23]

The γ-amino butyric acid (GABA) transporter γ-aminobutyric acid transporter (GAT)3 is usually only weakly expressed, if at all, on astrocytes. The expression of GAT3 on astrocytes in the sclerotic hippocampus is increased.[24] GAT3 expression is confined to cells resembling protoplasmic astrocytes, which are located in regions of relative neuronal sparing, such as the dentate gyrus and hilus of the sclerotic hippocampus.[24] In vivo microdialysis studies in human hippocampal seizure foci have demonstrated reduced levels of extracellular GABA in the epileptogenic seizure focus during the ictal state.[18] The increased expression of GAT3 in these hippocampi may contribute to excess removal of GABA and thus reduced extracellular levels in the ictal state.

Aquaporin 4 (AQP4) is a water transporter molecule that is found on astrocytes in the hippocampus. The distribution of these transporter molecules shows a distinct polarity on the astrocyte membrane being more densely expressed on the perivascular astrocytic end feet than on the membrane facing the neuropil (FIG. 2). In sclerotic hippocampi the expression of AQP4 on the perivascular membrane of the astrocyte is reduced, whereas its expression remains unchanged on the membrane facing the neuropil, thus suggesting a probable decrease in the expulsion of water from the neuropil out to the blood vessel lumen.[25]

MEMBRANE ION CHANNELS

Several studies involving patch and voltage-clamp techniques demonstrate the presence of voltage-dependent Na^+, K^+, and Ca^{2+} ion and anion channels on astrocytes.[26-31] Comparative patch-clamp studies were carried out on astrocytes in hippocampal specimens with and without significant sclerosis removed in epilepsy surgery. In a study from our laboratory, primary cultures of astrocytes established from the hippocampus (dentate gyrus and Ammon's horn) and parahippocampus (entorhinal region) of sclerotic hippocampi displayed much larger tetrodotoxin (TTX)-sensitive Na^+ currents with ~66-fold higher Na+ channel density than in astrocytes from nonsclerotic hippocampi and comparison temporal neocortex of the same patient[32] (FIG. 3). Two other studies have confirmed enhanced Na^+ current densities in human astrocytes *in situ* in sclerotic hippocampi.[33,34] A third study found no increase.[35]

The expression of voltage-dependent calcium channel a_1 subunits was examined by immunohistochemistry in sclerotic hippocampi from patients with TLE and compared to their expression in autopsy controls.[36] A signif-

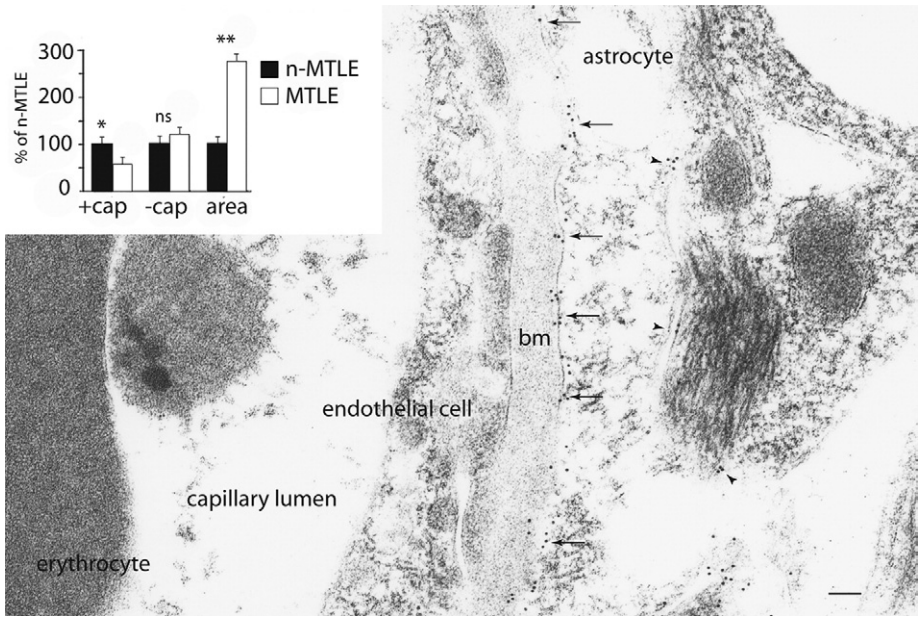

FIG. 2. Quantitative ImmunoGold electron microscopy reveals significant loss of AQP4 from the perivascular astrocyte membrane in mesial temporal lobe epilepsy (MTLE) *versus* non-MTLE hippocampi. ImmunoGold electron microscopy of the endothelial-astrocyte interface in CA1 of a representative non-MTLE hippocampus demonstrates that AQP4 (arrows) is enriched on the astrocyte membrane facing the endothelial cell. Considerably less labeling is present on the astrocyte membrane facing the neuropil (arrowheads). Inset: Quantitation of gold particle densities in random fields from area CA1 of six non-MTLE (n-MTLE) and six MTLE hippocampi. Gold particle counts for MTLE are given as percent of non-MTLE ± standard error of the mean: astrocyte membranes facing the endothelial cell (+cap; particles per μm), 56 ± 16% (*p = 0.01); astrocyte membranes facing the neuropil (-cap; particles per μm), no change. The number of gold particles per unit area of neuropil (particles per μm^2) was 273 ± 23% (**p = 0.013). A two-tailed Mann-Whitney U test was used for statistical analysis. bm = basal lamina; ns = not significant. (Scale bar, 100 nm.) (From T. Eid et al. [2005] PNAS 102: p. 1196).

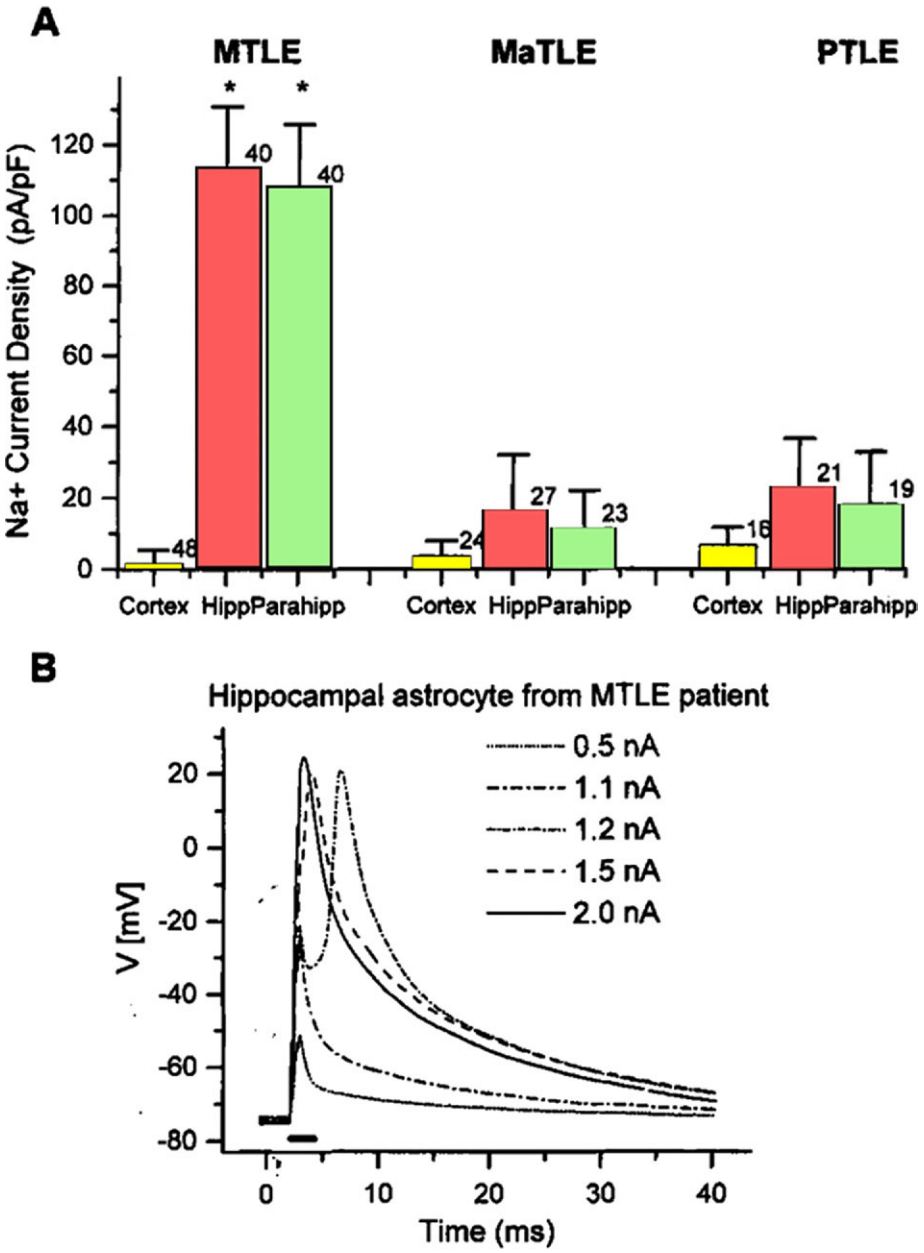

FIG. 3. Na^+ channel densities and action potential (AP)-like responses in human astrocyte cultures. A: Peak currents (at −40 to −20 mV) were divided by membrane capacitance, and mean values plotted. n = number of cells recorded. *$p < 0.01$ as evaluated by student's *t* test. B: AP-like response to current injection in a hippocampal astrocyte from a patient with mesial temporal lobe epilepsy (MTLE). Current injections of increasing value (0.6–2.0 nA) were applied to a cell with a resting potential of −52 mV (holding potential was −75 mV), and the change in membrane potential was recorded. Bar indicates current injection (2 ms). (From E. R. O'Connor et al. Epilepsia 1998;39:351). MaTLE = mass associated temporal lobe epilepsy; PTLE = paradoxical temporal lobe epilepsy.

icant finding of this study is the strong up-regulation of the a_{1C} subunit on astrocytes throughout the sclerotic hippocampus compared to controls in which only occasional white matter astrocytes were stained. The a_{1C} subunit contributes to the L-type calcium currents, suggesting the astrocytes in sclerotic hippocampi have a significant change in their current characteristics, although the functional significance of this change is unclear. It may suggest an enhanced astrocytic uptake of Ca^{2+}.

