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Critical review

Microglia modulate the structure and function of the hippocampus after early-life seizures

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ABSTRACT

The hippocampus is a brain region well-known to exhibit structural and functional changes in temporal lobe epilepsy. Studies analyzing the brains of patients with epilepsy and those from animal models of epilepsy have revealed that microglia are excessively activated, especially in the hippocampus. These findings suggest that microglia may contribute to the onset and aggravation of epilepsy; however, direct evidence for microglial involvement or the underlying mechanisms by which this occurs remain to be fully discovered. To date, neuron–microglia interactions have been vigorously studied in adult epilepsy models; such studies have clarified microglial responses to excessive synchronous firing of neurons. In contrast, the role of microglia in the postnatal brain of patients with epileptic seizures remain largely unclear. Some early-life seizures, such as complex febrile seizures, have been shown to cause structural and functional changes in the brain, which is a risk factor for future development of epilepsy. Because brain structure and function are actively modulated by microglia in both health and disease, it is essential to clarify the role of microglia in early-life seizures and its impact on epileptogenesis.

1. Introduction

Microglia are brain-resident immune cells that monitor and survey their surrounding environment through continuous movement of their highly-ramified processes.[1,2,3] When the brain experiences damage and inflammation, microglia contribute to the maintenance of brain homeostasis by producing and releasing inflammatory mediators and phagocytosing dead cells or pathogens that invade the brain parenchyma.[4] In recent years, researchers have actively studied the function of microglia both under pathological and physiological conditions. It has been shown that microglia interact with neurons and control various neuronal developmental stages including neurogenesis, neural circuit formation and synaptic plasticity.[5] During embryonic development, microglia migrate from the yolk sac into the brain where they mature morphologically and functionally by approximately postnatal day 14 (P14).[6,7,8] Recent developments in gene analysis techniques have revealed that microglial gene expression drastically changes during maturation.[9] In particular, it has been reported that the morphology, function and gene expression patterns of microglia during the developmental stage are characteristic of this stage and different from those observed in adulthood.[7,8,10] These data indicate that such stage-dependent differences may be necessary for microglia to regulate the robust formation and refinement of neural circuits during development.

The developing brain is vulnerable to a variety of environmental stimuli, which leads to stimuli-induced changes in brain function and can result in central nervous system disorders.[11] For example, hyperthermia-induced febrile seizures are frequent in infants; prolonged complex febrile seizures are associated with an increased risk for future development of temporal lobe epilepsy. The effects of complex febrile seizures on the hippocampus, the main seizure focus in temporal lobe epilepsy, have been extensively studied. Using animal models of complex febrile seizures, several structural and functional changes in the hippocampus such as ectopic granule cells,[12] enhanced neurogenesis, mossy fiber sprouting of granule cells[13] and hippocampal hyperexcitability[14] have been reported. Such changes have been also identified in adult animal models of temporal lobe epilepsy.[15] Further, the involvement of microglia in these structural changes has been elucidated using adult epilepsy animal models.[16,17,18] However, the contribution of microglia to structural and functional changes in

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the hippocampus after early-life seizures has been poorly investigated. Because both neuronal electrophysiological properties and microglial gene expression patterns are different between childhood and adulthood,[19,9] it is possible that neuron–microglia interactions in epilepsy are varied depending on the developmental phase of the brain. However, to date, only a small number of studies have focused on the properties of microglia in animal models of early-life seizures such as febrile seizures, the most common convulsive event in children,[20] and postnatal acute seizures induced by convulsant administration. Thus, in this review, we discuss the possible molecular mechanisms by which microglia modulate the structure and function of the hippocampus after early-life seizures by comparing with and based on the previous studies using adult epilepsy models including kainic acid- or pilocarpine-induced seizures. The elucidation of microglial involvement may help establish new interventions to prevent the development of epilepsy caused by early-life seizures by regulating microglial phenotypes.

