ISSN 0006-2979, Biochemistry (Moscow), 2017, Vol. 82, No. 3, pp. 380-391. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © L. G. Khaspekov, L. E. Frumkina, 2017, published in Biokhimiya, 2017, Vol. 82, No. 3, pp. 528-541.

REVIEW

Molecular Mechanisms Mediating Involvement of Glial Cells in Brain Plastic Remodeling in Epilepsy

L. G. Khaspekov* and L. E. Frumkina

Research Center of Neurology, Department of Brain Research, 105064 Moscow, Russia; E-mail: khaspekleon@mail.ru

Received October 29, 2016
Revision received November 28, 2016

Abstract—In this review we summarize published data on the involvement of glial cells in molecular mechanisms underlying brain plastic reorganization in epilepsy. The role of astrocytes as glial elements in pathological plasticity in epilepsy is discussed. Data on the involvement of aquaporin-4 in epileptogenic plastic changes and on participation of microglia and extracellular matrix in dysregulation of synaptic transmission and plastic remodeling in epileptic brain tissue are reviewed.

DOI: 10.1134/S0006297917030178

Keywords: glial cells, neurons, epilepsy, plasticity

Glial cells participate in many important physiological processes such as binding and/or redistribution of ions during neuronal activity, regulation of neurotransmitter functions, and delivery of energy substrates to neurons [1]. Neuroglia are also involved in neurotransmission plasticity. Recent studies showed that morphological and functional impairments in neuroglia promote the development of epilepsy [2]. Alterations in cell membrane channels and receptors are observed in glial cells in epileptic tissues [3]. Direct stimulation of astrocytes is sufficient to induce neuronal synchronization in epilepsy models [4]. Modulation of glial receptors and channels might become an efficient approach for therapy of epilepsy because it does not require direct action on neurons, and, therefore, will reduce many unwanted side effects [5].

Epilepsy belongs to a group of brain disorders characterized by spontaneous seizures often accompanied by loss of consciousness. The major electrographic manifestations of epilepsy are excessive synchronized bursts of neuronal activity generated by one or several populations of neurons (i.e. epileptic focus) [6]. The extent of deteriorations in the epileptic network electrical activity is determined by the degree of synaptic excitation/depression imbalance that results in neuronal hyperexcitation and hypersynchronization due to the activation of excitatory neurotransmission and suppression of inhibitory neurotransmission [7]. This imbalance impairs some of the major brain functions related to synaptic plasticity, such as learning and ability for behavioral modifications. Astrocytes are involved in synaptic plasticity modulation by regulating its excitatory and inhibitory components.

The results of numerous studies have demonstrated that glia are involved in brain plasticity, i.e. in morphofunctional remodeling of neurons and neuronal networks during learning and memory formation, as well as in reparative and compensatory processes in cerebral pathologies. However, the data published on the role of glial cells in neuroplasticity in epilepsy have not been sufficiently reviewed. In this article, we summarize data on the role of glial cells in the molecular mechanisms of brain plasticity in epilepsy.

Abbreviations: ADAMTS, a disintegrin and metalloprotease with thrombospondin motifs; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AQP, aquaporins; AQP4, aquaporin-4 receptor; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; CSPGs, chondroitin sulphate proteoglycans; ECM, extracellular matrix; ECS, extracellular space; EPSP, excitatory postsynaptic potential; GABA, gamma-aminobutyric acid; HAPLN1, hyaluronan and proteoglycan link protein 1; LPS, lipopolysaccharide; LTD, long-term depression; LTP, long-term potentiation; mGluRs, metabotropic glutamate receptors; MMP, matrix metalloproteases; NMDA, N-methyl-D-aspartate; PDS, paroxysmal depolarization shift; PNN, perineuronal net; PV^+, parvalbumin-positive inhibitory interneurons; SE, status epilepticus; SIC, slow inward currents; TLR4, toll-like receptor 4; tPA, tissue plasminogen activator.

* To whom correspondence should be addressed.

ASTROCYTES AS ELEMENTS OF PATHOLOGICAL NEUROPLASTICITY IN EPILEPSY

It has been proven to date that the onset, development, and establishment of status epilepticus (SE) are to a great extent regulated by mechanisms also involved in the long-term plasticity, learning, and memory and closely related to the changes in the factors that modulate synaptic plasticity of excitatory and inhibitory pathways [8]. Astrocytes form tripartite functional subunits with presynaptic and postsynaptic structures that modulate synaptic transmission and neuronal plasticity. The interactions between neurons and astrocytes make it possible for calcium-stimulated release of glutamate from astrocytes to strengthen glutamatergic synaptic transmission via metabotropic glutamate receptors (mGluRs) located on presynaptic terminals. Although the mechanisms of cerebral pathologies are reasonably believed to be of neuronal origin, increasing evidence has appeared that disturbances in the interactions between neurons and astrocytes underlie many brain disorders. Astrocytes mediate changes in the expression of nerve cell proteins such as transporters, enzymes, receptors, and cytoskeletal proteins in various neuropathological conditions including epilepsy. Moreover, proinflammatory molecules might induce the release of ATP from microglia, and ATP modifies synaptic efficiency by affecting secretion of gliotransmitters from neighboring astrocytes [9].