Astrocytes play a major role in K^+ homeostasis in the brain. They help move K^+ away from regions of high concentration to restore normal extracellular levels. During seizure activity, the $[K^+]_o$ significantly increases. The inwardly rectifying potassium ion (Kir) channels on astrocytes play a major role in the removal of K^+ from the extracellular space. Impaired K^+ buffering in astrocytes in sclerotic hippocampi from patients with TLE was first detected in studies of the effects of barium (a blocker of Kir channels) on stimulus induced changes in

$[K^+]_o$ in the dentate gyrus and area CA1 using potassium selective microelectrodes. Ba^{2+} augmented rises in $[K^+]_o$ in the dentate gyrus, but did not do so in the sclerotic CA1 region[37] suggesting defective Kir channels in the sclerotic region. Functional properties of astrocytes in acute hippocampal brain slices from patients with and without hippocampal sclerosis who were studied with the patch-clamp technique provided more direct evidence of defective Kir channels.[34,35,38] Hyperpolarizing voltages elicited inward rectifier currents that inactivated at membrane potentials −130 mV in astrocytes from nonsclerotic hippocampi, which were larger than those in the sclerotic tissue. Also the ratio of inward to outward K^+ conductances showed that they were significantly smaller in astrocytes from the sclerotic hippocampi compared to those from the nonsclerotic ones.[35] The Kir4.1 subunit is involved in this defect.[38]

ASTROCYTES SPECIFIC ENZYMES

Astrocytes play an important role in the metabolism of the brain through their unique degradation of both glucose and glutamate (reviewed[39]). The importance of the astrocyte in these processes lies in their possession of some key enzymes not normally found in neurons.

In the sclerotic hippocampus, there is a marked loss of glutamine synthetase immunoreactivity. This loss is particularly in prominent regions of major neuronal loss and increased glial density, such as CA1 and CA3. Western blot analysis confirmed these immunohistochemical findings, whereas *in vitro* enzymology confirmed that glutamine synthetase activity was reduced as well.[40,41] Glutamine synthetase catalyzes the conversion of glutamate to glutamine in astrocytes in a process that uses ammonia. The addition of ammonia, as ammonium chloride to *in vitro* slices of the sclerotic hippocampus only minimally increased glutamine labeling, but significantly increased labeling in slices of (normal) temporal neocortex from the same patients.[42] Thus, astrocytes in the sclerotic hippocampus seem to have a reduced capacity for glutamine synthesis and ammonia detoxification. Indeed, extracellular glutamine levels[43] and cellular glutamine levels[10,44] are reported reduced in the epileptogenic (sclerotic) hippocampus.

Malthankar-Phatak et al.[45] have also demonstrated that glutamate dehydrogenase (GDH) activity is increased while aspartate amino transferase activity is reduced in the sclerotic hippocampus when activity levels were normalized to the cortical levels.[45] Although neuron specific isoforms are known, GDH is expressed primarily in astrocytes. GDH catalyzes the conversion of glutamate to a-ketoglutarate and in the process produces ammonia. Nicotinamide adenine dinucleotide (NAD+) is a cofactor for this reaction. GDH can also synthesize glutamate, thereby removing toxic ammonia at the expense of α-ketoglutarate from the tricarboxylic acid cycle, which could be replaced by anaplerosis in astrocytes.[46] Astrocytes can use glutamate as a substrate for energy metabolism when extracellular glutamate levels increase. Aspartate amino transferase (also called aspartate transaminase) facilitates the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate and vice versa. The functional significance of its reduced levels in the sclerotic hippocampus is unclear.

Lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of nicotinamide adenine dinucleotide dehydrogenase (NADH) and NAD+. Lactate dehydrogenase activity levels, normalized to citrate synthase activity levels, are decreased in the sclerotic hippocampus.[45] However, interictal extracellular levels of lactate are elevated in the epileptogenic (sclerotic) hippocampus. Extracellular lactate levels measured by *in vivo* dialysis rise rapidly and clear slowly in the epileptogenic hippocampus during spontaneous seizures.[47,48] Due to the predominance of astrocytes rather than neurons in the sclerotic hippocampus, they may be the source of such increased lactate, even though LDH levels are reduced. Two distinct subunits, LDH1 and LDH5, combine to form the isoenzyme types of LDH. Of these LDH5 is found exclusively in astrocytes.[49] Whether these subunits are altered in the sclerotic hippocampus is not yet known. The changes in pyruvate carboxylase and cytosolic malic enzyme (two other enzymes exclusive to astrocytes in the sclerotic hippocampus) have not been investigated yet.

ASTROCYTES GENE EXPRESSION

Gene expression analysis studies have revealed the increased expression of several genes that may be associated with astrocytes in the sclerotic hippocampus.[50] These included the characteristic astrocytic marker glial fibrillary acidic protein (GFAP) as observed in other expression studies as well,[51,52] along with vimentin desmoyokin (AHNAK), CD44 antigen (CD44), crystallin alpha B, calpain 2, (m/II) large subunit (CAPN2), chondroitin sulfate proteoglycan 2 (versican), paladin (KIAA0992), moesin, presenilin 1, plectin 1, radixin (RDX), calcyclin (S100A6), neural (S100B), tenascin C (TNC), titin, villin, and AQP4. The up-regulation of astrocyte-related genes are consistent with the increased gliosis of the sclerotic hippocampus.[50] Some of the previously mentioned genes are involved in changing the morphology of the astrocytes.

Another group of genes whose up-regulation is interesting are those involved with immune and inflammatory responses.[50] These include those regulating chemokines and their receptors (chemokine C-C motif ligand 2 [CCL2], CCL3, CCL4, chemokine C-X-C motif receptor 4 [CXCR4]), cytokines, and their receptors (fibroblast

growth factor-1) (FGF1), FGF2, FGF3, tumor necrosis factor ligand superfamily member 7 (TNFSF7), signal transduction protein (calmodulin-1 [CALM1], CALM3, protein phosphatase 3, catalytic subunit alpha isoform (calcineurin A alpha), PPP3CA, PPP3R1, protein tyrosine phosphatase receptor type D (PTPRD), PTPRG, PTPRN, PTPRO, transcription factors (FK506 binding protein 1B, 12.6 kDa-FKBP1B, FKPB1A), transcription factor-related genes (AGT, COL1A1, COL21A1, NCAM1, VCAM1, CD44, IL11RA, IL13RA, IL15), complement (C1QB, C3, C4), and class II major histocompatibility complex antigen genes (HLA-DPA1, HLA-DQA1, HLA-DRB1, and HLA-DRB3). Astrocytes are shown to contribute to the inflammatory response of the central nervous system. *In vivo* and *in vitro* studies have shown that astrocytes can produce a range of immunologically relevant molecules, including class II major histocompatibility complex antigens, many cytokines, and chemokines, such as those seen in the sclerotic hippocampus.[53,54] Immunohistochemical analysis of sclerotic hippocampi from TLE patients has revealed increased expression of the NFkB-p65 subunit in astrocytes.[55] Several genes regulated through the NFkB pathway are among those up-regulated in the sclerotic hippocampus (S100B, ezrin/radixin/moesin, the chemokines [CCL2, CCL3, CCL4, CXCL1, and the chemokine receptor CXCR4]).

