Astrocytic TLR4 at the crossroads of inflammation and seizure susceptibility

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Astrocytes have been implicated in epilepsy development, but their contribution is under debate. In this issue, Shen et al. (2016. *J. Cell Biol.* https://doi.org/10.1083/jcb.201605046) demonstrate that early postnatal inflammatory stimuli activate toll-like receptor 4 signaling in astrocytes and promote excitatory synaptogenesis, thereby increasing seizure susceptibility in young and adult mice.

Intense research over the past 25 years has identified a multitude of mechanisms by which astrocytes rapidly modulate neuronal activity, in addition to their classical roles in potassium buffering and metabolic support. It is thought that astrocytes contribute to epileptic activity through two main mechanisms. First, physiological interactions between astrocytes and neurons could amplify aberrant network activity caused by neuronal dysfunction and thereby exacerbate epileptic activity (Henneberger, 2016). Second, it has been suggested that pathophysiological modifications of astrocytes represent the actual trigger for the development of epilepsy. For instance, impairment of the astrocyte glutamate/glutamine cycle reduces synaptic inhibition and thus renders a neuronal network more excitable (Ortinski et al., 2010). Indeed, astrocyte function is strongly altered in epileptic tissue and it is established that astrocytes are part of the pathophysiology of epilepsy. The potential causal role of astrocytes in the development of epilepsy is intensely studied (Ortinski et al., 2010; Bedner et al., 2015; Robel et al., 2015). The focus is often on temporal lobe epilepsy (TLE) because it is a very common form of epilepsy and often drug resistant (Engel, 2001; Schmidt and Löscher, 2005). TLE is thought to be triggered by an insult early in development that leads to the emergence of epileptic activity and seizures after a latent period, which in humans can last years. This delay requires a mechanism that after an initial injury persistently alters neuronal networks in, for instance, the hippocampus, which is a key region in the etiology of TLE. Febrile seizures in childhood are a risk factor for TLE (Engel, 2001), which suggests inflammation as one potential trigger of TLE. In this issue, Shen et al. show that early postnatal toll-like receptor 4 (TLR4) signaling in astrocytes promotes excitatory synaptogenesis and increases seizure susceptibility in mice, suggesting a possible direct role for astrocytes in shaping seizure development.

Several findings prompted Shen et al. (2016) to investigate the involvement of astrocyte TLR4 in mechanisms leading to enhanced seizure susceptibility in the context of early postnatal inflammation. First, TLR4 recognizes the bacterial membrane component lipopolysaccharide (LPS) and induces an immune response after detecting infection by specific bacteria (Okun et al., 2011). Second, activation of TLR4 is pro-convulsant and inhibition or knockout of TLR4 decreases the recurrence of acute and chronic seizures in mouse models of epilepsy (Maroso et al., 2010; Bedner et al., 2015). Third, TLR4 is expressed in microglial cells and also in astrocytes (Okun et al., 2011). Shen et al. (2016) found that low-dose intraperitoneal injections of the TLR4 ligand LPS in 2-wk-old mice prolonged the duration of seizures induced by the drug pentylenetetrazol (PTZ) in 3- and 10-wk-old mice (Fig. 1). Importantly, this prolongation of seizure activity was observed neither when 10-wk-old mice were injected with LPS nor in TLR4-deficient animals. These results demonstrate that TLR4 activation by LPS early after birth persistently increases seizure susceptibility. Shen et al. (2016) further tested the impact of LPS injection at the synaptic level. They observed that the frequency and amplitude of spontaneous, action potential–independent, excitatory postsynaptic currents and the number of dendritic spines of hippocampal CA1 pyramidal cells were increased when 2-wk-old mice were injected with LPS. The increases in excitatory currents and spine density suggest formation of new excitatory connections and strengthening of excitatory input received by hippocampal pyramidal cells. Again, LPS injection into older animals did not produce these effects. The differences in phenotypes between young and old mice indicate that LPS promotes excitatory synaptogenesis selectively in young animals, without a detectable effect on inhibitory synapses. These findings are generally in line with the earlier report of a critical period in the juvenile rat brain during which LPS increases adult seizure susceptibility (Galic et al., 2008). In addition, this previous study reported stronger excitatory postsynaptic field potentials and enhanced glial fibrillary acidic protein immunoreactivity in the adult hippocampal CA1 region after juvenile LPS administration, which is similar to the results reported by Shen et al. (2016).