The mGluRs might be the "molecular keys" to the changes in the synaptic plasticity of the epileptic network, in which glutamate-mediated gliotransmission acts as a signal for the increased excitability and neuronal hypersynchronization [10]. In several studies, group I and II mGluRs were found to be overexpressed in hippocampal astrocytes and neurons in patients with temporal lobe epilepsy or in its experimental models. These observations were confirmed in the kainate-induced epilepsy model in which mGluRs were also found to be expressed and localized in GFAP (glial fibrillar acid protein)-positive astrocytes of the hippocampus [11].

Kindling-induced enhancement of long-term potentiation (LTP) and population spike were abolished by selective antagonists of group I mGluRs [12]. Activation of tetrodotoxin-insensitive astrocytic calcium waves in an acute epilepsy model directly correlated with the increase in frequency of synchronous neuronal depolarization waves [13]. These waves preceded paroxysmal depolarization shift (PDS) or originated simultaneously with it. Some anticonvulsants block astrocytic calcium signaling and abolish epileptiform activity attenuated by NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonists that strongly indicates an important role of astrocytes as additional sources of glutamatergic excitation [4]. Therefore, glutamate release from astrocytes mediates glutamatergic imbalance in the epileptic network by maintaining high glutamatergic tone and providing excitatory neurotransmission at the seizure threshold level.

Recently, functional changes in astrocytes and effects of these changes on the plasticity of excitatory synaptic transmission in hippocampal slices of rats with rapid kindling-induced epilepsy have been studied [14]. These astrocytes exhibited increased spontaneous calcium-dependent signaling that manifested as long-term calcium transients and was associated with astroglial hyperexcitability mediated by hyperactivation of metabotropic purinergic receptors (P2Y1Rs). Such hyperexcitability activated gliotransmission mediated by astrocyte-secreted glutamate and, as a result, modified the efficiency of synaptic transmission by increasing the probability of neurotransmitter release due to the activation of group I mGluR, mGluR5. Therefore, increase in astroglial hyperexcitability and gliotransmission indicates that upregulation of glutamatergic transmission by astrocytes might decrease the excitation threshold in the epileptic brain.

At the postsynaptic level, glutamate release from astrocytes induces slow inward currents (SICs) that are mediated by activation of NMDA receptors and enter neighboring neurons [15]. These currents promote synchronization of neuronal networks and, due to the ability to induce cell depolarization at a considerable distance (up to 100 μm), simultaneously control the excitability of a whole group of pyramidal neurons. However, glutamate release from astrocytes is not necessary for the generation of interictal activity, but might modulate the strength of ictal seizures. Moreover, an increase in astrocytic calcium currents during acute epileptiform activity directly correlates with an increase in SIC frequency and either precedes PDS or occurs simultaneously with it. At the same time, the exact contribution of SICs to the hypersynchronized neuronal bursts typical for both ictal and interictal activities has not yet been established.

It was found that astrocytes are involved in the functioning of the endogenous cannabinoid system and respond to exogenous and endogenous cannabimimetics upon activation of type 1 cannabinoid receptors (CB1) [16]. This activation leads to calcium mobilization from internal stores, an increase in intracellular calcium levels, and stimulation of glutamate release by astrocytes. Released glutamate modulates synaptic transmission and plasticity. CB1 antagonists significantly reduce the maintenance of epileptiform discharge by preventing an increase in the calcium levels in astrocytes [17], which shows that epileptiform activity in the hippocampus involves gliotransmission induced by such increase.

The role of astrocytes in GABAergic transmission is less studied. Gamma-aminobutyric acid (GABA) causes $GABA_B$ receptor-mediated calcium oscillations in astrocytes [18]. Glutamate release from astrocytes can result in either depression or potentiation of inhibitory neuro-

transmission by promoting excitation/inhibition imbalance in target neurons. It is possible that the inhibitory effect of glutamate on GABAergic interneurons is mediated by several mechanisms, including decrease in the amplitude of miniature IPSPs (inhibitory postsynaptic potentials) and action potential-dependent GABA release caused by activation of kainate receptors. Besides, activation of group III mGluRs might suppress GABAergic transmission not only to individual interneurons, but to hippocampal pyramidal cells as well [19, 20].

These data suggest that activation by astrocyte-secreted glutamate of presynaptic group III mGluRs on GABAergic terminals together with activation of presynaptic group I mGluRs on glutamatergic terminals might be at least partially responsible for synchronous excitation/inhibition imbalance in the epileptic brain. The plastic modifications of synaptic efficiency and excitability of neuronal networks can involve also other astrocyte-secreted gliotransmitters. Thus, ATP causes an increase in the calcium levels in astrocytes and depolarization of interneurons. Similarly, D-serine released by astrocytes modulates NMDA receptor-mediated synaptic potentiation. Since interaction between neurons and astrocytes represents one of the varieties of synapse-specific cell-to-cell communications, it is possible that astrocytes serve as a source of glutamate that modulates the excitation/inhibition imbalance. However, it remains uncertain if astrocyte-secreted glutamate, D-serine, or ATP can regulate both glutamatergic and GABAergic plasticities [21].