Immunohistochemical localization of interleukin (IL)-1β and IL-1R in hippocampi of TLE patients with hippocampal sclerosis reveals increases IL-1β and IL-1R immunoreactivity in astrocytes in areas of prominent gliosis and neuronal loss (CA1–CA3 and hilus) with expression in perivascular end feet.[56] No such immunoreactivity is observed in nonsclerotic hippocampi. Immunohistochemical localization of complement C1q, C3c, C3d, and C5b–C9 in sclerotic and nonsclerotic hippocampi from TLE patients show no expression of complement component proteins in nonsclerotic hippocampi. However, in the sclerotic hippocampi immunoreactivity for complement component proteins was increased in regions of neuronal loss (CA1–CA3 and hilus). Strong C1q, C3c, and C3d immunoreactivity is observed in vimentin positive astrocyte-like cells. Astrocytes are not stained for C5–C9 protein components.[57] These immunohistochemical observations have been confirmed by quantitative polymerase chain reaction.[57]

ASTROCYTES AND VASCULAR CHANGES

Astrocytes in the normal brain have a close association with the microvasculature. Astrocyte end feet wrap around the blood-brain barrier formed by the tight coupling of endothelial cells. Astrocyte end feet ensheathing blood vessels release signals that support the formation and maintenance of tight junctions between endothelial cells, as well as the expression of endothelial transport molecules. They also play a role in the movement of water and other molecules between the blood and brain parenchyma.[58] It was reported more than 100 years ago that there is a proliferation of the microvasculature in the sclerotic hippocampus,[59] an observation that has been confirmed since then.[60,61] More recently, it has been reported that the blood-brain barrier may become leaky during seizures, resulting in the passage of substances from the blood to the brain. Immunohistochemical localization of albumin in resected hippocampi from TLE patients revealed strong albumin immunoreactivity in the parenchyma throughout the hippocampus next to blood vessels. Neurons and astrocytes located around the vessels were also albumin positive. Such extravasations of albumin are not observed in hippocampi of autopsy controls.[62] Studies on experimentally induced status epilepticus in animals confirm that the vasculature became permeable shortly after SE and continued into the latent and chronic epileptic phase.[62] Albumin released into the brain through vascular permeability is reported in animal studies to be taken up by astrocytes through transforming growth factor-b receptors (TGF-bR), and the transcriptional activation of downstream pathways[63] resulting in the down regulation of inward rectifying potassium (Kir 4.1) channels in astrocytes, astrocytic activation, increased inflammation, and reduced inhibitory transmission.[64]

Several changes in molecular expression have been reported in the blood-brain barrier, the astrocyte endothelial cell interface. The erythropoietin receptor (EPO-r) is strongly expressed on the capillaries of the sclerotic hippocampus,[60] particularly in regions of extensive neuronal loss and gliosis (CA3, CA1, and dentate hilus). High resolution immunogold electron microscopy revealed that the capillary EPO-r was localized to the luminal and abluminal plasma membranes of endothelial cells to endosome-like structures of these cells and to pericapillary astrocytic end feet.[60] The enrichment of EPO-r in these locations suggests a highly efficient uptake of plasma EPO into the hippocampus. Such expression of EPO-r is not found in the nonsclerotic hippocampus.

The multidrug resistance gene-1 (MDR1) encodes for P-glycoprotein, an energy-dependent efflux pump that exports planar hydrophobic molecules from the cell. A $>$ 10-fold ratio of multiple drug resistance gene-1 mRNA is reported in 9 of 14 medically intractable TLE patients. Immunohistochemistry for P-glycoprotein shows increased staining in the capillary endothelial cells in hippocampi in all TLE patients with high mRNA ratios and in comparison to normal autopsy controls and in astrocytes of 8 of 9 patients with high mRNA ratios.[65]

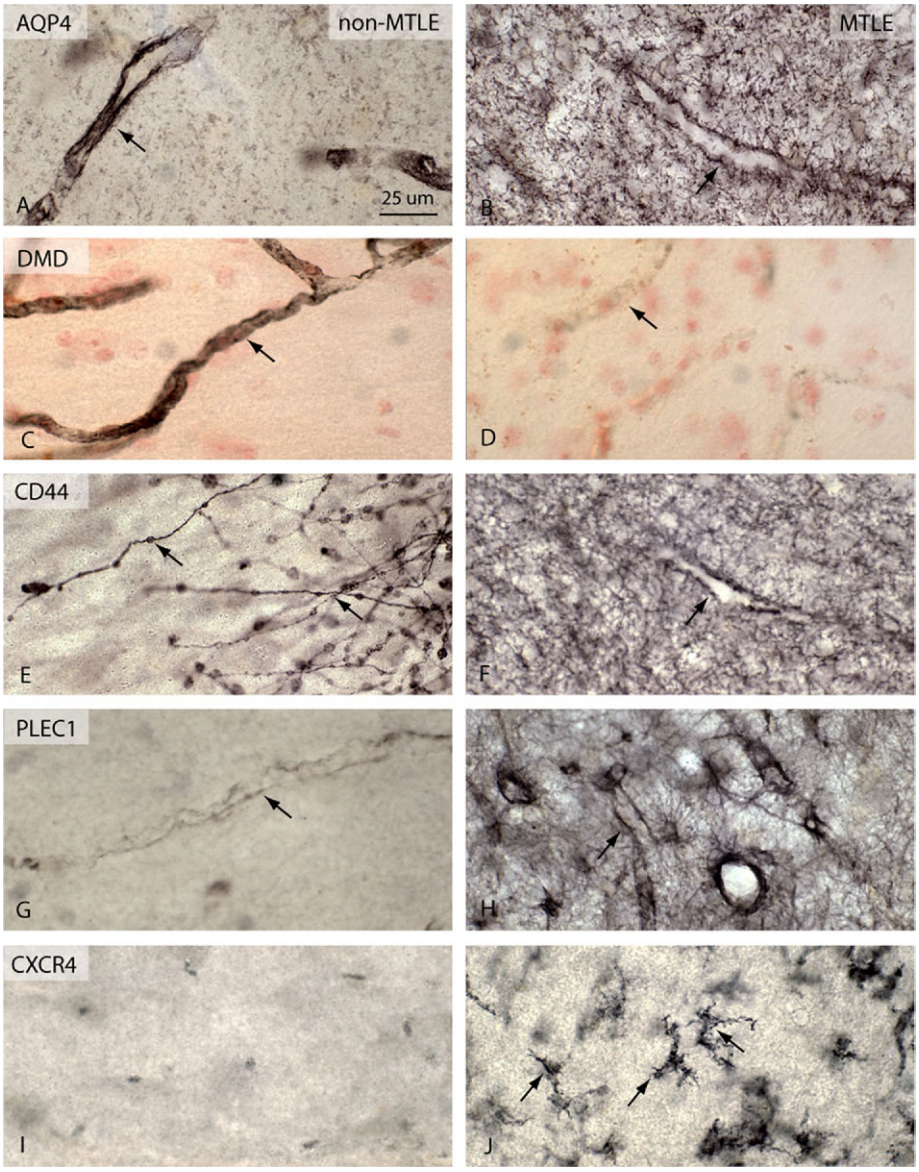

FIG. 4. Photomicrographs of the localization in area CA1 pyramidal layer of non-sclerotic (non-mesial temporal lobe epilepsy [non-MTLE]) (A, C, E, G, and I) and sclerotic (MTLE) (B, D, F, H, and J) hippocampi of some proteins whose genes showed changes in expression in this study. A, B: Immunolocalization of Aquaporin 4 (AQP4). Note the reduction in immunoreactivity in astrocytic foot processes around blood vessels in MTLE and increase in astrocytic processes throughout the pyramidal layer. C, D: Reduction in Dystrophin (DMD) around blood vessels in MTLE. E, F: Localization of CD44 antigen. In the nonsclerotic hippocampus (E), immunoreactivity is seen on fiber-like processes (arrow) extending into the pyramidal layer from the stratum oriens. Scattered among these fibers are CD44 positive astrocyte cell bodies (not shown). In MTLE, many more astrocytes are immunoreactive and form a dense network throughout the region with more intense staining in perivascular foot processes (arrow in F). G, H: Plectin 1 (pectin 1, intermediate filament binding protein 500 kDa) immunoreactivity, which is increased in expression in astrocyte cell bodies and perivascular foot processes (arrow). I, J: Increase in CXCR4 (Chemokine [C-X-C motif] receptor 4) expression on microglia in the sclerotic hippocampus (arrow). CXCR4 is increased on a small proportion of astrocytes as well. (From T.S. Lee et al. Mol Med 2007;13:10.)

Several other molecules located on the perivascular astrocyte end feet also show changes in the sclerotic hippocampus; AQP4 is reduced along with dystrophin, whereas CD44 and plectin 1 expression are increased[50] (FIG. 4).