Looking for the cellular mechanism underlying TLR4-mediated excitatory synaptogenesis, Shen et al. (2016) noted that injection of LPS leads to a TLR4-dependent activation of astrocytes, but not microglia, in young animals. This is a surprising result because microglia express TLR4 (Okun et al., 2011),

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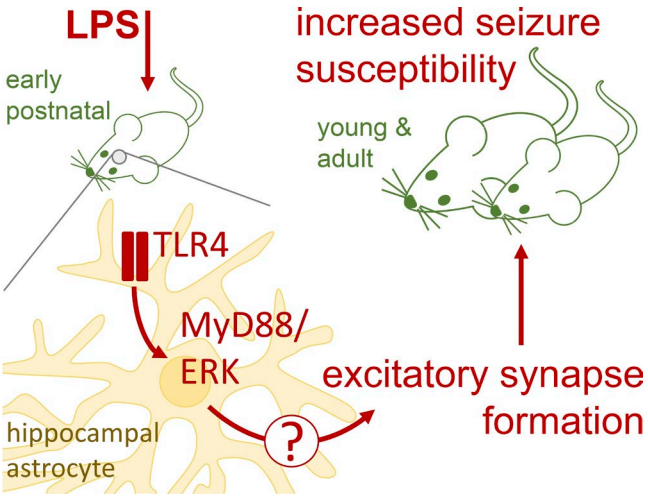

Figure 1. **Effects of an early postnatal inflammatory stimulus (LPS, intraperitoneal injection) on hippocampal synapse formation and seizure susceptibility.** Shen et al. (2016) find that LPS injection in young animals promotes the formation of excitatory, but not inhibitory, synapses between hippocampal neurons. The researchers further uncover that this process relies on astrocyte TLR4, MyD88, and extracellular signal regulated kinases (ERK) signaling and secretion of an unidentified factor. Importantly, the increased formation of excitatory synapses after early postnatal LPS injection was associated with higher seizure susceptibility in young and adult mice. These results suggest that an early inflammatory stimulus could increase seizure susceptibility in adult animals through astrocyte-mediated promotion of excitatory synapse formation.

and it is also at odds with the aforementioned study (Galic et al., 2008). It will be of interest to investigate the switch from astroglia to microglia activation, which Shen et al. (2016) only observed when older animals were treated with LPS.

Further, the researchers delved into the mechanisms of TLR4 signaling regulating synapse dynamics in vitro. LPS increased the growth of dendrites and their branching and the density of excitatory synapses detected by immunohistochemistry in mixed cultures, but not in pure neuronal cultures. Again, the increase of morphologically identified synaptic contacts was accompanied by an increased frequency and amplitude of excitatory, but not inhibitory, spontaneous postsynaptic currents. An important intracellular mediator of TLR4 signaling is myeloid differentiation factor 88 (MyD88; Okun et al., 2011). Growing neurons in the presence of astrocytes obtained from TLR4 or MyD88 knockout mice prevented the LPS-induced increase of excitatory synapse formation. Together, these results elegantly demonstrate that LPS-mediated excitatory synaptogenesis in vitro is mediated by astrocyte TLR4 and MyD88 signaling. Additional experiments revealed that culture medium from LPS-treated astrocyte cultures is sufficient to promote synapse formation, which therefore depends on a factor secreted from astrocytes into the medium. Further characterization of the intracellular signaling cascade uncovered an LPS-induced and astrocyte-specific up-regulation of ERK1/2 expression and phosphorylation, which also required astrocyte TLR4 and MyD88. Importantly, medium conditioned by astrocytes in which ERK1/2 was constitutively activated also induced excitatory synapse formation independently of MyD88, demonstrating that astrocytic ERK1/2 activity is sufficient to induce synapse formation.

Together, these culture experiments reveal that activation of an astrocyte TLR4–MyD88–ERK pathway increases the number of functional excitatory synapses via secretion of a factor that has yet to be identified, though several candidates exist. Accumulating evidence indicates that astrocytes are profoundly involved in the formation and elimination of synapses, also by secretion of several factors (Chung et al., 2015). Among those, thrombospondins (TSP1 and 2) are potent modulators of synapse formation (Christopherson et al., 2005; Chung et al., 2015). They are of particular interest in the context of the work by Shen et al. (2016) because they are expressed early during development and the antiepileptic drug Gabapentin inhibits TSP-induced synapse formation (Chung et al., 2015). However, TSP1 and TSP1/2 knockout mice unexpectedly display an increased sensitivity to PTZ-kindling, whereas TSP2 knockout mice are unaffected (Mendus et al., 2015). Therefore, it remains to be established if acute rather than chronic absence of TSP1/2 prevents LPS-induced synaptogenesis and increased seizure susceptibility, or if a different signaling molecule is involved.