The efficiency of learning and memory formation at the cellular level is now believed to depend on the duration of modulatory actions that mediate synaptic plasticity. Most probably, the changes in strength and plasticity of excitatory synaptic transmission are involved in the mechanisms of memory and learning. Recent studies showed that inhibitory neurotransmission not only undergoes plastic remodeling, but also modulates the efficiency and limits of excitatory synaptic plasticity. Moreover, in some brain regions manifestations of excitatory and inhibitory synaptic plasticities can be observed simultaneously [22]. Activation patterns that induce LTP in excitatory synapses can also induce short-term and long-term plasticity in inhibitory synapses. This functional balance between excitatory and inhibitory synapses, that is essential for normal brain functioning, is established during development and then maintained throughout life.

Astrocytes are located close to excitatory and inhibitory synapses, which allows them to integrate surrounding synaptic activity via release of gliotransmitters and control of synchronous depolarization of neuronal groups, lowering the threshold for synaptic plasticity, and inhibiting synaptic transmission. Due to these coordinating efforts, astrocytes help to maintain the balance between excitation and inhibition and specifically modulate neuronal network activity and plasticity [8]. As mentioned above, epilepsy is associated with dysregulation of excitation/inhibition balance of synaptic transmission. It is still unknown whether this imbalance is the background of epilepsy or the consequence of pathological patterns of neuronal activity. The astrocyte–neuronal network is a new pathogenetic key for explaining changes in synaptic plasticity that result in excitation/inhibition imbalance. Future experimental studies will explain the role of the astroglia–neuronal network in brain functioning and may reveal new therapeutic targets for treatment of convulsive disorders of the nervous system.

ROLE OF AQUAPORIN-4 IN EPILEPTOGENIC PLASTICITY

Changes in water and potassium homeostasis significantly affect an organism's susceptibility to seizures. The excitability of brain tissue strongly depends on the osmolarity and volume of the extracellular space (ECS). A reduction in ECS volume caused by hypoosmotic treatment induces hyperexcitability and enhances epileptiform activity as a result of elevated extracellular concentrations of neurotransmitters and ions and strengthening of ephaptic interactions between neurons due to the increased ECS resistance [23]. Increasing ECS volume by hyperosmotic treatment, on the other hand, results in the opposite effect, attenuating epileptiform activity [24]. These experimental results are in good agreement with clinical data that hypoosmolar states (e.g. hyponatremia) decrease the seizure threshold, while hyperosmolar states increase it [25]. Millimolar, or even submillimolar increase in the extracellular potassium concentrations considerably enhances epileptiform activity in the hippocampus; high potassium concentrations induce this activity in hippocampal slices of patients with pharmacoresistant temporal lobe epilepsy [26].

Aquaporins (AQPs) are a family of membrane proteins that function as water channels in many types of cells and tissues, in which water transport plays an important role. AQPs are small hydrophobic integral membrane proteins (monomer molecular weight, ~30 kDa) that facilitate bidirectional water transport by osmotic gradient [27]. AQP4 (aquaporin 4 receptor) is of great interest in neurobiology because it is expressed in the brain and spinal cord on specialized membrane domains of glial cells, primarily on astroglial endfoot membranes contacting with brain microvessels, and on astrocyte membranes that ensheath glutamatergic synapses [28]. It was found that radial water fluxes induced in the neocortex by neuronal activity represent water movement through AQPs in response to physiological activity [29]. Morphologically, AQP4s are structural components of orthogonal arrays of intramembranous particles that can be seen in electron micrographs obtained by the freeze-fracturing technique [30]. Being a potential regulator of edema, AQP4s might become a therapeutic target in the treatment of brain and

spinal cord injuries, ischemia, and seizures [31]. In the variety of pathological conditions, the alterations in AQP4s expression levels, water balance, water transport, and ion homeostasis might play an important role in the development of epilepsy.

Restoration of water and ion homeostasis is a new concept in epilepsy therapy that can be complementary to the standard methods of neuroprotection. Numerous experiments in animals and human brain tissues showed that epilepsy is accompanied by alterations in water and potassium homeostasis of glial cells [32]. In particular, AQP4 is downregulated in animals with SE state and in brain tissue of patients with epilepsy. This downregulation not only increases neuronal excitability as a result of pathological changes in water and potassium homeostasis, but also directly impairs synaptic plasticity (both LTP and LTD (long-term depression)) [33]. This might explain the fact that changes in astrocytes not only promote seizures but cause cognitive deficit. Indeed, patients with temporal lobe epilepsy suffer deterioration of cognitive functions, especially spatial memory [34].

As mentioned above, synaptic plasticity is strongly associated with epilepsy. The development of chronic spontaneous seizures after stroke or trauma (post-ischemic or post-traumatic epileptogenesis) is related to different types of plasticity, such as potentiation of glutamatergic pathways in the hippocampus, reorganization of neuronal circuits, and changes in postnatal neurogenesis. Since astrocytic AQP4s are involved in these plastic processes, they might also directly participate in the regulation of epileptogenesis [35]. Therefore, AQP4s ablation might be beneficial in epilepsy therapy. However, it was found that the absence of AQP4s in AQP4-knockout mice might exacerbate epilepsy by making the seizures less frequent but more prolonged [36]. AQP4s are downregulated in the hippocampus during epileptogenesis [32], but their contribution to synaptic remodeling and neurogenesis in adult epileptic brain is not understood. Thus, the role of AQP4 in the mechanisms of epileptogenesis requires further experimental studies.