2. Microglial involvement in early-life seizures

Kong and colleagues examined the relationship between febrile seizures and microglia.[21] The authors specifically examined the involvement of microglial transient receptor potential vanilloid type 1 (TRPV1) using a mouse model of febrile seizures. TRPV1 is a cation channel that is activated by a variety of exogenous and endogenous stimuli, such as capsaicin and hyperthermia (>43 °C). TRPV1 expression in cortical microglia was increased 1 day after the induction of febrile seizure at P14. Additionally, the microglial expression levels of Iba1 and ED1 (lysosomal marker) and the proportion of microglia with ameboid morphology were increased, suggesting that microglia are activated after febrile seizures. These febrile seizure-induced changes were not detected in TRPV1 knockout (KO) mice. Further, in TRPV1 KO mice, the seizure latency was prolonged and the seizure duration was reduced, suggesting that microglial TRPV1 may contribute to both the development and exacerbation of febrile seizures. Activation of TRPV1 in cultured microglia enhanced microglial migration and proliferation, and decreased TGF-β1 signaling; TGF-β1 signaling normally suppresses the inflammatory activation of microglia. Based on these results, the authors speculated that febrile seizures promoted the inflammatory response of microglia through TRPV1 activation, leading to neural circuit degeneration and induction of spontaneous seizures.

Whether susceptibility to febrile seizures varies during development remains unknown. Kim and colleagues compared the degree of microglial activation in the hippocampus after febrile seizures at P5, P10, P15 and P20 in CX3CR1-GFP mice.[22] First, the authors examined the number and morphology of microglia in the hippocampus from P0 to P60, reporting that the number of microglia increased and the cell bodies of microglia became larger from P0 to P15, then both decreased rapidly from P15 to P20 and finally became constant. Next, the authors found that the rate of generalized seizures and mortality, as well as the rate of increase in the number and percent area of microglia in the hippocampus were highest when febrile seizures were induced at P15. They also measured expression levels of microglial cytokines 30 min or 4 h after febrile seizures, finding that inflammatory cytokines such as TNF-α, IL-1β and NOS-2 were elevated. From these results, the authors proposed that increased susceptibility to febrile seizures at P10–P15 may be mediated by microglial activation.

Using a two-hit rat model of kainic acid-induced seizures, Somera-Molina and colleagues showed that microglial activation is involved in epileptogenesis.[23] For this study, kainic acid or phosphate-buffered saline (PBS) was intraperitoneally administered at P15 and P45. Seizure latency after the P45 administration of kainic acid was significantly reduced and neuronal cell death was significantly greater in mice that were administered kainic acid at P15. These results indicated that kainic acid administration during development increased susceptibility to epileptic seizures. 24 h after the first kainic acid administration, the expression levels of IL-1β and clusterin increased in the hippocampal CA1, indicating that microglia were activated. Next, 24 h after the second administration of kainic acid, hippocampal CA1 expression level of Iba1, a microglial activation marker, was examined by immunostaining. The expression level of Iba1 was significantly increased in mice receiving kainic acid in both the developmental and adult periods, compared to mice that received a single administration of kainic acid in adulthood. These results suggest that early-life seizure-induced activation of microglia may contribute to increased seizure susceptibility in adulthood.

Also using a two-hit mouse model of kainic acid-induced seizures, Abraham and colleagues showed that microglial activation is involved in epileptogenesis during young adulthood.[24] Kainic acid or PBS was intraperitoneally administered to CX3CR1-GFP mice at P25 and P39. They reported that seizure latency after the P39 kainic acid administration was significantly reduced in mice that had received kainic acid at P25. 24 h after the first kainic acid administration, the fluorescence intensity of CX3CR1-GFP (microglial-derived) was increased compared to PBS-treated control animals in the dentate hilus, suggesting microglial activation. Next, 24 h after the second administration of kainic acid, the expression level of CX3CR1-GFP in the hippocampal CA3 was examined by immunostaining. The CX3CR1-GFP expression level was significantly increased in mice that were administered kainic acid at both P25 and 39, compared to mice that received a single administration of kainic acid in adulthood. To examine the involvement of microglia, the authors administered minocycline daily for 1 week (first dose 3 h after the first kainic acid administration) to suppress microglial activation. In the minocycline-treated group, the seizure latency after the second administration of kainic acid was increased compared to the PBS-treated group. The results indicated that microglial activation increased subsequent seizure susceptibility, similar to what was reported by Somera-Molina et al., 2007.[23] However, it should be noted that the above two studies[23,24] that utilized two hit models did not clearly show if microglia respond differently in early life since they did not compare two hit models with early-life and adult phases and two adult phases. Thus, it remains unclear if microglia respond to the second stimulation differently or it is due to the microglial response in early life.

