



# Staving Off the Heat With Microglia

## Microglial Displacement of GABAergic Synapses Is a Protective Event During Complex Febrile Seizures

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Complex febrile seizures (FSs) lead to a high risk of intractable temporal lobe epilepsy during adulthood, yet the pathological process of complex FSs is largely unknown. Here, we demonstrate that activated microglia extensively associated with glutamatergic neuronal soma displace surrounding GABAergic presynapses in complex FSs. Patch clamp electrophysiology establishes that the microglial displacement of GABAergic presynapses abrogates a complex-FS-induced increase in GABAergic neurotransmission and neuronal excitability, whereas GABA exerts an excitatory action in this immature stage. Pharmacological inhibition of microglial displacement of GABAergic presynapses or selective ablation of microglia in CD11b<sup>DTR</sup> mice promotes the generation of complex FSs. Blocking or deleting the P2Y<sub>12</sub> receptor (P2Y<sub>12</sub>R) reduces microglial displacement of GABAergic presynapses and shortens the latency of complex FSs. Together, microglial displacement of GABAergic presynapses, regulated by P2Y<sub>12</sub>R, reduces neuronal excitability to mitigate the generation of complex FSs. Microglial displacement is a protective event during the pathological process of complex FSs.

### Commentary

A common cause for seizures in infants is febrile seizures (FSs). Although FSs are often simple and do not recur, complex FS lasting longer than 15 minutes or recurring within a day are associated with high risk of developing refractory temporal lobe epilepsy in adulthood.<sup>1</sup> The mechanism for how this occurs is not well understood. Wan et al set out to understand mechanisms for how complex FS result in epileptogenesis in their study.<sup>2</sup> They were especially interested in a role for microglia as microglia are innate immune cells associated with inflammation, microglia are activated following complex FS,<sup>3</sup> and inflammation is involved in the development of FS.<sup>4</sup>

They employed a powerful combination of molecular tools, electrophysiology, and transgenic animals. Mice were subjected to one of several FS paradigms to mimic the duration and repeated seizure features of complex FS, exposing animals to a heat challenge and then continuing the exposure for 5, 15, or 30 minutes after the initial seizure, or exposing animals to heat challenges to induce 1, 3, or 6 repeated seizures spaced 2 hours apart. Complex FS (15- or 30-minute exposures, or repeated exposures) led to increased cortical area occupied by microglia compared to control or simple FS. Some microglia soma changed morphology to a cup shape, but none took on the appearance of a phagocytic microglia. This is an important distinction since phagocytosis is a major function of activated microglia.

That the microglia assumed a cup shape suggested neuron–microglia interaction.<sup>5</sup> They examined this possibility via dual staining for microglia with Iba-1 and cell bodies with Nissl, and by using a mouse model that expresses green fluorescent protein (GFP) in microglia (CX3CR1<sup>GFP/+</sup>) and co-labeling for the neuronal nuclear marker, NeuN. Contact area between microglia, neuronal somas, and neuronal processes increased with exposure duration and number. Interestingly, most neurons with extensive microglial apposition were glutamatergic, while very few of the contacted neurons were GABAergic.

They found that the microglia predominantly congregated on neuronal somas. Since GABAergic presynapses are known to surround cortical neurons, they wanted to next evaluate the association between the presynapses and microglia. For this they labeled presynaptic terminals with anti-vesicular GABA transporter (VGAT) in control CX3CR1<sup>GFP/+</sup> mice or CX3CR1<sup>GFP/+</sup> mice exposed to 30-minute FS. They found that in control mice there was a small, but nonsignificant decrease in VGAT+ presynapses surrounding neuronal somas extensively associated with microglia. Thirty-minute FS caused a large increase in VGAT+ presynapses on neuronal somas not associated with microglia; however, the coverage of neuronal somas by VGAT+ presynapses was reduced to control levels when they were associated with microglia, suggesting that activated microglia displace GABAergic presynapses on neuronal somas.