INVOLVEMENT OF MICROGLIA IN PLASTIC REMODELING OF EPILEPTIC BRAIN TISSUE

Microglia play an important role in the functioning of normal brain and in various central nervous system (CNS) pathologies by closely interacting with neurons and modulating neurotransmission [37]. During development, microglia participate in the removal of apoptotic neurons [38], regulates neurogenesis and oligodendrogenesis [39], and promotes development of neuronal precursors and neuron survival [40]. In adult brain, microglia are involved in learning, memory, synaptic plasticity, and cognitive functions [41]. Microglia are activated in various pathologies and, depending on the pathology, may display either neurotoxic or neuroprotective effects. Therefore, microglia are important in both normal physiological functions and pathophysiological plasticity [42].

Morphological activation of microglia is very pronounced in epilepsy [43]. Thus, analysis of postmortem samples from patients with pharmacoresistant epilepsy revealed a multifold increase in reactivity toward specific microglial class II MHC (major histocompatibility complex) antigens in the CA1–CA3 region of the epileptic hippocampus. Similarly, an increase in microglial immunoreactivity was observed in patients with focal cortical dysplasia, a condition considered as epilepsy trigger. Moreover, the severity of epileptic seizures was found to be in direct correlation with the extent of microglial activation evaluated immunohistochemically from expression of specific antigens [43]. Therefore, persistent reactivity of microglia is a clinical index of epilepsy.

Pronounced morphological changes of microglia were found in mice with experimental epileptic seizures. Thus, 48 h after seizure induction by intracerebral injection of kainate, the number of microglial elements in the hippocampus increased, probably due to excitotoxic neuronal damage. When kainate was injected intraperitoneally, microglial activation was manifested as shortening and thinning of microglial processes with simultaneous increase in cell body size 24-48 h after the injection, which coincided in time with neuronal damage [44]. Therefore, microglial activation is delayed and might be a result of secondary response to the seizure-induced neuronal damage.

On the other hand, microglial activation might be initiated by seizures before neuronal damage starts, i.e. be induced by hyperactivity that precedes neuronal death. In the hippocampal CA1 region, microglia rapidly respond to seizures by increasing the number of processes and decreasing their arborization. This early response depends on the activation of neuronal NMDA receptors (NMDA-R), as confirmed in experiments that demonstrated rapid (within 5-10 min) response of microglia in brain slices to acute neuronal hyperactivity induced by NMDA-R activation [45]. The mechanism of this response might be related to the dependence of this activation on ATP release, since ATP stimulates microglial P2Y12 receptors, which results in the lengthening of cellular processes and formation of new ones.

Like kainate-induced seizures, pilocarpine-induced seizures also cause microglial activation in various brain regions 3 h to 3 days after seizure induction, at that intense arborization of processes along with their thickening is observed already 3 h after intraperitoneal injection of pilocarpine, while other manifestations of microglial hypertrophy develop later [46]. Therefore, numerous experimental models of epilepsy demonstrate rapid and persistent response of microglia to seizures, which represents strengthened ramification that precedes neuronal death and is sustained for days or even weeks.

Recently, it was found that microglia and neurons interact in the course of epileptiform activity induced by the removal of extracellular calcium. This interaction (so-called "convergence of microglial processes") is characterized by spontaneous directed local growth of microglial processes toward neuronal dendrites independently of the metabolic activity of astrocytes [47].

Resident microglia and peripheral monocytes/macrophages have common molecular markers [48]. Therefore, an increase in the number of microglial cell and macrophages after kainate or pilocarpine treatment might be a result of either cell migration/infiltration from other regions such as blood (a process that does not happen in healthy brain), or proliferation of the resident cell pool and *de novo* generation of microglia from neural, in particular nestin-positive precursors [49]. The microglial response is accompanied by activation of fractalkine receptor expression 1-6 h after the seizures [50]. Another important factor of neurogenesis is purinergic signaling, since expression of purinergic P2X7, P2X4, P2Y6, and P2Y12 receptors on microglial cells is upregulated after seizures [44]. Besides modulating microglial cell surface receptors, seizures activate expression of microglial cytokines such as TNF-α, TGFβ, and IL-1β and increase the activity of microglial proteases (cathepsins B, D, and S) [51].

Recently, experiments on genetic ablation of specific microglial proteins revealed that fine interactions between microglia and neurons allow the former to modulate plastic remodeling of neuronal activity. Genetic experiments were carried out on signal transmission mediated by fractalkine receptors, which are expressed by microglial cells in CNS parenchyma and bind fractalkine secreted by neurons. In fractalkine receptor knockout mice delayed functional maturation of synapses and impaired synaptic plasticity were observed [52]. The deficit of fractalkine signaling suppressed microglia propagation in the CNS and conversion of the NMDA receptor subunit GluN2B into GluN2A subunit during synapse development [53, 54]. Moreover, this deficit impaired neurogenesis, interneuronal connections, LTP, social behavior, and motor learning [55]. Genetic removal of another microglia-specific protein DAP12 caused LTP enhancement and activation of synaptic plasticity, probably due to alterations in the glutamate synaptic receptor composition that involve microglial brain-derived neurotrophic factor (BDNF) [56]. In *in vitro* experiments, microglia-conditioned nutrient medium (that contained glia-secreted proteins and glycine) potentiated EPSPs (excitatory postsynaptic potentials) and currents in cultured neurons and hippocampal slices [57].

