



# Astrocytes Keep It Under Wraps: Reconstructing Synapses in the Latent Phase of Epileptogenesis

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## Ultrastructural and Functional Changes at the Tripartite Synapse During Epileptogenesis in a Model of Temporal Lobe Epilepsy

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The persistent unresponsiveness of many of the acquired epilepsies to traditional antiseizure medications has motivated the search for prophylactic drug therapies that could reduce the incidence of epilepsy in this at risk population. These studies are based on the idea of a period of epileptogenesis that can follow a wide variety of brain injuries. Epileptogenesis is hypothesized to involve changes in the brain not initially associated with seizures but which result finally in seizure prone networks. Understanding these changes will provide crucial clues for the development of prophylactic drugs. Using the repeated low-dose kainate rat model of epilepsy, we have studied the period of epileptogenesis following status epilepticus, verifying the latent period with continuous EEG monitoring. Focusing on ultrastructural properties of the tripartite synapse in the CA1 region of hippocampus, we found increased astrocyte ensheathment around both the presynaptic and postsynaptic elements, reduced synaptic AMPA receptor subunit and perisynaptic astrocyte GLT-1 expression, and increased number of docked vesicles at the presynaptic terminal. These findings were associated with an increase in frequency of the mEPSCs observed in patch clamp recordings of CA1 pyramidal cells. The results suggest a complex set of changes, some of which have been associated with increasingly excitable networks such as increased vesicles and mEPSC frequency, and some associated with compensatory mechanisms, such as increased astrocyte ensheathment. The diversity of ultrastructural and electrophysiological changes observed during epileptogenesis suggests that potential drug targets for this period should be broadened to include all components of the tripartite synapse.

## Commentary

In recent years, we have come to appreciate the complex roles that glial cells play in epileptogenesis. Here, we focus on astrocytes, a glial cell type that directly shapes synaptic function via its ability to take up neurotransmitters, to buffer potassium, and to physically interact with synaptic structures as part of the “tripartite synapse.”<sup>1</sup> Astrocytes are particularly intriguing because reactive astrogliosis, a dramatic shift in astrocyte form and function, is a hallmark of the epileptic brain. Furthering our intrigue, experimentally inducing astrogliosis, without any other insult, is sufficient to cause hyperexcitability and seizures.<sup>2,3</sup> Changes associated with reactive astrogliosis include altered astrocyte morphology, remodeled physical interaction with synapses, initiation of inflammatory signaling, and an altered ability to take up neurotransmitters. Complicating the situation, we do not know whether these changes cause, or are caused by seizures, nor whether they are helpful or harmful. Therefore, we need a firm understanding of how the astrocyte-neuronal relationship changes during the progression of epilepsy.

Recently, Clarkson et al gave us a new perspective by reconstructing the ultrastructure of the tripartite synapse with electron microscopy (EM) during the latent phase of epileptogenesis.<sup>4</sup> Electron microscopy observations of epileptic brain tissue have been carried out before and provided fascinating insight.<sup>5</sup> Now, Clarkson et al report EM results for the first time during the latent phase of epileptogenesis, before spontaneous seizures emerge. Focusing on glutamate signaling, the authors monitor the relationship between astrocytic processes and synaptic neuronal structures at the hippocampal CA3-CA1 synapse 7 days after kainic acid (KA)-induced status epilepticus. This study was motivated by the group’s previous results showing more efficient astrocytic glutamate uptake following KA-induced epileptogenesis. Surprisingly, in that study they did not find a change in the expression of the astrocytic glutamate transporter 1 (GLT-1),<sup>6</sup> which mediates the vast majority of glutamate uptake in the brain. Intuitively, one would expect GLT-1 expression to be increased and cause the observed facilitation of astrocytic glutamate uptake. Instead,



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the authors hypothesized that changes in how astrocyte processes physically interact with synapses could enhance glutamate clearance.