Finally, though kainic acid is widely used for the purpose of inducing acute repetitive seizures that resemble status epilepticus in humans, it should be noted that kainic acid, which is a potent agonist for kainate-class ionotropic glutamate receptors could also modify glial properties in a way which is not necessarily related to seizures. In addition, although most studies have focused on the hippocampus alone, it will be important to investigate in the future studies whether and how the brain areas that sends axonal projections to the hippocampus could modulate microglial properties in early-life seizures via releasing several transmitters such as dopamine, serotonin and dopamine as well as glutamate and GABA.

3. Structural changes in the hippocampus are caused by early-life seizures and microglia play a role in facilitating these structural changes

3.1. Neurogenesis

Animal models of epilepsy have shown that adult neurogenesis is aberrantly enhanced in the hippocampal dentate gyrus after

epileptic seizures.[16,17,18] Excessive numbers of adult-born cells increase hippocampal excitability by forming ectopic neural circuits and subsequently triggering further epileptic seizures.[25] It has been suggested that microglia are not only involved in neurogenesis under physiological conditions but also after epileptic seizures.[16,17,18] Suppression of microglial activation with the tetracycline antibiotic minocycline or the fractalkine receptor inhibitor (rabbit anti-CX3CR1 antibody) has been shown to suppress neurogenesis after epileptic seizures.[26,27] Conversely, in the dentate hilus, microglia prevent neurogenesis by phagocytosing ectopic adult-born cells after kainic acid-induced seizures (Fig. 1).[28] Recently, it has been shown that the P2Y12 receptor, which is exclusively expressed by microglia in the brain, promotes neurogenesis after kainic acid-induced seizures (Fig. 1). The signals downstream of the P2Y12 receptor related to this observation remain unknown.[29] The P2Y12 receptor is activated by ATP released from neurons in an activity-dependent manner and promotes the motility of microglial processes.[2] RNA-Seq analysis of mouse brain tissue revealed that P2Y12 receptor gene expression is highest at P14.[10] In addition, because neuronal activity is also highly increased in experimental febrile seizures,[13] it is likely that the ATP-P2Y12 receptor signaling pathway is also activated during early-life seizures. Therefore, regulation of the P2Y12 receptor may also play a role in neurogenesis following early-life seizures. However, in a rat model of febrile seizures, the degree of neurogenesis at 3, 7 and 28 days following febrile seizures was similar to that in the control group.[13] It is not clear whether and how febrile seizures affect neurogenesis or what the involvement of microglia may be in this process. Developmental epileptic seizures in rats induced by pilocarpine increased neurogenesis, while induction by flurotyl decreased neurogenesis. Thus, it should be noted that differences in seizure induction methods may exert different effects on neurogenesis. Further, it is also possible that the modulation of neurogenesis by microglia may differ depending on the time frame when neurogenesis was investigated after epileptic seizures. For example, microglia could suppress neurogenesis by releasing inflammatory cytokines after acute epileptic seizures, while after status epilepticus, microglia could promote neurogenesis by secreting the neuroprotective factor IGF-1.[30,31]

3.2. Mossy fiber sprouting of granule cells

In the hippocampal dentate gyrus of both patients with refractory epilepsy and animal models of temporal lobe epilepsy, abnormal sprouting of mossy fibers (the axons of granule cells) has been observed.[32,33,34] Normally, granule cells project mossy fibers through the dentate hilus to hippocampal CA3, forming synapses with pyramidal cells. However, excessive mossy fiber branching in the dentate hilus and reverse projection to the molecular layer are observed in the epileptic brain. The sprouted mossy fibers are able to form excitatory recurrent circuits in the dentate gyrus, possibly exacerbating epileptic seizures.[35,36] The molecular mechanism underlying mossy fiber sprouting has been partially discovered: branching of mossy fibers is promoted by brain-derived neurotrophic factor (BDNF), and reverse projection of mossy fibers to the molecular layer is promoted by Netrin-1–UNC5 signaling.[37,38]