Since GABA exerts excitatory effects at this developmental stage,<sup>6</sup> microglial displacement prevented an increase in GABAergic neurotransmission and neuronal excitability that might otherwise have been triggered by FS. This was evidenced in whole-cell patch clamp recordings from pyramidal neurons by reduced frequency of spontaneous GABA-postsynaptic currents following FS when there was neuronal–microglial association versus not. There was no change in the amplitude of these currents. Similarly, there was a reduction in miniature GABA-postsynaptic currents, suggesting reduced vesicle release. Taken together with additional confirmatory experiments, the authors conclude that microglial displacement of GABAergic presynapses functionally abolishes the increase in unitary GABAergic synaptic contacts usually triggered by complex FS.


Pharmacologically preventing the microglial displacement of GABAergic presynapses via treatment with TRAM34, a blocker of Ca<sup>2+</sup>-dependent K<sup>+</sup> channel 3.1 activation, which promotes microglial activation, prevents microglial association with neuronal terminals, and impedes microglial displacement of GABAergic presynapses following a single 30-minute FS or 3 consecutive FS. Greater neuronal coverage by microglia correlated with longer latency to a third FS in the 3-seizure paradigm. Using a mouse model in which microglia express the human diphtheria toxin receptor (CD11b<sup>DTR</sup>), microglia were selectively ablated with diphtheria toxin treatment. Eliminating microglia in this manner caused an increase in GABAergic presynapses surrounding neuronal somas, indicating reduced presynapse displacement. This also led to a reduced latency to a third FS. Diphtheria toxin had no effect in control mice.

Given that purinergic receptors are known to control microglia activation, the authors next probed a role for the P2Y<sub>12</sub> receptor (P2Y<sub>12</sub>R) purinergic receptor. Blockade of P2 receptors with a broad P2 receptor antagonist, suramin, or P2Y<sub>12</sub>R receptors with selective P2Y<sub>12</sub>R antagonists, clopidogrel or MRS 2395, reduced microglial displacement of GABAergic presynapses, and shortened latency to complex FS. A broad P2 receptor antagonist that does not act on P2Y<sub>12</sub>Rs, pyridoxal-phosphate-6-azophenyl-2',4'-disulfonic acid, had no effect. P2Y<sub>12</sub> receptor was found to be highly expressed in microglia, especially those associated with neuronal somas. Deletion of P2Y<sub>12</sub> R in P2Y<sub>12</sub> R knockout mice resulted in a reduced microglial–neuronal interaction after 3 FS and an increase in surrounding GABAergic presynapses, suggesting that there was reduced microglial displacement of GABAergic presynapses in P2Y<sub>12</sub> R knockout mice. These mice also showed reduced latency to a third FS in the 3-seizure paradigm.


As the authors point out, while it is tempting to assume that activation of microglia after a complex FS would potentiate the effects of the seizure, the microglia actually serve to displace the GABAergic presynapses at glutamatergic neuronal somas and temper excitation. Thus, they keep the seizure in check by precisely modulating neuronal circuits. If this is part of the normal mechanisms to temper excitation, then it would be nice

to know what happens following complex FS in many patients that leads to the development of epilepsy later in life. Most rodents subjected to FS paradigms do not develop spontaneous seizures, but they are more susceptible to induced seizures.<sup>7</sup> Perhaps inhibiting GABAergic presynapse displacement would exacerbate susceptibility to induced seizures or would cause these animals to display spontaneous seizures. Rodent models of febrile status epilepticus do develop spontaneous seizures,<sup>8</sup> thus it would be interesting to know whether this presynapse displacement mechanism is somehow inhibited by the more severe insult, where there is also documented neuronal–glial interaction.<sup>9</sup>

Translational potential may lie in opportunities to positively drive this mechanism. Such an agent could be deployed as soon as it is apparent that an infant is having more than simple FS (ie, prolonged FS or multiple FS in 24 hours). This may stop the seizures in the present time and limit the development of epileptogenesis. Given that complex FS have such a high likelihood of leading to refractory temporal lobe epilepsy later in life, and refractory temporal lobe epilepsy carries a high rate of morbidity including high risk of sudden unexpected death in epilepsy, devising therapies to temper complex FS and prevent epileptogenesis has the potential to significantly impact patients with epilepsy.

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