This EM study suggests that their hypothesis is indeed correct! Using 3D-reconstructions of the tripartite synapse generated from serial EM imaging the authors show that the ensheathment of presynaptic terminals and postsynaptic dendritic spines by astrocyte processes was dramatically increased in KA-treated rats. Of all, ~27% of the presynaptic terminals and ~29% of the postsynaptic spines were enwrapped with astrocyte processes which amounted to 3.5-fold and 2-fold increases, respectively, compared to sham-treated rats. These structural changes could have a big effect on synaptic astrocytic glutamate uptake since astrocyte processes more closely interact with synapses. Glutamate transporter 1 transporters are better positioned to rapidly remove synaptically released glutamate from a tightened extracellular space. This morphological change alone could explain the previous results showing more efficient glutamate uptake without changes in GLT-1 expression. In fact, the authors now report a modest reduction in GLT-1 density by ~20% in synapse-facing astrocyte membranes, likely driven by the increased astrocytic surface area. This putative mechanism needs to be confirmed in future studies but highlights the strength of high-resolution EM in combination with electrophysiological and biochemical data. Together these approaches strongly support that astrocyte glutamate uptake is enhanced after KA-induced seizures due to ultrastructural changes of the tripartite synapse. Further interventional experiments, possibly incorporating modeling approaches, are necessary to definitively assess if the increased coverage is a compensatory, antiepileptogenic change limiting excitability of the network.

The reported increase in astrocytic coverage is particularly interesting since reactive gliosis is associated with both pro- and antiepileptogenic changes. For example, reactive astrocytes release molecules activating inflammatory cascades<sup>7</sup> can be neurotoxic and neuroprotective,<sup>8</sup> and can drive synaptogenesis and synaptic pruning.<sup>9,10</sup> Dividing reactive gliosis into subprocesses with potentially opposite effects on epileptogenesis is complicated, but critical to harnessing glial changes for translation into novel treatment approaches. It may be feasible to counteract astrogliosis in whole to halt epileptogenesis, but a more selective approach facilitating synaptic astrocytic ensheathment could also be effective and possibly more efficient. Of course, approaches to selectively attenuate known proepileptogenic subprocesses within astrogliosis would be equally desirable.


This EM study also revealed that in KA-treated rats presynaptic terminals were enlarged and contained more docked glutamatergic vesicles, with relatively little effect on the postsynaptic density (PSD). This results in a net increase in readily releasable vesicles per PSD area during KA-induced epileptogenesis. This change may be unique to epileptogenesis as previous work on the same synapse suggests that the number of docked vesicles and size of the PSD usually scale together.<sup>11</sup> Interestingly, AMPA receptor immunogold labeling showed a large reduction in GluA1, GluA2, and GluA3 subunits. Less postsynaptic AMPA receptors and more readily releasable

presynaptic glutamate will have opposing effects on synaptic excitability and potentially on epileptogenesis. So, what net effect do these EM observations have on glutamatergic neurotransmission? In parallel experiments, glutamatergic function was quantified by recording miniature excitatory postsynaptic currents (mEPSCs) in CA1 pyramidal cells. Miniature excitatory postsynaptic currents frequency was 3-fold higher in KA-treated rats, compared to shams. So, while glutamate receptors are less abundant, glutamatergic synaptic communication is enhanced, likely due to the increase in presynaptic docked vesicles seen using EM. This finding demonstrates the importance of subsynaptic organization, on the nanometer scale, for synaptic function. It is already clear that nanodomains in the pre- and postsynaptic membranes, and even across the synaptic cleft, govern synapses' dynamic properties.<sup>12</sup> In addition, the topographical arrangements of postsynaptic receptors in the PSD can produce vastly different synaptic properties.<sup>13</sup> Understanding how the structure of the tripartite synapse changes on this scale during epileptogenesis will be essential to our ability to manipulate synaptic function to improve the treatment of epilepsy.

In summary, Clarkson et al delivered an impressive study with novel insights into epileptogenic and compensatory processes by taking a snapshot during the latent phase of epileptogenesis. As with most new insights, more questions arise and further studies examining ultrastructural changes will be required to understand the complexity of the system. How do these changes evolve over time? What structural rearrangements occur after the first seizure? Certainly, NMDA receptors should be investigated as well as inhibitory synaptic inputs onto the same cell. Obviously, different synapses in other circuits are also of great interest. Unfortunately, EM experiments and their subsequent analysis are very labor-intensive and not trivial to conduct, but combined with functional experiments, they offer a great opportunity to better understand epileptogenesis and enable robust modeling studies.

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