In addition, microarray analysis demonstrates that molecules such as CCL2 and CCL3 are up-regulated in the sclerotic hippocampus. Brain microvessels from human hippocampi removed in epilepsy surgery express the chemokine receptors CCR1 and CCR2 on the parenchymal surface of the microvessels.[66] These receptor sites are also found on human astrocytes.[67] The ligands for these receptors CCL3 and CCL2 are up-regulated in the sclerotic hippocampus.[50] The binding of these chemokines is modulated

in reponse to astrocyte stimulation by pro-inflammatory cytokines IL-1b and tumor necrosis factor-α, indicating that these binding sites are subject to regulation.[67]

ASTROCYTE PHYSIOLOGY

Membrane physiology

Physiological studies of astrocytes from hippocampi surgically removed from patients with medically intractable epilepsy reveal several altered functional properties. Primary astrocyte cultures were established from the hippocampus (dentate gyrus and CA1–CA3), parahippocampus (entorhinal cortex) and temporal neocortex of each patient operated for intractable TLE. One group of patients had hippocampal sclerosis (MTLE) whereas two others (mass associated temporal lobe epilepsy and paradoxical temporal lobe epilepsy) had no sclerosis.[32] A large proportion (~60%) of astrocytes in primary cultures derived from sclerotic epileptogenic hippocampal seizure foci are capable of generating action potential-like responses after depolarizing currents in comparison to astrocytes from nonsclerotic foci and neocortex from which such action potential-like responses could not be elicited[32] (FIG. 3). Such responses in these astrocytes from sclerotic foci may be facilitated by their significantly depolarized resting membrane potentials (approximately −55 mV) compared to astrocytes from nonsclerotic foci (approximately −75 to −80 mV) and higher tetrodotoxin sensitive Na+ channel density. Whole cell patch clamp recordings from acute slices taken from similar patient tissue confirm the findings on cultured cells.[34]

Calcium release

LEE et al[68] carried out Ca^{2+} imaging using time-lapsed confocal scanning laser microscopy in primary astrocyte cultures from hippocampus, parahippocampus, and temporal neocortex of patients with both sclerotic and nonsclerotic hippocampi, before and after the application of glutamate. These studies showed that in astrocytes from the hippocampal region of nonsclerotic hippocampi and temporal neocortex of both groups, exposure to glutamate resulted in an initial peak of intracellular Ca^{2+}, with subsequent small oscillations and elevations of Ca^{2+} representing intracellular and intercellular Ca^{2+} waves.[68] Astrocytes from hippocampus and parahippocampus from patients with hippocampal sclerosis responded to glutamate with a strongly elevated intracellular Ca^{2+} response, which included an initial peak, followed by an increase in the number of intracellular Ca^{2+} oscillations. These oscillations continue even after glutamate is removed with superfusion of saline. Often spontaneous calcium spikes were seen in parahippocampal astrocytes prior to glutamate. In general, these responses were stronger in parahippocampal compared to hippocampal astrocytes, with both being stronger than cortical astrocytes. There were larger numbers of intercellular Ca^{2+} waves in the parahippocampal astrocytes cultures compared to hippocampal and cortical astrocytes.[68] The same investigators carried out time lapse imaging of fluorescence recovery after laser bleach on astrocytes loaded with 6-carboxyfluorescein diacetate succinimidyl ester, to assess functional gap junctional coupling in the cultures. Gap junction coupling was more pronounced in the parahippocampal (entorhinal cortex) cultures from sclerotic patients, having a faster and more complete fluorescence recovery after bleaching than astrocytes from the less excitable temporal neocortex from the same patient.[68]

Glutamate glutamine cycling

In the normally functioning brain, glutamate released by neurons into the extracellular space is cleared by uptake into astrocytes and converted to glutamine. This glutamine is released by the astrocyte into the extracellular space from where it is taken up by neurons and used to synthesize glutamate. This process is known as the glutamate-glutamine cycle.[69] The glutamate-glutamine cycle during the interictal period is significantly reduced in the sclerotic hippocampus, whereas it is normal in the nonsclerotic hippocampus.[70] *In vivo* dialysis studies show that glutamate release during a seizure is increased and its clearance prolonged, in the epileptogenic (sclerotic) hippocampus compared to the contralateral nonsclerotic one.[18] It was subsequently shown that interictal extracellular glutamate levels are also higher in the epileptogenic hippocampus compared to nonepileptogenic ones.[43] The increased interictal basal extracellular glutamate levels negatively correlate with hippocampal volume being significantly increased in the atrophic[43] hippocampus compared to nonatrophic ones.[71] The hippocampal volume negatively correlates with seizure frequency.[71] Magnetic resonance spectroscopic measurements show that cellular glutamate levels are also lower in the atrophic (sclerotic) hippocampus compared to the nonatrophic one.[72] However, tissue glutamate levels measured by proton magnetic resonance spectroscopy of perchloric acid tissue extracts show that the cellular glutamate content is increased above normal levels in the epileptogenic human hippocampus.[44] In this study, elevated glutamate content is found in hippocampi from TLE patients with normal clinical MRI appearance, and even higher levels in almost half the sclerotic cases with greatest neuron loss.[44] This suggests that in both groups in addition to neurons astrocytes may contain glutamate, and the release of this glutamate during a seizure contributes to the elevated extracellular glutamate levels measured.[18,43]

ASTROCYTE TYPES

Astrocytes are star-shaped cells that are normally characterized by the presence of GFAP. Three types of astrocytes have been recognized based on their morphological features (i.e., radial astrocytes, fibrous astrocytes, and protoplasmic astrocytes).[73] More recently, the coexistence of distinct types of cells with the astrocyte specific marker GFAP, but with diverse morphological, molecular, and functional profiles has been identified in the hippocampus. These were first identified in transgenic mice with GFAP-promoter controlled, enhanced green fluorescent protein expression combined with patch clamp recordings and single cell reverse transcription studies.[74] One group of cells was weekly fluorescent with short thin processes. Outward K+ currents dominate these cells, whereas the inward K^+ currents (I_{Kir}) were much less pronounced. Their resting potentials (Vr) were -31 ± 7 mV. Most of these cells have TTX-sensitive Na+ currents after depolarization beyond -50 mV, but no action potentials were produced in the current clamp mode. The second group of cells were more intensely fluorescent and resembled protoplasmic astrocytes having irregularly shaped somata bearing expanded branched nets of processes. They have a more negative resting potential (Vr = -70 mV). The inward potassium currents (I_{Kir}) were present in these cells.[74] The weakly fluorescent cells express AMPA-type glutamate receptors (GluRs), but not glutamate transporter currents, whereas the brightly fluorescent cells express glutamate transporters (GluT) but lack GluR currents.[74] These two types of enhanced green fluorescent protein-positive cells can be recognized also in acute slices from the hippocampus. These two types of astrocytes are nonoverlapping populations. The GluR cells completely lack gap junction coupling, whereas the GluT cells are extensively coupled.[75] Electron microscopic studies have identified synapse-like structures onto GluR cells and receive spontaneous synaptic inputs from GABAergic and glutamatergic neurons in acute hippocampal slice preparations.[76]

The GluR cell described previously also closely resembles a cell type identified as a third class of macroglia and variously named as neuron glia-2 (NG2) cells, oligodendrocyte precursor cells, polydendrocytes, synantocyte, or complex cells.[77] This star-shaped glial cell is characterized by the expression of the protein chondroitin sulphate proteoglycan, and does not express GFAP and does not have any glutamate transporters or gap junctions.[77] As with the GluR astrocytes, NG2 cells express AMPA glutamate receptors, and additionally express GABA receptors and receive synaptic terminals. The membrane properties of GluR cells and NG2 cells are indistinguishable.[77] Immunostaining of GluR cells show the presence of chondroitin sulfate proteoglycan (CSPG)-NG2 protein.[78] Most recently, Káradóttir et al.[79] have identified two classes of NG2 cells in the CNS white matter of the rat. One class of cells expressed voltage gated Na+ currents, which are reversibly blocked by TTX, along with voltage gated K^+ currents, whereas the other class of cells did not express I_{Na} currents. However, morphologically the two cell types are similar.[79] In those NG2 cells expressing voltage gated Na+ channels, current clamp recordings injecting depolarizing currents evoked action potentials, which were reversibly blocked by TTX. In a small number of cells spontaneous action potentials are observed.[79] Many cells expressing Na^+ channels receive synaptic inputs.[79]

In addition to Na^+ and K^+ channels, NG2 cells express voltage gated Ca^{2+} channels.[80] Neuronal activity can depolarize NG2 cells allowing influx of Ca^{2+} into the cell, with changes in intracellular Ca^{2+}, which can lead to changes in gene expression and glutamate release.

In the sclerotic hippocampus of TLE patients, several lines of evidence suggest the presence and accumulation of NG2 cells. cDNA microarray studies show elevated CSPG expression in area CA1 of the sclerotic hippocampus.[50] In primary cultures derived from sclerotic hippocampi, we observed two morphological types of cells. One cell type was strongly immunopositive for GFAP and possessed long fibrous processes, whereas the other cell type was only weakly positive for GFAP and was flatter and did not have fibrous processes (FIG. 5).