There has been no report that directly verified the relationship between mossy fiber sprouting in early-life seizures and microglia. However, all of the molecules mentioned below are expressed by microglia during development. Thus, it is possible that microglia modulate mossy fiber sprouting induced by early-life seizures. In a neonatal rat model of sepsis, microglial-produced IL-1β reduced

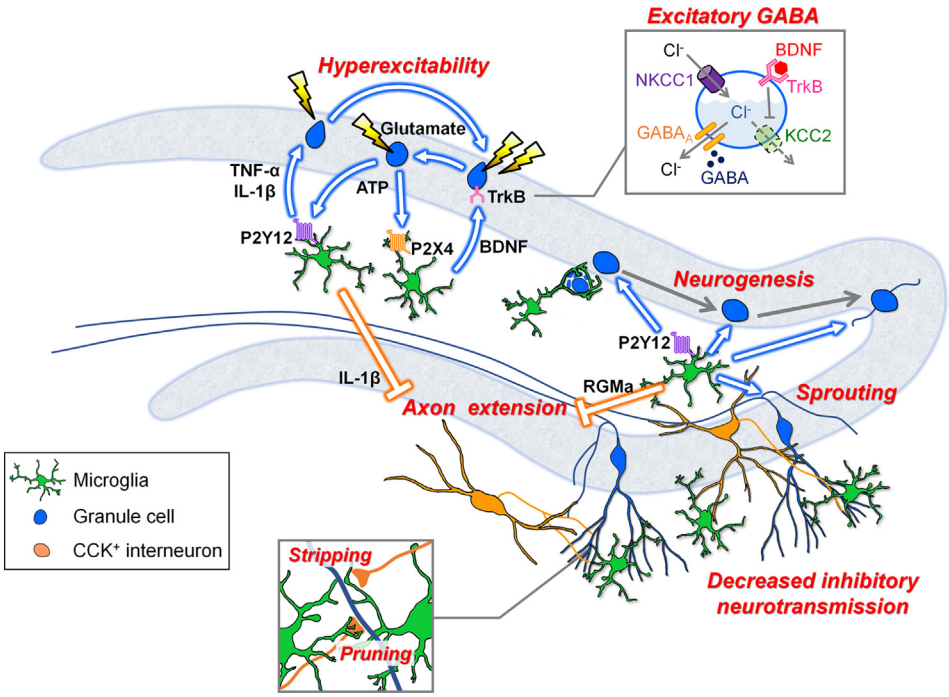

Fig. 1. Possible role of microglia in the hippocampus after early-life seizures. Neurogenesis: Microglia promote neurogenesis via P2Y12 receptor signaling; they may also prevent excessive neurogenesis by phagocytosing ectopic newborn cells. Mossy fiber sprouting and extension: Microglia promote sprouting via P2Y12 receptor signaling; however, they inhibit axon extension via secretion of IL-1β and RGMa. Hyperexcitability: Neuronal activity induces the release of IL-1β and TNF-α from microglia which further increases the excitability of neural circuits. Excitatory GABA: Neuronal activity-dependent release of microglial BDNF reduces KCC2 expression, increasing $[Cl^-]i$ which shapes excitatory GABA signaling. Decreased inhibitory neurotransmission: Microglia disrupt the E/I balance of hippocampal circuits by phagocytosing or stripping inhibitory inputs from newborn dentate granule cells. Abbreviations: BDNF, brain-derived neurotrophic factor; CCK, cholecystokinin; E/I, excitatory versus inhibitory; GABA, gamma-amino butyric acid; KCC2, K^+-Cl^- cotransporter; NKCCI, Na^+-K^+-$2Cl^-$ cotransporter; RGMa, repulsive guidance molecule A.

