EPILEPSY CURRENTS

Current Literature

In Basic Science

Developmental Inflammation Takes a Toll: Early Immune Responses Increase Seizure Susceptibility via Astrocytic TLR4 Signaling

Postnatal Activation of TLR4 in Astrocytes Promotes Excitatory Synaptogenesis in Hippocampal Neurons.

Shen Y, Qin H, Chen J, Mou L, He Y, Yan Y, Zhou H, Lv Y, Chen Z, Wang J, Zhou YD. *J Cell Biol* 2016;215:719–734.

Astrocytes are critical in synapse development, and their dysfunction in crucial developmental stages leads to serious neurodevelopmental diseases, including seizures and epilepsy. Immune challenges not only affect brain development, but also promote seizure generation and epileptogenesis, implying immune activation is one of the key factors linking seizures and epilepsy to abnormal brain development. In this study, we report that activating astrocytes by systemic lipopolysaccharide (LPS) challenges in the second postnatal week promotes excitatory synapse development, leading to enhanced seizure susceptibility in mice. Toll-like receptor 4 (TLR4) activation in astrocytes increased astrocytic extracellular signal-related kinase 1/2 (Erk1/2) and phospho-Erk1/2 levels in a myeloid differentiation primary response protein 88 (MyD88)-dependent manner. Constitutively activating Erk1/2 in astrocytes was sufficient to enhance excitatory synaptogenesis without activating TLR4. Deleting MyD88 or suppressing Erk1/2 in astrocytes rescued LPS-induced developmental abnormalities of excitatory synapses and restored the enhanced seizure sensitivity. Thus, we provide direct evidence for a developmental role of astrocytes in shaping a predisposition to seizure generation.

Commentary

It is well established that the developing brain has an increased propensity for seizure activity compared to the adult brain,[1] but the reasons for this vulnerability are not well understood. While this particular sensitivity for seizure susceptibility is likely due to many varying factors, one emerging hypothesis is that neural inflammation, particularly early in development, predisposes the brain towards seizure induction.[2] Astrocytes, the most abundant type of glial cell in the central nervous system, are critically involved in synaptic development.[3] These cells are also hypothesized to play crucial roles in epilepsy[4] and to become reactive and contribute to immune responses,[5] making them intriguing candidates to link early immune responses in the developing brain to increased seizure susceptibility. The studies in the highlighted manuscript address the question of how astrocytic Toll-like receptor 4 (TLR4) activation affects postnatal synaptic development in the hippocampus and how this relates to early-life epileptic seizures induced by a strong peripheral immune response.

The authors first investigated the effects of inducing a strong immune response at different developmental stages on seizure susceptibility. They mimicked a strong immune response with a 3-day treatment of lipopolysaccharide (LPS), a common model of inflammation,[6] in young and adult mice. Seizure susceptibility and severity were then measured in response to pentylenetetrazol (PTZ) injections, a method for seizure induction in mice. Similar to previous studies,[2] the authors observed that younger, but not older, mice treated with LPS had a more rapid onset of seizures with longer duration as well as increased glutamate signaling following PTZ. TLR4 activation stimulates an innate immune response in the brain and can be triggered by molecules such as LPS.[7] The authors found that LPS did not influence seizure susceptibility in young TLR4$^{-/-}$ mice, which did not express TLR4, suggesting that this signaling pathway played a role in the seizure susceptibility observed in young mice.

Glial cells are particularly sensitive to immune responses but are also imperative in supporting synaptic development and function.[3,8] Therefore, the researchers next sought to determine how LPS affects glial cell activation in young and older mice, and how this may affect synaptic development. Because microglia are a subtype of glial cell thought to act as the brain's resident immune responder, activation of both microglia and astrocytes was examined. Younger mice had an increase in astrocyte activation following LPS treatment compared to saline controls, with no change in microglial activation. Adult mice, by contrast, showed the opposite response, with an increase in microglial activation following LPS treatment, but no change in astrocyte activation. These data suggest that effects of LPS on seizure

Epilepsy Currents, Vol. 17, No. 6 (November/December) 2017 pp. 370–371

susceptibility in young mice may be related to the activation of astrocytes but not microglia. Once again, this effect appeared to be mediated by TLR4 signaling, as increased astrocyte activation was not observed in young TLR4$^{-/-}$ mice. In neuron-astrocyte hippocampal cell cultures, used to investigate how astrocytes influence synaptic development, increases in the number of neuronal synaptic terminals, dendrite length and number of branch points were observed following treatment with LPS for 48 hours. These effects could be blocked by the TLR4 antagonist LPS-Rs. Of note, the increase in synaptic terminals was not observed when neuron-only cell cultures were treated with LPS for 48 hours. Taken together, these data suggest that astrocytic TLR4 signaling may underlie increased excitatory synapse development following a strong immune response.

A major downstream adaptor to TLR4 activation is myeloid differentiation primary response protein 88 (MyD88). Following exposure to LPS treatment, astrocytes—but not neurons (either alone in culture or co-cultured with astrocytes)—exhibited an increase in MyD88 expression. Furthermore, neurons in culture with astrocytes lacking MyD88 did not show increased synapse formation or dendritic branching following LPS treatment. Deletion of astrocyte-specific MyD88 in mice also decreased the susceptibility to PTZ-induced seizures following LPS treatment. In addition, Erk1/2 activation—downstream in the TLR4-MyD88 pathway in astrocytes—was necessary to drive the increase in synapse number following LPS-treatment. Together, these data indicate that TLR4 activation in neurons and astrocytes engages different downstream signaling pathways. Astrocytes signal through MyD88 and subsequently Erk1/2, which leads to an increase in synapse formation and dendritic morphological changes that occur following a strong immune response and that likely underlie increased seizure susceptibility.

While there does appear to be a relationship between the astrocyte TLR4-MyD88-ERK1/2 signaling pathway and excitatory synapse development, it is still unclear how astrocytes directly mediate this effect. Data obtained using a co-culture system in which neurons and astrocytes were cultured together, but did not make physical contact with one another, would suggest that this effect could be underpinned by astrocyte-secreted factors that are widely reported to be necessary for the formation, maturation, and maintenance of synapses.[3] It will be interesting to learn how the astrocytic TLR4 signaling pathway may be associated with any number of these secreted factors to mediate this effect. This work also utilized PTZ as the sole method of inducing acute seizure activity in mice. It would be valuable to determine if different models for seizure induction (such as other chemoconvulsants or electrical kindling[9,10]) would yield a similar sensitivity to LPS challenges and astrocyte TLR4-mediated signaling, and whether suppression of the astrocyte TLR4-MyD88-ERK1/2 pathway ameliorates epileptogenesis or chronic epilepsy. Finally, while this work focused on effects on glutamatergic synaptic terminals and signaling, GABAergic signaling is also important for synapse formation and strengthening early in development. Furthermore, $GABA_A$ receptor activation switches from excitatory in the developing brain to primarily inhibitory in the adult brain. It would be worth investigating how strong immune responses affect GABAergic signaling in both young and adult mice and whether this is also mediated by the astrocytic TLR4 signaling pathway.

In conclusion, this work highlights how astrocytes regulate excitatory synapse development via the TLR4-mediated signaling pathway during postnatal development and the potential relationship to early life seizure susceptibility. These studies thus provide more insight into the complex nature of neuron–astrocyte interactions in both physiological and pathophysiological states.

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