In Ca^{2+} imaging studies with FURA 2, it was the flat cells that showed increases and oscillations in intracellular Ca^{2+} levels, which dramatically increase on application of glutamate, especially in hippocampal and parahippocampal cultures[50] (and unpublished observations). Our electrophysiological studies revealed that these astrocytes such as NG2 cells had significantly more depo-

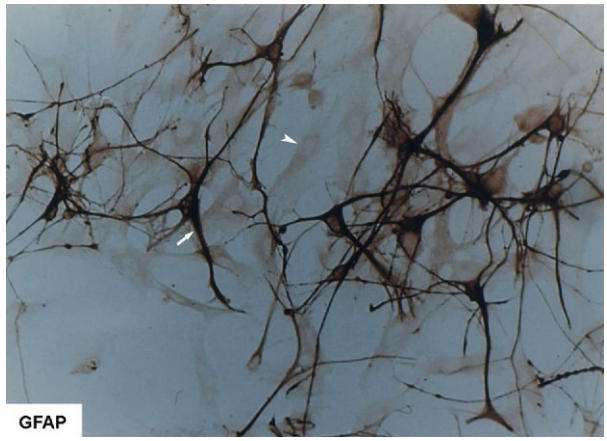

FIG. 5. A photomicrograph of a primary culture of astrocytes, made from a sclerotic hippocampus, immunostained for glial fibrillary acidic protein (GFAP). The strongly GFAP positive fibrous astrocytes (arrow) probably correspond to GluT type cells. The weekly stained cell (arrowhead) probably corresponds to the GluR type cells. These are the cells that show strong oscillations in intracellular Ca2+, spontaneously and increasing with the application of glutamate.

larized resting membrane potential (−57 ± 8.5 mV; range, −37 to −70 mV), increased Na^+ channel densities, and expressed large inward Na+ currents, which were blocked by the application of TTX.[32] Action potential like responses was obtained in approximately 60% of hippocampal and parahippocampal astrocytes from sclerotic hippocampi when depolarized by a series of current steps of increasing amplitude.[32] These observations strongly suggest the presence of NG2/GluR cells in the sclerotic hippocampus. Seifert et al.[14] provide more direct evidence. They found that cells similar to GluR and GluT are also present in the human hippocampus. However, although both types are found in the nonsclerotic hippocampi from TLE patients, they report an almost complete loss of GluT cells in the CA1 region of the sclerotic hippocampus.[35] Furthermore, it was the GluR cells in the sclerotic tissue but not in the nonsclerotic hippocampus that had increased levels of the Flip isoform of GluR1.[14]

WHAT ROLE DO ASTROCYTES PLAY IN THE HIPPOCAMPAL SEIZURE FOCUS?

Based on the information reviewed previously, astrocytes in the sclerotic hippocampus could influence seizure generation through several means. To place in perspective on how some of these changes in astrocytes in a sclerotic hippocampus may contribute to establishing an epileptic focus, it is helpful to understand the organization of the hippocampus and its unique pathology in TLE. The principal source of inputs to the hippocampus is from the entorhinal cortex, which is a part of the parahippocampal gyrus. Efferents from the entorhinal cortex project into the hippocampus as the perforant path and synapse on the apical dendrites of the dentate granule cells, with some collaterals to area CA1 via a temporo-amonic path. The axons of the granule cells (mossy fiber axons) extend to synapse on CA3 neurons with collaterals to hilar neurons, most prominently the mossy cells. Neurons in area CA3 project collaterals of their axons, known as the Schaffer collaterals, to area CA1 and synapse on neurons there. The axons from CA1 neurons in turn project to the subiculum, with the subiculum being the major source of efferents from the hippocampus to other regions of the brain. The "tri synaptic pathway" is a well understood system of projections through the hippocampus, but there are other less well understood anatomical interconnections between the hippocampal regions.[81] In the sclerotic hippocampus significant neuronal loss is reported in both the dentate gyrus and hippocampus proper (Ammon's horn).[9,82] Area CA1 neurons are the most susceptible to injury and was so noted more than a decade ago by Sommer.[83] The region is filled with reactive astrocytes, resulting in the hardening or sclerotic nature of the hippocampus. Recent studies report a series of reorganizational changes in the dentate gyrus that result in hyperexcitable dentate granular neurons.[8] The subicular region of the sclerotic hippocampus is remarkable in that it does not show significant neuronal loss,[84,85] although more subtle changes in receptor expression and synaptic inputs may occur. This is quite different from other neurological disorders involving the hippocampus, such as Alzheimer's disease, schizophrenia, and so forth, where the subiculum shows significant neuronal injury. The entorhinal cortex is also reported to have neuronal loss with some gliosis, especially in layers three and to a lesser extent in layer two.[86] Studies with depth electrodes in the hippocampus of TLE patients show that seizure activity originates from such hippocampi, and especially from the most sclerotic regions within them.[87] How does this happen? How does the hyperexcitability of the dentate granule cells[88] spread to the subiculum, through the neuron-depleted area CA1? What triggers the injury of the hippocampus? The altered function of astrocytes may provide answers to such questions.

Astrocytes may contribute to the high glutamate levels at the seizure focus

Several lines of information point to this conclusion. The down regulation of the enzyme glutamine synthetase activity in the astrocytes, may account for the increase the extracellular glutamate levels.[41] In addition, increase in GDH may lead to increase in cellular glutamate observed.[46] Evidence of intracellular Ca^{2+} release and Ca^{2+} oscillations in NG2-like cells in the sclerotic focus, as argued for previously, along with evidence of the up-regulation of synaptosome associated protein 23 expression gathered from microarray analysis studies[50] suggest the possibility that in the sclerotic hippocampus are cells that may be capable of Ca^{2+}-dependent exocytotic release of glutamate.[89] The intracellular Ca^{2+} release pathway may be activated by the transcription factor NFkB, which is increased in the sclerotic hippocampus.[50,55] Experimental demonstration of the activation of the NFkB activated cycloxygenase-2/prostoglandin 2 mediated Ca^{2+} release pathway in the human sclerotic hippocampus is still lacking. Another mechanism by which intracellular glutamate may be released is due to astrocyte swelling.[90] Alterations in the distribution of AQP4 on astrocytes in sclerotic hippocampi may result in accumulation of metabolic water within the astrocyte as AQP4 transporters on the cell body are increased, but the release of water into the blood through the astrocyte end feet may be reduced due to reduced expression of AQP4 on the perivascular astrocyte membrane, thus resulting in astrocyte swelling.

Diffusion of glutamate, released into the extracellular space, from the Ammon's horn region to the relatively intact subicular region could trigger excitation of the

neurons in the latter, and thus the spread of seizure activity out of the hippocampus resulting in behavioral seizures.

It is suggested that pathologic changes in the entorhinal cortex (EC) may underlie epileptogenesis in the sclerotic hippocampus. Abnormal epileptiform activity has been recorded from the EC region[91,92] and is reported to be the lead structure in the emergence of tonic discharges in mesial structures in a majority of patients with EC atrophy.[93,94] Some studies have reported neuronal loss and gliosis in superficial layers of the EC,[86] whereas others find no significant difference in neuronal densities in the EC of patients with and without hippocampal sclerosis.[95] However, gliosis was a common finding in the EC of sclerotic and nonsclerotic patients. The evidence for increased Ca^{2+} release and Ca^{2+} oscillations in NG2-like cells from the EC (parahippocampus) in TLE patients,[68] if associated with glutamate release, suggests a mechanism by which neurons in the entorhinal cortex may be excited and thus provide strong excitatory inputs to the hippocampus. Although such a mechanism has been postulated as the trigger for hippocampal seizure activity, how this occurred has remained a puzzle. The activity of astrocytes may provide the answer, and merits further investigation in this context.

Astrocytes may contribute to increased extracellular K+ in the seizure focus

K^+ buffering capacity is diminished in the sclerotic hippocampus compared to a nonsclerotic one, most prominently in areas such as CA1.[34] The impaired inwardly rectifying K^+ channels in sclerotic hippocampi may be one contributory factor.[38] The loss AQP4 from the astrocyte perivascular membrane may contribute to this. The buffering of K^+ by the inwardly rectifying Kir channel depends on a parallel flux of water through the plasma membranes of these cells. K^+ and water are taken up by the astrocyte membrane facing the neuropil and pushed into the blood and CSF through the end foot membranes under conditions of high neuronal activity.[96] A loss of AQP4 from the perivascular membrane could impair water movements[25] and lead to increased extracellular concentrations of K^+, which can depolarize neurons in adjacent regions such as the subiculum. This buffering of K^+ is probably performed by the GFAP positive subset of GluT-like astrocytes. Although very preliminary data presented by Seifert et al.[14] suggest that such cells are absent in the sclerotic regions of the hippocampus, they may be a reduced population rather than absent. The relative contribution of GluT-like cells to a human sclerotic hippocampal seizure focus needs further analysis.

Excitable astrocytes may contribute to the spread of excitation through the hippocampus

As discussed previously, the sclerotic hippocampus may also be populated with a large number of NG2-like cells. These cells in addition to contributing to Ca2+-dependent glutamate release may also directly contribute to the excitability of the seizure focus. NG2-like cells have been demonstrated in animal studies to be capable of being depolarized to yield action potentials, sometimes even producing spontaneous action potentials.[79] Cells capable of being depolarized to generate actions potentials[32] and GluR1 receptors with elevated flip to flop ratios, which would facilitate and prolong depolarization[14] are demonstrated in the human hippocampus. Such cells may facilitate the generation or spread of waves of depolarization from granule cells to the subiculum without synaptic pathways between these two regions. However, even in the most sclerotic of hippocampi there is a intricate network of neurons in the stratum oriens that strongly express the glutamate synthesizing enzyme phosphate activated glutaminase,[97] along with other transmitter molecules such as neuropeptide Y, somatostatin, and GABA among other molecules. It is interesting to speculate if this network of neurons, which runs along the CA1 region provides synaptic input to NG2-like neurons to excite them.[98] If so, a weak wave of depolarization from surviving granule cells in the sclerotic hippocampus could be transmitted along the stratum oriens neural network and "amplified" via the NG2 cell substrate into a stronger wave of depolarization then excites neurons in the subiculum to generate seizures.