the expression levels of axon elongation factors.[39] As previously mentioned, IL-1β expression in microglia is elevated after febrile seizures[22]; therefore, microglia may suppress mossy fiber sprouting after febrile seizures (Fig. 1). Another candidate molecule that suppresses mossy fiber sprouting is repulsive guidance molecule A (RGMa). Shibata and colleagues found that RGMa application prevented hyperexcitability-induced mossy fiber sprouting in hippocampal slice cultures.[40] It was also shown that the expression level of RGMa in the hippocampal CA3 region was reduced after pentylenetetrazole-induced seizures in rats.[41] In mice, RGMa expression was increased in activated microglia after spinal cord injury, suppressing axonal outgrowth.[42] These findings suggest that microglial expression of RGMa may decrease in the hippocampus after epileptic seizures, promoting mossy fiber elongation and branching (Fig. 1). The microglial P2Y12 receptor was recently shown to promote elongation and branching of mossy fibers after kainic acid-induced seizures, although the mechanistic details remain unclear (Fig. 1).[29] On the other hand, microglia have been shown to preferentially contact with highly active neurons, suppressing neuronal activity.[43] In this process, attraction of microglial processes to neurons requires neuronal pannexin-1 hemichannels through which ATP is secreted. Therefore, deletion of P2Y12 receptors may inhibit microglial detection of ATP and their contact to highly active neurons, which could result in the impairment of decreasing neuronal activity and the aggravation of epileptic seizures.[44] The findings that P2Y12 receptors support aberrant neurogenesis and mossy fiber sprouting[29] may conflict with the idea that P2Y12 receptor plays anti-epileptic roles in case neurogenesis and mossy fiber sprouting are pro-epileptogenic. This contradiction in terms of the role of P2Y12 receptors on epileptogenesis would result from the wide range of P2Y12 receptors' functions, suggesting that it is necessary to regulate the expression levels and functions of P2Y12 receptor in a time-specific manner after or during seizures to inhibit the deterioration of epilepsy.

3.3. Hyperexcitability: disrupted synaptic E/I balance

In the epileptic brain, the excitatory versus inhibitory balance (E/I balance) of neural circuits favors excitability. Therefore, it is possible that disruption of the E/I balance in the hippocampus after early-life seizures contributes to the future development of epilepsy. Three-month-old rats that experienced febrile seizures at P10 or P11 showed a shortened seizure latency (kainic acid-induced) and increased duration of epileptiform electroencephalographic (EEG) in the hippocampus compared to controls.[45,13] The disrupted E/I balance is shaped by enhanced excitatory neurotransmission and reduced inhibitory neurotransmission. In the following, we discuss the possibility that febrile seizures disrupt the E/I balance and the potential involvement of microglia in this process.

3.3.1. Enhanced excitatory neurotransmission

As mentioned, experimental febrile seizures in mice induce abnormal mossy fiber sprouting, which may enhance excitatory neurotransmission in the dentate gyrus and lead to hippocampal hyperexcitability.[13,35] It has recently been suggested that neuronal hyperexcitability is induced by inflammation. The inflammatory response of microglia is enhanced in multiple epileptic seizure models, including models of early-life febrile seizures. Zhao and colleagues examined the contribution of microglial activation to the development of temporal lobe epilepsy.[46] They treated cultured microglia with coriaria lactone, which is used to experimentally induce temporal lobe epilepsy. They subsequently found both an increase in microglial proliferation and a morphologic change to an amoeboid shape, suggesting that the microglia were activated. In addition, the concentrations of IL-1β and TNF-α in the culture medium were increased. When this spent supernatant was administered to the lateral ventricle of rats, epileptic seizures and epileptiform EEG were induced. Furthermore, the concentration of glutamate in the cerebral cortex, hippocampal CA1, CA3 and dentate gyrus was increased compared to controls. These results suggest that activated microglia release IL-1β and TNF-α, thus increasing neuronal excitability and resulting in the development of epilepsy (Fig. 1). Kim and colleagues reported that the density of microglia in mice was highest in the hippocampus at P15 and that the severity of induced febrile seizures was highest when the seizures were induced at P10 or P15.[22] They further reported that the increase of microglial percent area and density was observed only when febrile seizure was induced at P15. Additionally, mRNA expression of inflammatory cytokines such as TNF-α and IL-1β was increased both immediately after induction of the seizures and 4 h later. Taken together, these findings suggest that microglia are able to contribute to increased hippocampal excitability by enhancing inflammation after febrile seizures.