The control of neuronal activity by microglia was demonstrated in experiments with lipopolysaccharide (LPS), a component of the outer cell membrane layer of gram-negative bacteria. In the brain, LPS binds to toll-like receptor 4 (TLR4). This receptor is expressed by microglia cells of the healthy brain, which suggests microglia is an immediate participant of LPS signaling. LPS potentiates microglia-dependent spontaneous EPSPs in neurons in hippocampal slices by a mechanism mediated by TLR4 activation. This process is accompanied by ATP release and astrocyte P2Y1 receptor activation, which in turn regulates the frequency of neuronal EPSPs as a result of glutamate release that activates presynaptic mGluR5 [9]. LPS injection *in vivo* also exacerbates neuronal excitability and seizures via TLR4 activation and the subsequent IL-1β signaling with the involvement of microglia [58]. At the same time, LPS-induced activation of microglia in combination with hypoxia suppresses synaptic transmission by inducing LTD [59].

In mammalian brain, activation of neuronal NMDA receptors caused a pronounced P2Y12-dependent activation of microglial process growth promoting contacts of microglia with neurons. Suppression of microglial response by knockout of P2Y12 receptor was accompanied by deterioration of epileptic phenotype [45]. Hence, P2Y12-dependent contacts between microglia and neurons might provide neuroprotective effects in epilepsy. This suggestion was confirmed in experiments on the developing *Danio rerio* brain, in that, during physiological activity microglial processes strengthened their contacts with hyperactive neurons, leading to suppression of neuronal activity [60]. Therefore, microglia regulate neuronal plasticity via several mechanisms, and such regulation can also take place in epilepsy.

The contribution of microglia to the development of epileptic seizures was studied in microglial P2Y12 receptor knockout mice [45]. This receptor is involved in rapid chemotactic response of microglia to ATP presumably released by hyperactive neurons [45]. Epileptic seizures in mice lacking P2Y12 receptor were much more pronounced than in the control animals, which directly indicates the involvement of microglia in the P2Y12 receptor-mediated neuroprotective mechanisms in epilepsy. The data show that microglia undergo morphological and biochemical changes during seizures, while at the same time regulating plastic remodeling of neuronal activity

ROLE OF EXTRACELLULAR MATRIX AND PERINEURONAL NET IN DYSREGULATION OF SYNAPTIC TRANSMISSION AND PLASTICITY IN EPILEPSY

As mentioned above, epilepsy is characterized by recurrent seizures that cause motor, sensory, cognitive, psychic, or vegetative disturbances. By themselves, the seizures represent the clinical manifestation of transient disorders of neural activity, and the phenotypic expression of each seizure is determined by the location of the hyperexcitability focus and the extent of its propagation

in the brain. Molecules of the extracellular matrix (ECM) regulate multiple aspects of nerve system development and plasticity in various brain regions. It is therefore evident that disturbances in the ECM structure might associate with many forms of epilepsy. Moreover, seizures activate expression of numerous ECM molecules and extracellular proteases. Pathological ECM rearrangements in epilepsy might initiate plastic remodeling causing many long-term morphofunctional changes in the CNS affecting the development of the disorder [61].

The main constituents of ECM in the CNS are glycoproteins (laminins, fibronectin, thrombospondins, tenascins) and also various proteoglycans (syndecan, glypican, agrin, aggrecan, versican, phosphacan), in which glycosaminoglycans, such as hyaluronan, heparan sulfate, chondroitin sulfate proteoglycans (CSPGs), etc. are covalently bound to the core protein [62]. Most of these glycoproteins and proteoglycans are synthesized by glial cells, predominantly by astrocytes.

Activation of astrocytes after brain injury leads to changes of the ECM composition. In addition to its supporting function, ECM is involved in the regulation of synaptic plasticity by modulating the structure, maturation, and functioning of synapses [63]. By surrounding the neurons, ECM affects the mobility of AMPA receptors and synaptic function. Changes in the ECM composition because of proteolysis are critical for structural remodeling of synapses. For example, it is known that tissue plasminogen activator (tPA) mediates experience-dependent pruning of dendritic spines in the visual cortex during development [64].

The role of ECM in synaptic plasticity is especially important regarding the perineuronal net (PNN), a specialized ECM structure that envelops neurons, primarily those generating fast spikes, such as parvalbumin-positive (PV^+) inhibitory interneurons [65]. The PNN is enriched with some types of proteoglycans, including neurocan, aggrecan, tenascin, and hyaluronan, and with proteoglycan link proteins [61]. This net tightly enwraps synapses at the cell body, proximal dendrites, and axon hillock [66], which allows it to efficiently regulate the processes of synaptic development, stabilization, and plasticity. For example, in hippocampal cell culture, enzymatic cleavage of PNN by chondroitinase ABC (ChABC) or hyaluronidase increases the number of interneuronal synaptic contacts. Moreover, enzymatic removal of PNN *in vitro* increases the diffusion constant of the AMPA receptor GluR1 subunit and the area occupied by these receptors at the cell surface [67]. Treatment with ChABC after formation of a posttraumatic scar in the spinal cord promotes axon regeneration and restoration of locomotor responses [68].