Alternatively, the NG2 cell action potentials may just serve as Na^+ ion influx to increase astrocytic $[Na^+]_I$ that stimulates the activity of Na/K ATPase. An increase in Na/K ATPase in astrocytes may play a role in buffering extracellular K^+, compensating to some degree for the decreased Kir function in these astrocytes.

Astrocytes modulate and are modulated by the microvasculature

Immunohistochemical and gene expression studies reviewed above demonstrate that a variety of molecules are reorganized in the perivascular end feet on blood vessels. Some of these such as AQP4 and dystrophin are down regulated and associated with water and K+ buffering. Others, such as plectin 1, CD44, CXCR4, and erythropoietin receptor, are up regulated. Their functions are not clear. The monocyte chemoattractant protein-1 (MCP-1 or CCL2) and macrophage chemoattractant protein-1a (MIP-1α or CCL3) are up regulated in the sclerotic hippocampus. Both these molecules are synthesized by astrocytes and are expressed in astrocyte perivascular membranes that contact the abluminal surface of brain microvessels.[67] The receptors for these compounds

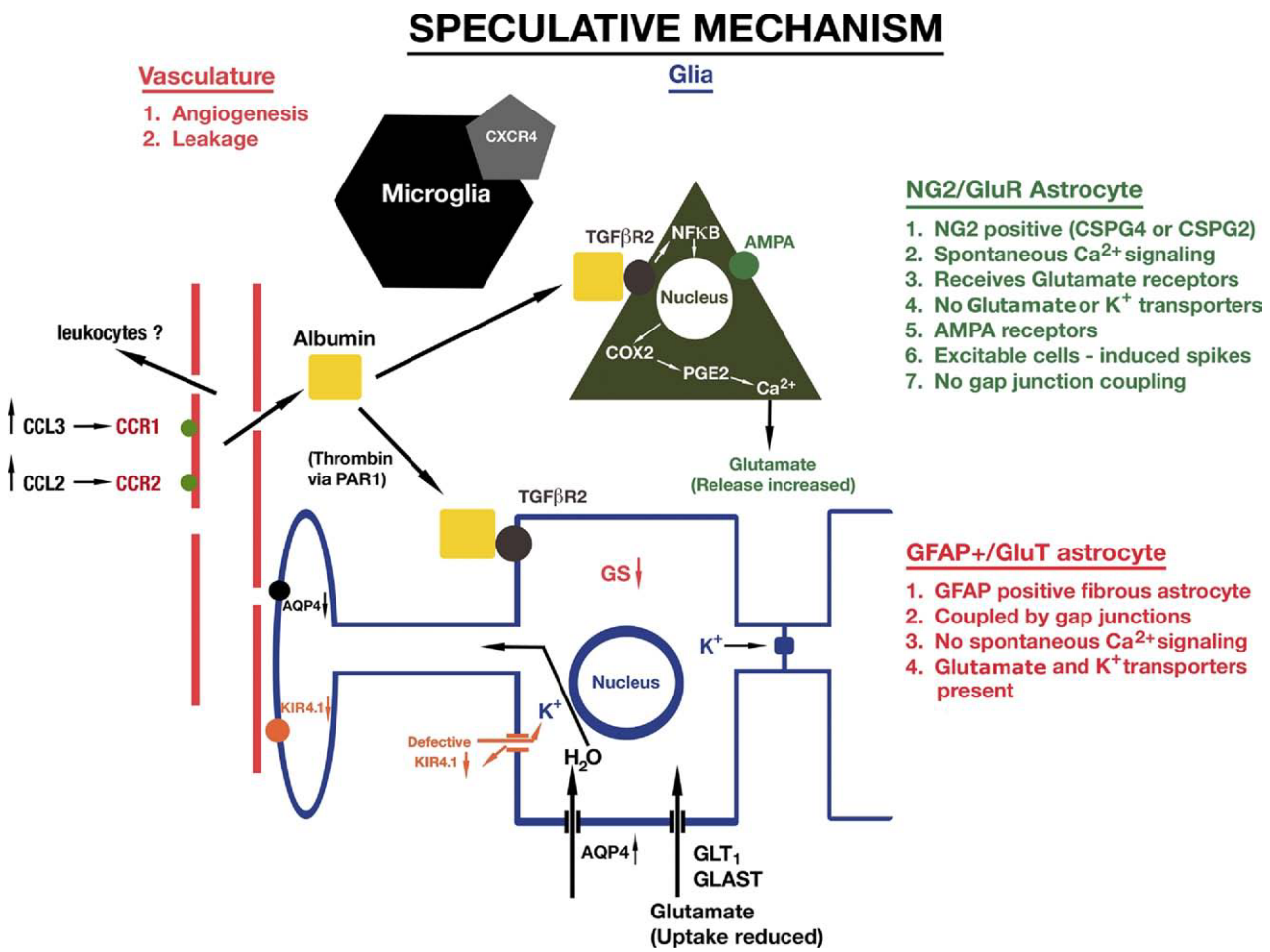

FIG. 6. A diagram to represent the postulated mechanisms involving astrocyte-like cells in the human sclerotic hippocampus, showing their altered functional states. (Downward small arrow = down regulated; long arrows indicate direction of flow.)

CCR2 and CCR1, respectively, have been identified on microvessels from epileptogenic brain tissue.[66] It is believed that these glial-derived chemokines guide circulating leukocytes through endothelial junctions into underlying brain tissue. They may also facilitate the extravasation of molecules, such as albumin into the brain through a leaky blood-brain barrier. Albumin released into the brain parenchyma binds to TGFβR2 receptors on astrocytes and modulates genes associated with the TGFβ pathway,[99] including the COX2/PGE2 pathway for Ca2+ release and the down regulation of the Kir channels.

Astrocytes and immune and inflammatory factors

The association of inflammatory and immune markers (chemokines, cytokines, class II major histocompatibility complex markers and complement) in association with astrocytes, pose the question as to their role in epilepsy. Factors such as IL-1β is shown to be able through binding to IL receptors on astrocytes activate them to produce several of the factors observed in micro-array studies to be regulated in astrocytes.[54] Many of these are transcribed by the transcription factor NFkB, and the resultant molecules may be involved in several functions, such as changing astrocyte morphology (GFAP, vimentin, Ezrin, Radixin, Moesin), triggering intracellular Ca^{2+} release (S100B, CXCR4, COX2/PGE2 pathway), and influencing the functioning of the microvasculature (CCL2, CCL3, EPOR) and down regulating critical membrane channels and transporters (Kir 4.1, AQP4).[50] Components of the complement cascade increase vascular permeability.[100] The role in epilepsy of these factors associated with immune functions is still poorly understood, but may have a significant place in the process of epileptogenesis.

FIG. 6 is a summary of the mechanisms involving astrocytes that we speculate are operative in the human sclerotic hippocampal seizure focus. Astrocytes may play several roles in epilepsy, which include participating in the genesis of a seizure focus by structural alterations to brain regions that become epileptogenic to mechanisms that maintain seizure activity through release of glutamate and poor clearance of K^+. The careful

study of each of these roles of the astrocytes during the process of epileptogenesis may offer insight for more targeted pharmacotherapy for epilepsy.

Acknowledgements: We thank our collaborators, especially Tore Eid, Jung Kim, Michael Brines Idil Cavus, Anne Wiliamson, and Hitten Zaveri, who participated in the many studies on the pathophysiology of human temporal lobe epilepsy, which have contributed to this review. We thank Dr. Ognen Petroff for many stimulating discussions, which have helped us greatly in clarifying our own understanding of the role of astrocytes in epilepsy. Ilona Kovacs has provided outstanding technical support for many years. The National Institutes of Health and the Lee Foundation supported our research.