In contrast to the above findings, it should be noted that increased inhibitory neurotransmission in the hippocampus has been also reported after febrile seizures.[47] Several mechanisms such as rebound depolarization and firing of neurons via an activation of a hyperpolarization-activated cyclic nucleotide-gated (HCN) channel[48] and the depolarization-induced suppression of inhibition (DSI)[49] have been suggested to underlie resulting enhanced excitatory neurotransmission. Whether or not microglia contribute to these phenomena remain to be studied.

One of the characteristic neurophysiological functions in the early developmental brain is excitatory gamma-amino butyric acid (GABA) signaling.[50] The intracellular Cl^- concentration ($[Cl^-]_i$) in immature neurons is higher than that in mature neurons, and activation of $GABA_A$ receptors by GABA causes Cl^- efflux which results in depolarization $[Cl^-]_i$ is mainly controlled by the Na^+-K^+-$2Cl^-$ cotransporter (NKCC1), which allows Cl^- to flow into cells, and the K^+-Cl^- cotransporter (KCC2), which allows Cl^- to flow out of cells. The timing of expression for these cotransporters is different: NKCC1 is highly expressed during development and expression decreases as cells mature, whereas KCC2 expression is low during development and increases as cells mature.[50] It has been reported that KCC2 expression is essential for GABA to function as an inhibitory neurotransmitter, and its expression is reduced in patients with temporal lobe epilepsy.[51] Given that GABA levels increase in the hippocampus of rats 2–6 h after febrile seizures are induced,[52] it is possible that febrile seizures cause hyperexcitation of the hippocampus by enhancing excitatory GABA signaling. The possible involvement of microglia in promoting excitatory GABA signaling in febrile seizures has been partially elucidated. In spinal cord injury models, it has been shown that ATP released from the injury site activates microglial P2X4 receptors, promoting BDNF release from microglia.[53,54,55] BDNF has been shown to reduce KCC2 expression by acting on TrkB receptors.[56] Microglial P2X4 receptor expression is enhanced in the mouse hippocampus following kainic acid-induced status epilepticus.[57] Since ATP is released from neurons in an activity-dependent manner, it is possible that the ATP concentration also increases in the hippocampus after febrile seizures. Taken together, it is possible that the neuronal activity-dependent release of microglial BDNF reduces KCC2 expression which subsequently increases $[Cl^-]_i$ and shapes excitatory GABA signaling (Fig. 1).

3.3.2. Reduction of inhibitory neurotransmission

It is likely that inhibitory neurotransmission is at least transiently enhanced in the hippocampus after febrile seizures, but the presence of long-term changes remains unclear. If inhibitory

