



Glial Cell Collaboration in Space and Time Contributes to Epileptogenesis

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Reactive Astrocyte-Driven Epileptogenesis Is Induced by Microglia Initially Activated Following Status Epilepticus

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Extensive activation of glial cells during a latent period has been well documented in various animal models of epilepsy. However, it remains unclear whether activated glial cells contribute to epileptogenesis, that is, the chronically persistent process leading to epilepsy. Particularly, it is not clear whether interglial communication between different types of glial cells contributes to epileptogenesis because past literature has mainly focused on 1 type of glial cell. Here, we show that temporally distinct activation profiles of microglia and astrocytes collaboratively contributed to epileptogenesis in a drug-induced status epilepticus model. We found that reactive microglia appeared first, followed by reactive astrocytes and increased susceptibility to seizures. Reactive astrocytes exhibited larger Ca²⁺ signals mediated by IP3R2, whereas deletion of this type of Ca²⁺ signaling reduced seizure susceptibility after status epilepticus. Immediate, but not late, pharmacological inhibition of microglial activation prevented subsequent reactive astrocytes, aberrant astrocyte Ca²⁺ signaling, and the enhanced seizure susceptibility. These findings indicate that the sequential activation of glial cells constituted a cause of epileptogenesis after status epilepticus. Thus, our findings suggest that the therapeutic target to prevent epilepsy after status epilepticus should be shifted from microglia (early phase) to astrocytes (late phase).

Commentary

Although glial cells, including astrocytes and microglia, are purported to greatly outnumber neurons in the brain, investigations into mechanisms of epileptogenesis, and for that matter, treatment of epilepsy with anti-seizure medications, has focused primarily on neuronal targets. Recently, however, there has been an explosion of research exploring the influence of glial cells in neuronal and network excitability, and in epileptogenic processes. In addition to being a major source of pro-inflammatory cytokines, the release of which can promote epileptogenesis,¹ microglial cells can also act as negative regulators of neuronal excitability.² Astrocytes, too, are perfectly placed to govern neuronal excitability, forming the tripartite synapse with pre- and post-synaptic neurons. In addition to regulating ionic and neurotransmitter levels at synapses, astrocytes are excitable in their own right. These cells are hyperpolarized at rest, allowing them to respond biochemically to fluctuations in the synaptic environment and in turn regulate synaptic transmission. Glial cells, therefore, have *a priori* potential to contribute to epilepsy.

The molecular and pathophysiological cascades which occur following epileptogenic injury, such as status epilepticus (SE), are yet to be completely understood. It is also unclear which cascades are critical mediators of

epileptogenesis and which are inconsequential to disease development. Activation of glial cells is a well-described phenomenon occurring acutely after SE, and persisting into the chronic phase of epileptogenesis. Although both microglia and astrocytes proliferate, several studies report that the astrocytes account for a small proportion of new glial cells in the epileptogenic zone following SE, suggesting a delayed proliferative response.³ In contrast, the intense microglial activation that occurs acutely after injury often subsides with relatively small number of activated microglia observed in the chronic period, whereas activated astroglial “scars” are prominent features in the chronic disease phase in animal models of epilepsy. This differential pattern of pathological activation may suggest communication between different types of glial cells which collaboratively contribute to epileptogenesis. To date, this has not been extensively studied.

The current report by Sano et al. utilized the mouse pilocarpine-induced SE model to investigate activation and cross-talk between glial cells, and how these pathologies contribute to the development of disease.⁴ They first characterized the temporal evolution of glial cell activation following SE. Consistent with previous reports, they demonstrate early onset of microgliosis, followed by delayed astrogliosis. Activation of astrocytes, but not microglia, was sustained into the chronic disease period. To explore a



contributory role of these cells to epileptogenesis, using transgenic mice and calcium imaging, they then studied the excitability of astrocytes activated in the chronic phase. The authors identified signs of hyperexcitability within the astrocytes—evidenced by enhanced calcium dynamics—an effect mediated by release of internal stores of Ca^{2+} through IP_3 receptor 2 ($\text{IP}_3\text{-R}_2$). These increased calcium currents were found to be independent of neuronal activity, suggesting that such pathology may directly contribute to neuronal network instability. These illuminating data suggest that glial activation is temporally coordinated following SE, and culminates in sustained activation of hyperexcitable astrocytes.

The authors next explored the direct contribution of these hyperexcitable astrocytes to disease development using $\text{IP}_3\text{-R}_2$ knock out (KO) mice. Following SE, microglia were equally activated in KO and WT mice, although somewhat surprisingly, the authors made no mention of whether subsequent activation of astrocytes was also triggered in KO mice. They then assessed two surrogate measures of epileptogenesis in the chronic phase—interictal spikes, and sensitivity to additional treatment with pilocarpine—both of which were reduced in $\text{IP}_3\text{-R}_2$ KO mice. It should be noted here that the authors did not report whether spontaneous seizures were observed in any mice, which is surprising since EEG recordings were conducted for the spike analyses. They conclude that $\text{IP}_3\text{-R}_2$, therefore, mediates sustained hyperexcitability of astrocytes, and that this is essential for the epileptogenic consequences after SE.