It seems logical that the generation of new excitatory but not inhibitory synapses leads to network hyperexcitability and increased seizure susceptibility. How long perturbations of the balance between synaptic excitation and inhibition last is less clear, because such imbalances can be counteracted by, for instance, homeostatic plasticity (Turrigiano et al., 1998). Independent of these considerations, the findings from Shen et al. (2016) are in line with recent work reporting that activation of astrocyte-dependent albumin/TGF-β signaling is associated with increased excitatory but not inhibitory synapse formation and generation of spontaneous seizures in a model of post-injury epilepsy (Weissberg et al., 2015). Importantly, both Shen et al. (2016) and past work demonstrate that inhibiting synaptogenesis prevents an increase of seizure susceptibility/seizure occurrence (Weissberg et al., 2015; Shen et al., 2016). Thus, selective stimulation of excitatory synapse formation appears to be tightly associated with increased seizure susceptibility.

To directly establish the relevance of astrocyte MyD88 signaling in vivo, Shen et al. (2016) deleted MyD88 from about half of astrocytes using in utero electroporation of piggyBac plasmids targeting astrocytes in MyD88$^{flox/flox}$ mice. Under conditions of reduced astrocyte MyD88 expression, LPS injections into young mice did not shorten the time to onset of PTZ-induced seizure and did not prolong seizure duration, in contrast to control experiments. Similarly, the frequency and amplitude of spontaneous excitatory synaptic currents were not altered by LPS injections after deletion of astrocyte MyD88. These experiments demonstrate that both LPS-induced excitatory synapse formation and LPS-induced increased seizure susceptibility require astrocytic MyD88 signaling.

It will be of interest to explore if disruption of MyD88 signaling also reduces seizure susceptibilityor prevents/dampens seizure activity in other models of epilepsy, such as the post-injury epilepsy model or models of TLE (Bedner et al., 2015). Additionally, it will be important to determine if activation of astrocyte TLR4/MyD88 in young animals leads not only to an increased duration of PTZ-induced seizures but also to the occurrence of spontaneous seizures in older animals or if a second stimulus/insult is needed for seizures to emerge. In either case, hippocampal histology of adult animals will reveal to what extent low-dose LPS injection followed by excitatory synaptogenesis reproduces the histopathology of, for instance, human TLE.

Similarly, it will be of interest to test if LPS injections increase seizure susceptibility by triggering excitatory

synapse formation alone. There are several cellular targets, pathways, and mechanisms through which inflammation contributes to epilepsy (Vezzani et al., 2011). Therefore, it may be challenging to dissociate LPS effects on synaptogenesis from other mechanisms such as modulation of inwardly rectifying K^+ channels (Ivens et al., 2007) or gap junction coupling (Bedner et al., 2015). Importantly, Shen et al. (2016) demonstrate that astrocyte-specific deletion of MyD88 is sufficient to prevent LPS-induced formation of excitatory synapses and the increase of seizure susceptibility, which reduces the set of relevant mechanisms to those that involve astrocyte MyD88. Establishing the causal contribution of astrocyte TLR4–MyD88–ERK–dependent synapse formation to epileptogenesis will be helped by identifying the secreted factor or the corresponding receptor that promotes synaptogenesis, as disruption of their interaction could be used to dissociate the specific role of synaptogenesis and other LPS-mediated effects in seizure susceptibility.

Finally, the experimental approach for inducing inflammation may also require some consideration in the future. Systemic LPS administration and inflammation, for example, do not only activate TLR4 but also compromise the integrity of the blood–brain barrier (Mayhan, 1998) and lead to extravasation of albumin (Kovács et al., 2012). Under such conditions, astrocytes are activated, down-regulate the inwardly rectifying potassium channels (Kir4.1), lose gap junction communication, and acquire an impaired competence for K^+ buffering (Braganza et al., 2012; Kovács et al., 2012). These changes also lead to enhanced neuronal excitability; future work will reveal how and under which specific conditions the different inflammatory pathways are activated or converge to render the brain prone to epilepsy.

In summary, Shen et al. (2016) elegantly demonstrate that stimulation of an astrocyte TLR4–MyD88–ERK pathway early in development triggers excitatory synaptogenesis and increases seizure susceptibility (Fig. 1). Irrespective of the questions outlined here, Shen et al. (2016) provide novel and important experimental evidence on how an early inflammatory stimulus persistently increases neuronal network function via astrocytes and thereby increase seizure susceptibility. Thus, this study opens up novel routes for the investigation and manipulation of the disease process.

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