transmission is enhanced after febrile seizures, this should work together with the developmental attenuation of excitatory GABA signaling to reduce neuronal excitability in adult individuals that experienced febrile seizures. Contrary to this idea, seizure susceptibility during adulthood is increased in experimental febrile seizures, indicating that the E/I balance of neural circuits tips toward excitation. Because the density of both excitatory pyramidal cells and inhibitory interneurons were comparable to the control in the rat hippocampus 3 months after febrile seizures were induced,[13] changes may occur at the synaptic level; however, few studies have performed detailed investigations of synaptic properties after epileptic seizures. Jackson and colleagues induced status epilepticus using electrical stimulation of the hippocampus of adult rats to examine the effect of seizures on synaptic changes. Seven days after inducing status epilepticus, a retroviral vector coding GFP was injected into the hippocampal dentate gyrus to label newborn neurons.[58] In the status epilepticus group, the number of excitatory postsynaptic protein (PSD95)-reactive puncta on the spines of 6-week-old neurons was decreased in the molecular layer, while the number of puncta positive for neuroligin (NL)-1, an excitatory postsynaptic adhesion factor, was unchanged. Decreased PSD95 expression may be a compensatory mechanism for increased excitability. In contrast, the number of gephyrin-reactive puncta, an inhibitory postsynaptic protein, on the dendrites of 3- and 6-week-old neurons was increased in the inner and outer molecular layers. This may also be a compensatory mechanism for increased excitability. However, in the status epilepticus group, the number of NL-2 clusters, an inhibitory post-synaptic adhesion factor, on the dendrites of 6-week-old neurons was significantly reduced, indicating that the function of inhibitory synapses was attenuated. In addition, the number of presynapses provided by cholecystokinin (CCK)-positive interneurons,[59] which are the main interneurons projecting to the inner molecular layer, was reduced. This decrease in NL-2 was not seen on the dendrites of 12-week-old neurons, suggesting a temporary decrease in inhibitory inputs to the inner molecular layer. At the same time the decrease in presynapses from CCK-positive interneurons was confirmed, it was observed that the density of CCK-positive interneurons in the hippocampal dentate hilus was similar between the status epilepticus and control groups,[60] indicating that seizures affect the structure of neural circuits at a synaptic level.

The involvement of microglia in seizure-induced synaptic changes has also been examined.[61] In rats with electrical stimulation-induced status epilepticus, the percentage of microglia that were in contact with the basal dendrites of 3-week-old neurons in the inner molecular layer was significantly higher than in control animals. Because the basal dendrites of the inner molecular layer mainly receive inhibitory synaptic input, this result suggests that microglia may temporarily reduce inhibitory synapses that project to newborn neurons following status epilepticus. Microglia are known to alter neural circuits by phagocytosing synapses both during brain development and in neurodegenerative diseases.[62] In addition, microglia block neuronal inputs by removing synapses on the neuronal cell body; this is referred to as synapse stripping.[63] Thus, it is possible that activated microglia disrupt the hippocampal E/I balance by phagocytosing or stripping inhibitory inputs from newborn dentate granule cells after early-life seizures (Fig. 1).

4. Conclusion

The role of microglia in the epileptic brain has mainly been studied using adult temporal lobe epilepsy models. Previous studies have suggested that microglia affect inflammation, neuronal death and neurogenesis after seizures. However, in epilepsy and seizure models using animals in the early postnatal developmental period, the properties of neurons, but not glial cells, have been the main target of study. In recent years, researchers have focused on the role of microglia in febrile seizure models and two-hit seizure models using animals in the early postnatal developmental period. Early-life seizures may cause neurodevelopmental disorders, cognitive dysfunction and epilepsy.[64] Therefore, elucidating the structural and functional effects of epileptic seizures on the developing brain and the involvement of microglia in these processes is expected to contribute to the discovery of the underlying mechanisms of these complications and to help facilitate the development of new therapies for epilepsy.

Up to 30% of patients with intractable epilepsy are resistant to existing anti-epileptic drugs, suggesting the need for therapeutic strategies other than the suppression of neuronal hyperactivity. In this review, we have discussed both the involvement of microglia and the molecular mechanisms underlying the structural and functional changes of the hippocampus after early-life seizures. Both the electrophysiological properties of neurons and the genetic and functional properties of microglia are distinct during development, emphasizing the need to understand the role of microglia in early-life seizures and making such studies an important component of basic biological research. The field of neuroscience has experienced recent advances in techniques that allow researchers to manipulate microglia spatiotemporally. For example, the $CX3CR1^{CRE/ERT2}$ mouse line allows researchers to investigate the necessity of microglia in the development of epilepsy by eliminating microglia both in a specific region of the brain and for a limited window of time. Furthermore, it is possible to verify the properties of human microglia in epilepsy by implanting induced pluripotent stem cell-derived microglia from epileptic patients into the brains of mice. These novel strategies will help to elucidate the involvement of microglia in early-life seizures.

Declaration of competing interest

The authors declare no conflict of interests.

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