Due to the high content of inhibitory CSPGs, the PNN can suppress formation of new synapses and restrict synaptic reorganization [69]. Genetic ablation of link proteins activates plastic reorganization in the visual cortex of adult animals [70], and enzymatic cleavage of ECM together with PNN erases fear memories in the amygdala [71]. The appearance of PNN in postnatal ontogenesis coincides with the end of the critical period during which neurons undergo stabilization after plastic remodeling induced by functional activity [69].

During development, synapses, after setting at target cells, undergo a period of activity-dependent remodeling, while some of them are stabilized and others are eliminated. This period of CNS development is characterized by the predominance of structural plasticity. Mature ECM inhibits activity-dependent plasticity; however, damage to already formed nerve tissue might reactivate the mechanisms that act during brain development. Prolonged seizures or SE lead to synaptic reorganization in the hippocampus, including axonal sprouting and increase in the number and size of spines, probably due to activation of plastic processes [72]. These changes are accompanied by rearrangements in the extracellular environment, including the ECM, and promote plasticity during epileptogenesis.

Impairments of GABAergic signaling induce and sustain brain predisposition to SE. Since PNN closely surrounds inhibitory neurons, changes in the expression of PNN components might affect the morphofunctional state of GABAergic signaling. In inhibitory basket cells of fascia dentata that are most closely surrounded by the PNN among all hippocampal cells, pilocarpine-induced SE causes a reduction of synaptic inputs and outputs. Considering the role of PNN in synaptic stability and its location around GABAergic interneurons, it is possible that PNN promotes epilepsy [73].

Neurocan and phosphacan, which are dominating CSPGs in the developing hippocampus, were also found in PNN. It is believed that their expression is associated with the formation of axonal pathways, cell adhesion, lengthening and arborization of neurites, synaptic development, and synaptic plasticity [74-76]. Neurocan, which is expressed around developing mossy fibers, presumably acts as a barrier to prevent their uncontrolled promotion. The highest levels of neurocan expression are observed in the developing brain; however, in the adult epileptic hippocampus it is reexpressed [77]. Elevated neurocan reexpression might promote sprouting of mossy fibers and formation of new synapses in the granular layer of fascia dentata, as observed in rats of the Ihara line with congenital spontaneous epilepsy accompanied by the development of SE [78].

Neurocan is expressed not only in the PNN, but also in the ECM over the entire neuropil. Although ECM contains all members of the GSPG family, aggrecan, unlike other GSPGs and lecticans, is expressed only in the PNN, mostly around hippocampal PV^+ interneurons [79]. SE induction with kainic acid at early stages of development, i.e. before PNN formation, resulted in transient accelerated expression of aggrecan-positive PV^+

neurons that decreased by postnatal day 21. It is possible that such early PNN expression affects synaptic plasticity, although this assumption needs experimental testing. At the same time, SE induction at early developmental stages resulted in lesser neuronal damage, increased sensitivity to seizures, diminished mossy fiber sprouting, and less pronounced epilepsy [59].

In contrast, SE induction in adult animals resulted in serious neuronal damage, cell death, active sprouting of mossy fibers, and epilepsy. SE induced in adult animals, i.e. after PNN formation, caused stable (up to two months) decrease in the aggrecan-containing PNN, but did not suppress PV expression, which remains the same and even increased during the first week of SE [79]. Therefore, the absence of PV^+ cells does not cause decrease in the aggrecan-containing PNN. The disappearance of PNN two months after SE induction directly correlated with the decrease in aggrecan mRNA levels, thereby suggesting that the mechanism of this phenomenon is different from that with which decreased aggrecan transcription might promote alterations in the PNN one week after SE. Perhaps not only the content of the aggrecan-expressing PNN decreases, but also PNN loses its structural integrity with the formation of PNN degradation products.

One of the reasons for PNN destruction and appearance of its degraded form in the hippocampus after SE might be changes in the PNN-supporting structures. The disappearance of aggrecan is preceded by a decrease in the immunoreactivity of hyaluronan and proteoglycan link protein 1 (HAPLN1) and hyaluronan synthase 3 (HAS3), which are components of the PNN known to stabilize the connection between aggrecan and hyaluronan. It is known that the content of hyaluronan in the brain of patients with temporal lobe epilepsy and animal models of this disorder is increased. This increase, as well as the decrease in the contents of aggrecan, HAPLN1, and HAS3, might result in the elevation of extracellular unbound hyaluronan [79, 80]. Such changes in the extracellular space that accompany SE might activate neuron growth and promote synaptic plasticity.

In mature CNS, epilepsy induces changes in the extracellular space composition, such as activation of neurocan expression, disturbances in phosphacan biosynthesis, downregulation of aggrecan expression. These changes are similar to those that take place in immature CNS. In adults, SE produces deteriorating effect on the aggrecan-expressing PNN. One of the major functions of PNN is to bind hyaluronan in the extracellular space. Suppression of proteoglycan expression, which impairs binding of excessive hyaluronan, might enhance the plasticity of a neuronal network and promote epileptogenesis [79].

