Guardians of the Frequency: Neuronal Regulation by Microglia

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Microglial G(i)-Dependent Dynamics Regulate Brain Network Hyperexcitability

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Microglial surveillance is a key feature of brain physiology and disease. Here, we found that G_i-dependent microglial dynamics prevent neuronal network hyperexcitability. By generating Mg^{PTX} mice to genetically inhibit G_i in microglia, we show that sustained reduction in microglia brain surveillance and directed process motility-induced spontaneous seizures and increased hypersynchrony after physiologically evoked neuronal activity in awake adult mice. Thus, G_i-dependent microglia dynamics may prevent hyperexcitability in neurological diseases.

Negative Feedback Control of Neuronal Activity by Microglia

Badimon A, Strasburger HJ, Ayata P, et al. *Nature*. 2020;586(7829):417-423. doi:10.1038/s41586-020-2777-8

Microglia, the brain's resident macrophages, help to regulate brain function by removing dying neurons, pruning nonfunctional synapses, and producing ligands that support neuronal survival.[1] Here, we show that microglia are also critical modulators of neuronal activity and associated behavioral responses in mice. Microglia respond to neuronal activation by suppressing neuronal activity, and ablation of microglia amplifies and synchronizes the activity of neurons, leading to seizures. Suppression of neuronal activation by microglia occurs in a highly region-specific fashion and depends on the ability of microglia to sense and catabolize extracellular adenosine triphosphate (ATP), which is released upon neuronal activation by neurons and astrocytes. Adenosine triphosphate triggers the recruitment of microglial protrusions and is converted by the microglial ATP/ADP hydrolysing ectoenzyme CD39 into AMP; AMP is then converted into adenosine by CD73, which is expressed on microglia and other brain cells. Microglial sensing of ATP, the ensuing microglia-dependent production of adenosine, and the adenosine-mediated suppression of neuronal responses via the adenosine receptor A_1R are essential for the regulation of neuronal activity and animal behavior. Our findings suggest that this microglia-driven negative feedback mechanism operates similarly to inhibitory neurons and is essential for protecting the brain from excessive activation in health and disease.

Commentary

Microglia are known as the guardians of the brain: they continually survey their surroundings, can rapidly direct their processes to active neurons, and respond to tissue disturbances.[1-3] Microglia sense trauma or pathogen invasion, remove apoptotic cells, and promote inflammation or repair in the healthy brain and during disease.[2-4] Under pathological conditions, microglia are activated, their shape becomes amoeboid, and they produce pro-inflammatory mediators, such as interleukins and chemokines. Microglia play a key role in the neuroinflammation which has been associated with epileptogenesis. Seizures in turn promote alterations in phagocytosis, proliferation, and other microglial functions. However, more recent studies show that microglia also exert neuroprotective and antiepileptogenic effects, for example, via maintenance of glutamate homeostasis.[3]

Microglia can contact neurons directly to enable more effective and dynamic regulation of neuronal activity. An important messenger that attracts microglial processes to neurons is adenosine triphosphate (ATP). Adenosine triphosphate and its metabolite, ADP, are detected by microglial purinergic receptors, including P2Y purinoceptor 12 ($P2Y_{12}R$).[5-7] Purinergic-based chemoattraction is a first step in the process of phagocytosis[3] but is also important for microglia–neuron connections that protect neurons after acute injury.[6] Two new studies led by Anne Schaefer (Badimon et al[8]) and Katerina Akassoglou (Merlini et al[9]) now reveal a new function of microglia—protecting the *healthy* brain against abnormal neuronal activation. The study by Merlini et al focused on microglial surveillance, whereas Badimon and colleagues explored in depth the microglia–neuron connection itself. Both teams combined genetic murine models with advanced imaging techniques to address their questions.

Merlini et al wanted to know why microglia survey their environment. Because the above-mentioned $P2Y_{12}R$ plays a role in microglial motility to injury sites but not in baseline surveillance,[5] the team looked for another suspect in the non-injured brain. They hypothesized that baseline microglial surveillance in $P2Y_{12}R$-depleted mice is compensated by other G protein–coupled receptors. Accordingly, they generated a new murine model, Mg^{PTX} mice, in which they inhibited the G_i subunit of microglial G proteins—the master behind the activity of not only $P2Y_{12}R$ but also other microglial receptor types. The Mg^{PTX} mice had fewer microglia with degenerated processes that formed fewer contacts onto neurons. Two-photon imaging confirmed reduced microglial baseline surveillance and diminished response to laser ablation as compared to control mice. Unexpectedly, these mice also presented a pathological phenotype: they developed spontaneous seizures that decreased their survival.