REFERENCES

1. Steinhäuser C, Haydon PG, de Lanerolle NC. Astroglial mechanisms in epilepsy. In: Engel JJ, Pedley TA, eds. Epilepsy: a comprehensive textbook. Philadelphia: Lipincott, Williams & Wilkins, 2008:277–288.
2. Penfield W, Humphreys S. Epileptogenic lesions of the brain. A histologic study. Arch Neurol Psychiatry 1940;43:240–259.
3. Foerster O, Penfield W. The structural basis of traumatic epilepsy and results of radical operations. Brain 1930;53:99–119.
4. Ward AA. Glia and epilepsy. In: Schoffeniels E, Frank G, Towers DB, Hertz L, eds. Dynamic PROPERTIES OF GLIA CELLS. New York: Pergamon, 1977:413–427.
5. Pollen DA, Trachtenberg MC. Neuroglia: gliosis and focal epilepsy. Science 1970;167:1252–1253.
6. Harris AB. Cortical neuroglia in experimental epilepsy. Exper Neurol 1975;49:691–715.
7. Tiffany-Castiglioni E, Castiglioni AJJ. Astrocytes in epilepsy. In: Fedoroff S, Vernadarkis A, eds. Astrocytes: cell biology and pathology of astrocytes. New York: Academic Press, 1986:401–424.
8. de Lanerolle NC, Lee TS. New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. Epilepsy Behav 2005;7:190–203.
9. de Lanerolle NC, Kim JH, Williamson A, et al. A retrospective analysis of hippocampal pathology in human temporal lobe epilepsy: evidence for distinctive patient subcategories. Epilepsia 2003;44:677–687.
10. Petroff OA, Errante LD, Kim JH, Spencer DD. N-acetyl-aspartate, total creatine, and myo-inositol in the epileptogenic human hippocampus. Neurology 2003;60:1646–1651.
11. Cohen-Gadol AA, Pan JW, Kim JH, Spencer DD, Hetherington HH. Mesial temporal lobe epilepsy: a proton magnetic resonance spectroscopy study and a histopathological analysis. J Neurosurg 2004;101:613–620.
12. Verkhratsky A. Neurotransmitter receptors in astrocytes. In: Parpura V, Haydon PG, eds. Astrocytes in (patho)physiology of the nervous system: Springer Science, 2009.
13. Seifert G, Schroder W, Hinterkeuser S, Schumacher T, Schramm J, Steinhauser C. Changes in flip/flop splicing of astroglial AMPA receptors in human temporal lobe epilepsy. Epilepsia 2002; 43(Suppl. 5):162–167.
14. Seifert G, Hüttmann K, Schramm J, Steinhäuser C. Enhanced relative expression of glutamate receptor 1 flip AMPA receptor subunits in hippocampal astrocytes of epilepsy patients with Ammon's horn sclerosis. J Neurosci 2004;24:1996–2003.
15. Steinhäuser C, Seifert G. Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity. Eur J Pharmacol 2002;447:227–237.
16. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 2005;6:626–640.
17. Fiacco TA, Agulhon C, Taves SR, et al. Selective stimulation of astrocyte calcium in situ does not affect neuronal excitatory synaptic activity. Neuron 2007;54:611–626.
18. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. Lancet 1993;341:1607–1610.
19. Cavus I, Abi-Saab WM, Cassadey M, et al. Basal glutamate, gamma-aminobutyric acid, glucose , and lactate levels in the epileptogenic and non-epileptogenic brain site in neurosuergery patients. Epilepsia 2002;43:247.
20. Proper EA, Hioogland G, Kappen SM, et al. Distribution of glutamate transporters in the hippocampus of patients with pharmaco-resistant temporal lobe epilepsy. Brain 2002;125:32–43.
21. Mathern GW, Mendoza D, Lozada A, et al. Hippocampal GABA and glutamate transporter immunoreactivity in patients with temporal lobe epilepsy. Neurology 1999;52:453–472.
22. Tessler S, Danbolt NC, Faull RLM, Storm-Mathisen J, Emson P. Expression of the glutamate transporters in human temporal lobe epilepsy. Neuroscience 1998;88:1083–1091.
23. Bjørnsen LP, Eid T, Holmseth S, Danbolt NC, Spencer DD, de Lanerolle NC. Changes in glial glutamate transporters in human epileptogenic hippocampus: inadequate explanation for high extracellular glutamate during seizures. Neurobiol Dis 2006;25: 319–330.
24. Lee T-S, Bjornsen LP, Paz C, et al. GAT1 and GAT3 expression are differently localized in the human epileptogenic hippocampus. Acta Neuropathol 2006;111:351–363.
25. Eid T, Lee T-SW, Thomas MJ, et al. Loss of perivascular aquaporin 4 inderlie deficient water and K+ homeostasis in the human epileptogenic hippocampus. Proc Natl Acad Sci U S A 2005;102: 1193–1198.
26. Tse FW, Fraser DD, Duffy S, MacVicar BA. Voltage-activated K+ currents in acutely isolated hippocampal astrocytes. J Neurosci 1992;12:1781–1788.
27. Sontheimer H, Waxman SG. Expression of voltage-activated ion channels by astrocytes and oligodendrocytes in the hippocampal slice. J Neurophysiol 1993;70:1863–1873.
28. Sontheimer H, Ransom B, Cornell-Bell A, Black J, Waxman S. Sodium current expression in rat hippocampal astrocytes in vitro: Alterations during development. J Neurophysiol 1991;65:3–19.
29. Bevan B, Chiu S, Gray P, Ritchie J. The presence of voltage gated sodium, potassium, and chloride channels in rat cultured astrocytes. Proc R Soc Lond 1985;225:299–313.
30. Barres BA, Chun LLy, Corey DP. Ion channels in vertebrate glia. Ann Rev Neurosci 1990;13:441–474.
31. Barres BA, Chun LLY, Corey DP. Ion channel expression by white matter glia: I. type 2 astrocytes and oligodendrocytes. Glia 1988;1:10–30.
32. O'Connor ER, Sontheimer H, Spencer DD, de Lanerolle NC. Astrocytes from human hippocampal epileptogenic foci exhibit action potential-like responses. Epilepsia 1998;39:347–354.
33. Bordey A, Spencer DD. Distinct electrophysiological alterations in dentate gyrus versus CA1glial cells from epileptic humans with temporal lobe sclerosis. Epilepsy Res. 2004;59:107–122.
34. Bordey A, Sontheimer H. Properties of human glial cells associated with epileptic tissue. Epilepsy Res 1998;32:286–303.
35. Hinterkeuser S, Schröder W, Hager G, et al. Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. Eur J Neurosci 2000;12: 2087–2096.
36. Djamshidian A, Grassl R, Seltenhammer M, et al. Altered expression of voltage-dependent calcium channel a1 subunits in temporal lobe epilepsy with Ammon's horn sclerosis. Neuroscience 2002;111:57–69.
37. Gabriel S, Eilers A, Kivi A, et al. Effects of barium on stimulus induced changes in extracellular potassium concentration in area CA1 of hippocampal slices from normal and pilocarpine treated rats. Neurosci Lett 1998;242:9–12.
38. Schroder W, Hinterkeuser S, Seifert G, et al. Functional and molecular properties of human astrocytes in acute hippocampal slices obtained from patients with temporal lobe epilepsy. Epilepsia 2000;41:S181–184.
39. Hertz L, Dringen R, Schousboe A, Robinson SR. Astrocytes: glutamate producers for neurons. J Neurosci Res 1999;57:417–428.