Another reason for the disappearance of PNN and appearance of its degradation products in the hippocampus is PNN enzymatic cleavage. It is believed that lectican-hydrolyzing proteases, such as ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) and matrix metalloproteases (MMPs), are activated by seizures. SE is followed by the appearance of structurally damaged PNN resulting from its degradation exacerbated by the deficit of HAPLN1 and HAS3. In the CNS, lecticans are degraded by ADAMTS4/5. *ADAMTS4* mRNA is actively expressed in various CNS regions including the hippocampus. Kainate-induced seizures dramatically upregulate *ADSMTS4* mRNA transcription, which directly correlates with an increase in the hippocampal content of the degradation products of brevican, a member of the lectican family [81].

MMPs are membrane-bound or secreted zinc-dependent proteases involved in ECM remodeling. Moreover, MMPs cleave lecticans and participate in many pathological processes in the CNS. MMP-9 plays an important role in the pathogenesis of epilepsy [80]. *MMP-9* knockout mice displayed lower excitability, and seizures induced by pentylenetetrazole (PTZ) kindling were less severe in these animals. On the contrary, mice overexpressing MMP-9 were more susceptible to PTZ kindling. Moreover, MMP-9 activity after kainate-induced SE increased in the areas close to hippocampal synapses. Expression of MMP-9 was activated 24 h after SE, reached its maximum after 72 h, and remained elevated for the next 7 days. The MMP-9 deficit prevented seizure-induced elimination of dendritic spines after kainate-induced SE. MMP-9 is also involved in PNN degradation in epileptogenesis.

Tissue plasminogen activator (tPA) is a serine protease that is only insignificantly expressed in the CNS. The substrates of tPA are CSPGs, and tPA activation results in site-specific lectican degradation mediated by MMP activation. The biosynthesis of tPA is upregulated in events that require synaptic remodeling or activation of neuronal activity, e.g. motor learning, kindling, and seizures [82]. In tPA knockout mice, the sprouting of mossy fibers and delayed seizure progression are reduced. Seizures promote the activity of tPA that either directly cleaves CSPGs and activates MMPs, or mediates CSPG degradation. Simultaneously, increased secretion of ADAMTS might cause lectican degradation.

In mice with modeled fragile X syndrome (Martin–Bell syndrome), aberrantly high tPA expression in glial cells was accompanied by impairments in cell migration and activity-dependent calcium responses [83]. Proteases of ADAMTS family regulate morphological properties of synapses in neuromuscular junctions of *Caenorhabditis elegans*. Proteolysis of proteoglycans by ADAMTS is associated with neuronal growth [84]. MMP-9 has been repeatedly demonstrated to play a crucial role in the regulation of synaptic structure and LTP, especially in aberrant synaptic remodeling in rodent models of epilepsy [85].

Epileptogenesis is a unique phenomenon that takes place in adult brain and involves active synaptic remodel-

ing, including ECM reorganization tightly associated with epileptic pathology [86]. For example, changes in the receptor expression of urokinase-type plasminogen activator are observed in humans with frontal lobe epilepsy and in animal models of this disorder [87, 88]. Mutations in reelin, an ECM component that regulates cortex stratification, cause spontaneous seizures in mice [89]. These data indicate the leading role of ECM in the regulation of the synaptic microenvironment in epileptogenesis.

Despite the demonstrated ability of ECM enzymatic cleavage to restore the critical period of plasticity and to increase the excitability of PV^+ cells *in vitro*, ECM degradation or removal of its key constituents result in neural network dysfunction [90]. For example, knockout of glycoprotein tenascin-C or CSPG brevican alters LTP in mouse synapses. Hippocampal neurons lacking tenascin-C, tenascin-R, brevican, and neurocan display abnormal synaptic structure and functions *in vitro* [91]. However, since enzymatic degradation or genetic ablation of ECM components affect not only the PNN around neurons, but also other PNN types including those surrounding excitatory synapses, PNN deprivation may not be the only reason for aberrant synaptic plasticity. Nevertheless, the above data suggest that ECM molecules, including its main component PNN, play a central role in the fine regulation of plasticity and functional integration of neuronal networks.

Because the underlying cause of epilepsy is excitation/inhibition imbalance, most anticonvulsive treatments are aimed at modulation of inhibitory signaling [92]. Considering that PNN is associated primarily with inhibitory, rapidly discharging PV^+ interneurons, it is possible that alterations in the ECM affect synaptic plasticity of these cells. In normal conditions, PV^+ neurons generate synchronized gamma oscillations, which makes possible coordinated activation of major cell assemblies required for information processing [93]. In *in vivo* models of epilepsy (e.g. post-traumatic epilepsy), alterations in rapidly discharging GABAergic interneurons are accompanied by functional deficiency and structural reorganization [94]. Optogenetic activation of PV^+ neurons in mouse cortical slices revealed functional dichotomy of these cells [95]. Selective activation of PV^+ neurons in the epileptic focus not only did not block generation of ictal activity, but rather triggered it by inducing post-inhibitory spikes of pyramidal neurons. On the other hand, activation of PV^+ cells in regions distant from the epileptic focus delayed propagation of seizures and shortened their duration in the focus. Besides, activation of PV^+ interneurons initiated low-voltage fast-onset seizures [96]. Although the question whether the effect of this activation on seizure generation is sufficient for the onset of chronic epilepsy remains to be elucidated, the critical role of PV^+ interneurons in neuronal synchronization is obvious.

