ASTROCYTE NETWORKS AND EPILEPSY: WHEN STARS COLLIDE

Loss of Astrocytic Domain Organization in the Epileptic Brain. Oberheim NA, Tian GF, Han X, Peng W, Takano T, Ransom B, Nedergaard M. *J Neurosci* 2008;28(13):3264–3276. Gliosis is a pathological hallmark of posttraumatic epileptic foci, but little is known about these reactive astrocytes beyond their high glial fibrillary acidic protein (GFAP) expression. Using diolistic labeling, we show that cortical astrocytes lost their nonoverlapping domain organization in three mouse models of epilepsy: posttraumatic injury, genetic susceptibility, and systemic kainate exposure. Neighboring astrocytes in epileptic mice showed a 10-fold increase in overlap of processes. Concurrently, spine density was increased on dendrites of excitatory neurons. Suppression of seizures by the common antiepileptic, valproate, reduced the overlap of astrocytic processes. Astrocytic domain organization was also preserved in APP transgenic mice expressing a mutant variant of human amyloid precursor protein despite a marked upregulation of GFAP. Our data suggest that loss of astrocytic domains was not universally associated with gliosis, but restricted to seizure pathologies. Reorganization of astrocytes may, in concert with dendritic sprouting and new synapse formation, form the structural basis for recurrent excitation in the epileptic brain.

Astroglial Metabolic Networks Sustain Hippocampal Synaptic Transmission. Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. *Science* 2008;322(5907):1551–1555. Astrocytes provide metabolic substrates to neurons in an activity-dependent manner. However, the molecular mechanisms involved in this function, as well as its role in synaptic transmission, remain unclear. Here, we show that the gap-junction subunit proteins connexin 43 and 30 allow intercellular trafficking of glucose and its metabolites through astroglial networks. This trafficking is regulated by glutamatergic synaptic activity mediated by AMPA receptors. In the absence of extracellular glucose, the delivery of glucose or lactate to astrocytes sustains glutamatergic synaptic transmission and epileptiform activity only when they are connected by gap junctions. These results indicate that astroglial gap junctions provide an activity-dependent intercellular pathway for the delivery of energetic metabolites from blood vessels to distal neurons.

COMMENTARY

In astronomy, the collision of two stars, once believed to be extremely rare, recently has been recognized to be a common, yet spectacular event that results in explosions of colossal magnitude. In neuroscience, the star-like cell, the astrocyte, increasingly has been recognized as having a critical role in normal brain function and neurological disorders, including epilepsy. Similar to stars colliding, it may be the collective interactions of astrocytes within networks—more than the properties of individual astrocytes—that promote explosive electrical discharges of seizures in the brain.

Astrocytes were traditionally viewed as uninteresting, passive brain cells with primarily housekeeping functions, such as structural integrity and metabolic maintenance. However, recent research clearly demonstrates that astrocytes have an impressive diversity of functions and play active, central roles in brain physiology (1). For example, astrocytes regulate brain excitability by controlling extracellular neurotransmitter and ion levels through specific transporters and pumps. Furthermore, in the so-called tripartite synapse, astrocytes directly participate in brain signaling by responding to neurotransmitters through discrete astrocyte receptor sites and, in turn, modulating neurons and other astrocytes by releasing their own gliotransmitters (2).

Given the newly recognized roles of astrocytes in brain physiology under normal conditions, it is not surprising that astrocyte dysfunction increasingly is implicated in contributing to epileptogenesis under pathological conditions (3,4). Impairment of various astrocyte membrane proteins, including glutamate transporters, potassium channels, and water-permeable aquaporins, may lead to neuronal hyperexcitability and seizures via dysregulation of extracellular glutamate, potassium, or osmotic homeostasis, respectively. Glutamate released from astrocytes may trigger paroxysmal epileptiform discharges in neurons (5). Furthermore, reactive gliosis, which is a stereotypic morphological and biochemical conversion of astrocytes into an abnormally activated state, occurs commonly in the context of brain injuries and neurodegenerative disorders (6,7). While reactive gliosis is often viewed as a beneficial, compensatory response to brain injury, some of these functional alterations

Epilepsy Currents, Vol. 9, No. 4 (July/August) 2009 pp. 113–115
Wiley Periodicals, Inc.

likely have detrimental consequences that predispose the patient to developing epilepsy.

Although isolated cellular and molecular alterations in individual astrocytes can promote epileptogenesis, the physiological and pathological implications of large, synergistic networks of astrocytes have recently commanded more attention. Astrocytes can directly or indirectly communicate with hundreds of neighboring astrocytes via connexin protein-mediated gap junctions or nonsynaptic mechanisms. These large syncytia of astrocytes may operate in a coordinated fashion to increase the range and diversity of physiological and biochemical functions of the network (8). For example, the capacity to buffer extracellular potassium is enhanced by spatial redistribution of potassium, absorbed at one part of the network, to astrocytes in other areas of the network with lower potassium concentration. Furthermore, physiological signaling may occur across extensive networks of astrocytes through gliotransmission or gap-junction–mediated communication. Glial calcium waves, a unique form of astrocytic signaling initiated by neurotransmitter-receptor activation, travel great distances along astrocyte networks *in vitro*, although it is not established whether they occur *in vivo*.

Despite recent insights into astrocyte network function under normal conditions, the mechanisms by which astrocyte networks influence epilepsy are poorly understood. Beyond the modulatory mechanisms of individual astrocytes that promote epileptogenesis, the collective properties of astrocyte networks likely add an extra level of complexity in regulating brain excitability in the epileptic state. Taking different approaches, the two papers reviewed here have investigated the role of astrocyte interactions in epilepsy; each focuses on separate structural organization and metabolic properties of astrocyte networks in epilepsy models.