40. van der Hel WS, Notenboom RG, Bos IW, van Rijen PC, van Veelen CW, de Grann PN. Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. Neurology 2005;64:326–333.
41. Eid T, Thomas MJ, Spencer DD, et al. Loss of glutamine synthetase in the human epileptogenic hippocampus: a possible mechanism for elevated exttracellular glutamate in mesial temporal lobe epilepsy. Lancet 2004;363:28–37.
42. Eid T, Williamson A, Lee T-S, Petroff OA, de Lanerolle NC. Glutamate and astrocytes: key players in human mesial temporal lobe epilepsy. Epilepsia 2008;49 42–52.
43. Cavus I, Kasoff WS, Cassaday MP, et al. Extracellular metabolites in the cortex and hippocampus of epileptic patients. Ann Neurol 2005;57:226–235.
44. Petroff OA, Errante LD, Rothman DL, Kim JH, Spencer DD. Neuronal and glial metabolite content of the epileptogenic human hippocampus. Ann Neurol 2002;52:635–642.
45. Malthankar-Phatak GH, de Lanerolle NC, Eid T, et al. Differential glutamate dehydrogenase (GDH) activity profile in patients with temporal lobe epilepsy. Epilepsia 2006;47:1292–1299.
46. Petroff OAC. Metabolic biopsy of the brain. In: Waxman SG, ed. Molecular neurology. New York: Elsevier, 2007:77–100.
47. During MJ, Itzhak F, Leone P, Katz A, Spencer DD. Direct measurement of extracellular lactate in the human hippocampus during spontaneous seizures. J. Neurochem. 1994;62:2356–2361.
48. Cendes F, Stanley JA, Dubeau F, Andermann F, Arnold DL. Proton magnetic resonancenspectroscopic imaging for discrimination of absence and complex partial seizures. Ann Neurol 1997; 41:74–81.
49. Bittar PG, Charnay Y, Pellerin L, Bouras C, Magistretti PJ. Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. J Cereb Blood Flow Metab 1996;16:1079–1089.
50. Lee T-S, Mane S, Eid T, et al. Gene expression in temporal lobe epilepsy is consistent with increased release of glutamate by astrocytes. Mol Med 2007;13:1–13.
51. Özbas-Gerceker F, Redeker S, Boer K, et al. Serial analysis of gene expression in the hippocampus of patients with mesial temporal lobe epilepsy. Neuroscience 2006;138:457–474.
52. Becker AJ, Chen J, Paus S, et al. Transcriptional profiling in human epilepsy: expression array and single cell real-time qRT-PCR analysis reveal distinct cellular gene regulation. NeuroReport 2002;13:1327–1333.
53. Dong Y, Benveniste EN. Immune function of astrocytes. Glia 2001;36:180–190.
54. John GR, Lee SC, Song X, Rivieccio M, Brosnan CF. IL-1-Regulated responses in astrocytes: relevance to injury and recovery. Glia 2005;49:161–176.
55. Crespel A, Coubes P, Rousset M-C, et al. Inflammatory reactions in human medial temporal lobe epilepsy with hippocampal sclerosis. Brain Res 2002;952:159–169.
56. Ravizza T, Gagliardi B, Noé F, Boer K, Aronica E, Vezzani A. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: Evidence from experimental models and human temporal lobe epilepsy. Neurobiol Dis 2008;29:142–160.
57. Aronica E, Boer K, van Vilet EA, et al. Complement activation in experimental and human temporal lobe epilepsy. Neurobiol Dis 2007;26:497–511.
58. Abbott NJ. Astrocyte-endothelial interactions and blood-brain barrier permiability. J Anat 2002;200:629–638.
59. Bratz E. Ammonshornbefunde bei Epileptikern. Arch. Psychiatr Nervenkr 1899;32:820–835.
60. Eid T, Brines M, Cerami A, et al. Increased expression of erythropoietin receptor on blood vessels in the human epileptogenic hippocampus with sclerosis. J Neuropathol Exptl Neurol 2004; 63:73–83.
61. Rigau V, Morin M, Rousset M-C, et al. Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. Brain 2007;130:1942–1956.
62. Van Vliet EA, da Costa Araújo S, Redeker S, van Schaik R, Aronica E. Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. Brain 2007;130:521–534.
63. Cacheaux LP, Ivens S, David Y, et al. Transcriptome profiling reveals TGF-b signalling involvement in epileptogenesis. J Neurosci 2009;29:8927–8935.
64. Ivens S, Kaufer D, Flores LP, et al. TGF-b receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. Brain 2007;130:535–547.
65. Tishler DM, Weinberg KI, Hinton DR, Barbaro N, Annett GM, Raffel C. MDR1 gene expression in brain of patients with medically intractable epilepsy. Epilepsia 1995;36:1–6.
66. Andjelkovic AV, Pachter JS. Characterization of binding sites for chemokines MCP-1 and MIP-1a on human brain microvessels. J Neurochem 2000;75:1898–1906.
67. Andjelkovic AV, Kerkovich D, Shanley J, Pulliam L, Pachter JS. Expression of binding sites for b-chemokines on human astrocytes. Glia 1999;28:225–235.
68. Lee SH, Magge S, Spencer DD, Sontheimer H, Cornell-Bell A. Human epileptic astrocytes exhibit increased gap junction coupling. Glia 1995;15:195–202.
69. Petroff OAC, Spencer DD. MRS studies of the role of altered glutamate and GABA neurotransmitter metabolism in the pathophysiologyof epilepsy. In: Shulman RG, Rothman DL, eds. Brain energetics and neuronal activity:Applications to fMRI and medicine. New York: Wiley, 2004.
70. Petroff OA, Errante LD, Rothman DL, Kim JH, Spencer DD. Glutamate-glutamine cycling in the epileptic human hippocampus. Epilepsia 2002;43:703–710.
71. Cavus I, Pan JW, Hetherington HP, et al. Decreased hippocampal volume on MRI is associated with increased extracellular glutamate in epilepsy patients. Epilepsia 2008;49:2358-1366.
72. Pan JW, Venkatraman T, Vives KP, Spencer DD. Quantitative glutamate spectroscopic imaging of the human hippocampus. NMR Biomed 2006;19:209–216.
73. Privat A, Gimenez-Ribotta M, Ridet J-L. Morphology of astrocytes. In: Kettenmann H, Ransom BR, eds. Neuroglia. New York: Oxford University Press, 1995:3–22.
74. Matthias K, Kirchhoff F, Seifert G, et al. Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. J Neurosci 2003;23:1750–1758.
75. Wallraff A, Odermatt B, Willecke K, Steinhäuser C. Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. Glia 2004;48:36–43.
76. Jabs R, Seifert G, Steinhäuser C. Astrocytic function and its alteration in the epileptic brain. Epilepsia 2008;49:3–12.
77. Paukert M, Bergles DE. Synaptic communication between neurons and NG2 cells Curr Opinion in Neurobiol 2006;16:515-521.
78. Schools GP, Zhou M, Kimmelberg HK. Electrophysiolohically "complex" glial cells fresshly isolated from the hippocampus are immunopositive for chondroitin sulphate proteoglycan NG2. J Neurosci Res 2003;73:765–777.
79. Káradóttir R, Hamilton NB, Bakiri Y, Attwell D. Spiking and non-spiking classes of oligodendrocyte precursor glia in CNs white matter. Nature Neurosci 2008;11:450–456.
80. Akiopian G, Kressin K, Derouiche A, Steinhäuser C. Identified glial cells in the early postnatal mouse hippocampus display different types of Ca2+ currents. Glia 1996;17:181–194.
81. Witter MP, Groenewegen HJ, Lopes da Silva FH, Lohman AHM. Functional organization of the extrinsic and intrinsic circuitry of the parahippocampal region. Prog Neurobiol 1989;33:161–253.
82. Babb TL, Brown WJ, Pretorius J, Davenport C, Lieb JP, Crandall PH. Temporal lobe volumetric cell densities in temporal lobe epilepsy. Epilepsia 1984;25:729–740.
83. Sommer W. Erkrankung des Ammonshorns als aetiologisches Moment der Epilepsie. Arch Psychiatr Nervenkr 1880;10:631–675.
84. Fisher PD, Sperber EF, Moshe SL. Hippocampal sclerosis revisited. Brain Dev 1998;20:563.
85. Kim JH, Guimaraes PO, Shen M-Y, Masukawa LM, Spencer DD. Hippocampal neuronal density in temporal lobe epilepsy with and without gliomas. Acta Neuropathol 1990;80:41–45.
86. Du F, Eid T, Lothman EW, Kohler C, Schwarcz R. Preferential neuronal loss in layer III of the medial entorhinal cortex in rat

models of temporal lobe epilepsy. J Neurosci 1995;15: 6301–6313.
87. Babb TL, Lieb JP, Brown WJ, Pretorius J, Crandall PH. Distribution of pyramidal cell density and hyperexcitability in the epileptic human hippocampal formation. Epilepsia 1984;25: 721–728.
88. Williamson A, Spencer SS, Spencer DD. Depth electrode studies and intracellular dentate granule cell recordings in temporal lobe epilepsy. Ann Neurol 1995;38:778–787.
89. Malarkey EB, Parpura V. Mechanisms of glutamate release from astrocytes. Neurochem International 2008;52:142–154.
90. Kimelberg HK, Mongin AA. Swelling-activated release of excitatory amino acids in the brain: relevance for pathophysiology. Contrib Neprhol 1998;123:240–257.
91. Bragin A, Wilson CL, Almajano J, Mody I, Engel J. High-frequency oscillations after status epilepticus: epileptogenesis and seizure genesis. Epilepsia 2004;45:1017–1023.
92. De Guzman P, D'Antuono M, Avoli M. Initiation of electrtoencephalographic seizures by neural networks in entorhinal and perirhinal cortices in vitro. Neurosci 2004;123:875–886.
93. Bar-Peled O, Ben-Hur H, Biegon A, et al. Distribution of glutamate transporter subtypes during human brain development. J Neurochem 1997;69:2571–2580.
94. Bartolomei F, Khalil M, Wendling F, et al. Entorhinal cortex involvement in human mesial temporal lobe epilepsy: an electrophysiologic and volumetric study. Epilepsia 2005;46:677–687.
95. Dawodu S, Thom M. Quantitative neuropathology of the entorhinal cortex region in patients with hippocampal sclerosis and temporal lobe epilepsy. Epilepsia 2005;46:23–30.
96. Paulson OB, Newman EA. Does the release of potassium from astrocyte endfeet regulate cerebral blood flow. Science 1987;237: 896–898.
97. Eid T, Hammer J, Runden-Pran E, et al. Increased expression of phosphate activated glutaminase in hippocampal neurons in human mesialtemporal lobe epilepsy. Acta Neuropathol 2007;113: 137–152.
98. Jabs R, Pivneva T, Hüttmann K, et al. Synaptic transmission onto hippocampal glial cells with hGFAP promoter activity. J Cell Sci 2005;118:3791–3803.
99. Friedman A, Kaufer D, Heinemann U. Blood-brain barrier breakdown-inducing astrocytic transformation: Novel targets for the prevention of epilepsy. Epilepsy Res 2009;85:142–149.
100. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. Br J Pharmacol 2006;147:232–240.