Further studies revealed that the mice whose microglial surveillance was blocked were more susceptible to pilocarpine-induced seizures, with hypersynchrony and distinct γ oscillatory patterns as compared to controls. These experiments proved that microglial surveillance is important for suppressing seizure activity. In vivo observations in awake control mice demonstrated that repetitive whisker stimulation increased microglia brain surveillance and that the microglia contacted primarily active neurons. This preferential interaction was lost in mice deficient of the microglial G_i, in which whisker stimulation resulted in hypersynchronized neuronal activity. These findings suggested a new role for microglial surveillance: preventing neuronal hypersynchronization and hyperexcitability during periods of heightened excitatory neuronal responses.

The second study by Badimon et al solves another piece of the puzzle of the microglia–neuron interactions. The team first demonstrated by a transcriptomic analysis that microglia sense changes in neuronal activity and respond by distinct alterations in gene expression. Next, microglia in adult mice were ablated by inhibition of colony-stimulating factor 1 receptor. This manipulation did not affect the animal behavior at baseline, but they became hyperresponsive to neurostimulants: a subthreshold dose of kainic acid resulted in seizures in 90% of microglia-depleted mice compared to only 11% of controls. Similarly, injection of the $GABA_A$ antagonist picrotoxin or an experimental dopaminergic D_1 agonist prolonged seizures in mice lacking microglia. Two-photon in vivo imaging of neuronal calcium responses in the dorsal striatum demonstrated increased neuron synchrony and simultaneous firing in microglia-depleted mice. Vice versa, neuronal activation increased the extension of microglial processes toward active neurons, which was prevented by blocking ADP sensing. So, neurons which release ATP lure microglial processes, but what happens then?

The authors found that isolated microglia can convert ATP to adenosine which acts on adenosine receptors in active neurons and suppresses their activity. In vivo, microglia depletion was associated with reduced striatal extracellular adenosine levels. Finally, microglial negative feedback mechanisms were not restricted to the striatum: they occurred in vivo and ex vivo in cortical neurons stimulated by kainic acid or glutamate, respectively.

Taken together, these studies suggest that the microglial ability to sense and respond to mediator release from active neurons is key for controlling neuronal function in the healthy brain. But can microglia prevent hyperexcitability and hypersynchrony in epilepsy? In inflamed brain tissue, microglia retract rather than extend their processes in response to purinergic stimuli, likely due to a switch in receptor expression. In a recent study in epileptogenic brain tissue from rodents and humans, low-intensity purinergic stimuli triggered process extension, whereas high-intensity stimuli led to process withdrawal, mediated by $P2Y_1$ and $P2Y_{13}$ receptors.[10] The question then becomes—how do the microglial "brakes" that control neuronal activity change during epileptogenesis? Is it a cause of seizures, a result, or both? Clarifying the downstream signaling pathways that are associated with such changes and the relationships of neuronal inhibition with other microglial functions is pivotal for treatment development. The importance of microglia for neuroprotection was proven through their selective depletion in rat models of epilepsy, which aggravated seizures, increased neuronal degradation in the hippocampus, and was associated with higher mortality rates.[11] In addition, unless microglia-targeted therapies are applied locally to the brain, they might affect the activity of monocyte-like cells in the blood, liver, lungs, and other organs and lead to peripheral immunomodulation. Better understanding of the roles microglia play in epileptogenesis is therefore required for advancing microglia-targeted therapies to later phases of clinical development while avoiding failure related to off-target effects that may cause harm.

A key player in the microglia–neuron feedback system, adenosine signaling, has already been targeted in epilepsy. Adenosine-releasing implants exerted anti-seizure and antiepileptogenic activity in animal models. These effects were mostly attributed to neuronal targets and signaling mechanisms, including inhibition of DNA methylation. An inhibitor of adenosine kinase, the astrocytic clearance mechanism of adenosine, is under evaluation for the treatment of epilepsy.[12] Interestingly, increased purine levels are present in the cerebrospinal fluid (CSF) of rats following seizure, in the blood of patients with epilepsy when compared with healthy controls and in mice after status epilepticus.[13] The source of purines in blood is unknown but may not necessarily involve the brain itself. However, this raises the question of whether simply enhancing purinergic activity in the brain can help control seizures. Beyond purinergic transmission, the range of therapeutic targets can be extended to additional ligands of G_i protein–coupled receptors, including metabotropic glutamate receptors and cannabinoid receptors.

Clearly, there is more work to be done for translating the findings of Badimon, Merlini, and their colleagues to new therapies. Yet, the results of their studies are exciting in that they reveal a brand-new role of microglia in the intact brain, which can go wrong during epileptogenesis. Together, these

findings reshape the character of microglial processes to better remind the *Guardians of the Galaxy*'s Star-Lord: they go around space, hanging out with hot neurons . . . and through teaming up with other cells of the brain, find a higher purpose for themselves.

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