The study by Oberheim et al. examined the structural interactions and overlap between astrocytes in the epileptic state. The study is based on the recent finding that cortical astrocytes are normally organized into unique spatial domains with minimal overlap of processes between neighboring astrocytes (9,10). This revelation emerged from advances in cellular imaging techniques that allow individual astrocytes and their processes to be visualized in their entirety. So, while astrocytes do communicate with each other extensively in large networks through gap junctions, these interactions normally occur in a limited portion at the periphery of astrocytic processes, with the majority of each astrocyte occupying its own unique territory. The functional implications of the nonoverlapping domains are not entirely clear, but a simple theory is that this organization permits individual astrocytes to compartmentalize physiological functions of large groups of neurons or synapses (each astrocyte domain may exclusively control over 100,000 synapses), thus exerting a more local measure of control or regulation across the larger area covered by astrocyte networks.

Oberheim et al. studied astrocyte domain organization in three different rodent models of epilepsy: the intracortical iron injection model of posttraumatic epilepsy, the pharmacological kainate epilepsy model, and an inbred genetic epilepsy strain. In each case, they observed typical signs of reactive astrocytosis in the cortex, with hypertrophy of processes and increased glial fibrillary acidic protein (GFAP) expression in astrocytes. More remarkably, based on diolistic labeling—a method for ballistically delivering membrane-adhering dyes to tissue to visualize individual cells in their entirety—there was a consistent loss of the nonoverlapping domain organization between neighboring astrocytes located around the epileptic focus. Compared to controls, the overlap of processes between adjacent astrocytes in epileptic animals increased by about 10-fold. The loss of domain organization was observed acutely within a week of the initial injury and persisted chronically over months. In contrast, in a genetic mouse model of Alzheimer's disease without seizures, cortical astrogliosis was prominent, as reflected by increased GFAP expression, but there was no significant hypertrophy or loss of domain organization of astrocytes.

Although these structural changes in astrocyte networks in epilepsy models are striking, their pathophysiological significance and consequences remain uncertain. The study by Oberheim and colleagues did not address the important issue of whether increased astrocytic overlap is causally involved in epileptogenesis. Suppression of seizures with valproate in the intracortical iron model partially prevented the loss of domain organization, indicating that seizures themselves, in part, cause the structural changes in astrocytes. However, this finding does not rule out the possibility that the astrocytic reorganization nonetheless contributes to an ongoing, progressive process of epileptogenesis. The study also showed correlative data of altered dendritic size and spine density of neurons that paralleled the astrocytic reorganization, but again, no cause–effect relationship was established. Future studies are needed to understand the specific implications and mechanisms of these findings, but it is attractive to speculate that the increased astrocytic overlap translates into abnormal connectivity and communication within astrocyte networks, which secondarily influences neurons to promote seizure generation.

In contrast to structural changes in astrocyte networks in epilepsy, the study by Rouach et al. focused on metabolic functions of astrocyte networks in regulating normal synaptic transmission and epileptiform activity. As glucose is the main fuel for neuronal activity, this study began by demonstrating that fluorescently labeled glucose (loaded into single perivascular astrocytes through a patch pipette) is transported extensively throughout astrocyte networks of hippocampal slices,

delivering glucose from blood vessels to distant astrocytes and ultimately to neurons. The glucose trafficking through astrocyte networks was: 1) mediated by gap junctions (blocked by pharmacological or genetic antagonism of connexins) and 2) activity dependent (augmented by a zero magnesium solution and picrotoxin-induced epileptiform bursts). Remarkably, while glucose-free extracellular solution abolished epileptiform activity, intracellular glucose delivery to astrocyte networks partially restored epileptiform bursting of neurons despite extracellular glucose deprivation. These findings demonstrate a direct role for astrocyte networks in supporting epileptiform activity and suggest a novel approach for treating epilepsy by interrupting the metabolic communication of the networks. Inhibiting neuronal gap junctions is a proposed treatment for preventing seizure propagation between neurons; thus, blocking astrocyte gap junctions, similarly, may have an antiepileptic action. However, interrupting astrocyte communication also may have proconvulsant effects, such as impaired spatial potassium buffering. Although the two highlighted papers are significant in implicating astrocyte interactions in epilepsy, future studies are clearly needed to determine whether preventing collisions of the star-like cells in the brain may have benefits for epilepsy patients.

by Michael Wong, MD, PhD

References

1. Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* 2005;6:626–640.
2. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci* 1999;22:208–215.
3. Binder DK, Steinhauser C. Functional changes in astroglial cells in epilepsy. *Glia* 2006;54:358–368.
4. Wetherington J, Serrano G, Dingledine R. Astrocytes in the epileptic brain. *Neuron* 2008;58:168–178.
5. Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M. An astrocytic basis of epilepsy. *Nat Med* 2005;11:973–981.
6. Pekny M, Nilsson M. Astrocyte activation and reactive gliosis. *Glia* 2005;50:427–434.
7. Sofroniew MV. Reactive astrocytes in neural repair and protection. *Neuroscientist* 2005;5:400–407.
8. Theis M, Sohl G, Eiberger J, Willecke K. Emerging complexities in identity and function of glial connexins. *Trends Neurosci* 2005;28:188–195.
9. Bushong EA, Martone ME, Jones YZ, Ellisman MH. Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci* 2002;22:183–192.
10. Halassa MM, Fellin T, Takano H, Dong JH, Haydon PG. Synaptic islands defined by the territory of a single astrocyte. *J Neurosci* 2007;27:6473–6477.