The authors then returned to the question of the nature of inter-communication between microglia and astrocytes in disease development, proposing that initial activation of microglial cells after SE was necessary to set in chain the pathological transformation of astrocytes. Colony-stimulating factor 1 (CSF-1) receptors are essential for microglial survival,⁵ and recent studies have utilized a variety of strategies targeting this receptor to explore the influence of these cells on epileptogenic processes.⁶ Here, using a pharmacological inhibitor of CSF-1 receptors, the authors depleted microglia at 2 distinct timepoints—at the time of SE, and 3 weeks after SE induction. Early elimination of microglia suppressed astrocytic activation over the ensuing 3 weeks, normalized the pathological increase in astrocytic Ca^{2+} currents, and suppressed the resultant neuronal circuit hyperexcitability and increased seizure susceptibility. On the contrary, elimination of microglial cells during the chronic disease state was not able to affect the established astrocyte activation or subsequent epileptogenic events, despite a prominent reduction in Iba1^+ microglial cells in the epileptic brain. Together, these findings suggest that early microglial activation is necessary for subsequent activation and hyperexcitability of astrocytes. However, once activated, astrocytes are beyond the influence of microglia, as well as neighboring neurons, and independently mediate hyperactivity, and the resultant epileptogenic consequences of SE.

Suppression of microglia activation has been previously studied, with contrasting outcomes: some show early depletion

worsens pathological and epileptogenic consequences of SE,⁷ whereas others demonstrate that inhibition of CSF-1 in the chronic disease phase suppresses seizures.^{8,9} These conflicting results may be related to the disease stage at which microglial cell depletion was initiated: the phenotype of these cells appears to change over time, initially contributing protective anti-inflammatory features, and switching to pro-inflammatory and epileptogenic consequences in the chronic disease phase.¹⁰ Because the precise timings of these phenotypic switches in different models are not fully characterized, it is somewhat difficult to align the findings of the current report with these previous studies.

Nevertheless, the current report provides further evidence of a role for glial cells in epileptogenesis. This work describes a collaboration between glial cells in the immediate aftermath of SE, and identifies a critical role of activated and hyperexcitable astrocytes in mediating the enhanced seizure susceptibility in the chronic disease phase. This study expands our knowledge of how glial cells communicate in space and time following brain injury, and how this can ultimately lead to epilepsy development. Future studies should explore whether this pattern of collaboration exists in other models of epileptogenic brain injury, such as traumatic brain injury. Furthermore, novel therapeutic approaches can be developed that differentially target microglia (early) or astrocytes (late) at different stages of epileptogenesis. It is especially enticing to consider development of strategies to suppress $\text{IP}_3\text{-R}_2$ signaling and therefore hyperexcitability of astrocytes as novel anti-epileptic therapies which target glial cells.

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References

1. Vezzani A, French J, Bartfai T, Baram TZ. The role of inflammation in epilepsy. *Nat Rev Neurol*. 2011;7(1):31-40.
2. Badimon A, Strasburger HJ, Ayata P, et al. Negative feedback control of neuronal activity by microglia. *Nature*. 2020;586(7829):417-423.
3. Gibbons MB, Smeal RM, Takahashi DK, Vargas JR, Wilcox KS. Contributions of astrocytes to epileptogenesis following status epilepticus: opportunities for preventive therapy? *Neurochem Int*. 2013;63(7):660-669.
4. Sano F, Shigetomi E, Shinozaki Y, et al. Reactive astrocyte-driven epileptogenesis is induced by microglia initially activated following status epilepticus. *JCI Insight*. 2021;6(9):e135391.
5. Elmore MRP, Najafi AR, Koike MA, et al. Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. *Neuron*. 2014;82(2):380-397.
6. Jones NC. Microglial cells in epilepsy: not that bad after All? *Epilepsy Current*. 2021;21(1):54-56.



7. Wu W, Li Y, Wei Y, et al. Microglial depletion aggravates the severity of acute and chronic seizures in mice. *Brain Behav Immun.* 2020;89:245-255.
8. Srivastava PK, van Eyll J, Godard P, et al. A systems-level framework for drug discovery identifies Csf1R as an anti-epileptic drug target. *Nat Commun.* 2018;9(1):3561.
9. Di Nunzio M, Di Sapia R, Sorrentino D, et al. Microglia proliferation plays distinct roles in acquired epilepsy depending on disease stages. *Epilepsia.* 2021;62(8):1931-1945.
10. Therajaran P, Hamilton JA, O'Brien TJ, Jones NC, Ali I. Microglial polarization in posttraumatic epilepsy: potential mechanism and treatment opportunity. *Epilepsia.* 2020;61(2):203-215.