It has been shown that seizures can be induced by changes in some ECM components, in particular by upregulation of tenascin-C and neurocan expression or downregulation of aggrecan and HAPLIN1 expression [73]. Impairment of PNN structural integrity was observed in rat brain in pilocarpine-induced SE [80]. Enzymatic treatment of ECM with ChABC decreased the threshold of myoclonic PTZ-induced seizures, but at the same time delayed their onset and decreased their duration. Mice deficient in tenascin-R, one of the main PNN components, displayed decreased perisomatic inhibition, increased excitatory neurotransmission, impaired LTP, and slowed kindling progression [97, 98]. All these facts are clear indications of the important plastic role of PNN in pathophysiology of seizures and epilepsy.

Close interactions between different cell types within the limits of neurovascular modules are the basis of the full-fledged activity of neuronal networks. Various types of brain damage, such as brain trauma or stroke, cause blood–brain barrier deterioration followed by astrocyte activation, which initiates a cascade of pathological reactions resulting in epilepsy. Because astrocytes are key components in ECM regulation, and ECM modulation is crucial for synaptic plasticity during CNS development or upon its damage, it is reasonable to suggest that the response of astrocytes to destructive processes can modify the extracellular space in a way that induces epilepsy [99]. Impairments in the composition and structural integrity of the ECM in general and PNN in particular directly affect neuronal activity and synaptic stabilization, especially in fast-spiking inhibitory interneurons, which in turn causes imbalance between excitation and inhibition. Such sequence of pathological events might underlie delayed seizure onset that is often observed in acquired post-traumatic or post-stroke epilepsy.

Until recently, it had been commonly believed that epilepsy is related mostly to the pathology of neurons. Only during the last decade, epilepsy has been associated with dysfunction of glial cells, primarily astrocytes. In normal brain, astrocytes are essential for maintaining water and ion balance. Besides, due to close interaction with neurons, astrocytes modulate synaptic plasticity via producing components of the extracellular matrix. Recent studies *in vivo* and *in vitro* have demonstrated that astrocytes and microglia are active participants of plastic brain tissue remodeling in epilepsy. Although neurons are principal brain cells that generate convulsive discharges, an increasing amount of experimental data indicate that deteriorations of the homeostatic functions of glia in epilepsy promote induction, development, and maintaining of pathological plastic rearrangements in epileptic nervous tissue.

However, our knowledge about the functional interactions between glial cells and neurons in conditions of epileptic pathology is still very limited. It remains unclear

how significant is the contribution of glia-mediated plastic processes to the development of epilepsy and how these processes could be corrected. It is not entirely clear whether plastic reconstructions mediated by glia is a contributing factor to the development of epilepsy, or they are its consequence. It remains to be seen whether glia mediate plastic reconstruction of neurons and interneuronal connections in epilepsy not only in experiment but also in humans. Search for answers to these questions may be the subject of future research whose results should contribute to the development of new approaches to treatment of epilepsy.

Acknowledgements

This work was supported by the Russian Foundation for Basic Research (project No. 14-04-1263).

REFERENCES

1. Ransom, B., Behar, T., and Nedergaard, M. (2003) New roles for astrocytes (stars at last), *Trends Neurosci.*, **26**, 520-522.
2. Halassa, M. M., and Haydon, P. G. (2010) Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior, *Annu. Rev. Physiol.*, **72**, 335-355.
3. De Lanerolle, N. C., and Lee, T. (2005) New facets of the neuropathology and molecular profile of human temporal lobe epilepsy, *Epilepsy Behav.*, **7**, 190-203.
4. Tian, G. F., Azmi, H., Takano, T., Xu, Q., Peng, W., Lin, J., Oberheim, N., Lou, N., Wang, X., Zielke, H. R., Kang, J., and Nedergaard, M. (2005) An astrocytic basis of epilepsy, *Nat. Med.*, **11**, 973-981.
5. Binder, D. K., and Carson, M. J. (2013) Glial cells as primary therapeutic targets for epilepsy, *Neurochem. Int.*, **63**, 635-637.
6. Meldrum, B. S. (1992) Excitatory amino acids in epilepsy and potential novel therapies, *Epilepsy Res.*, **12**, 189-196.
7. Khazipov, R., Valeeva, G., and Khalilov, I. (2015) Depolarizing GABA and developmental epilepsies, *CNS Neurosci. Ther.*, **21**, 83-91.
8. Bonansco, C., and Fuenzalida, M. (2016) Plasticity of hippocampal excitatory-inhibitory balance: missing the synaptic control in the epileptic brain, *Neural Plast.*, 28607038.
9. Pascual, O., Ben Achour, S., Rostaing, P., Triller, A., and Bessis, A. (2012) Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission, *Proc. Natl. Acad. Sci. USA*, **109**, E197-E205.
10. Tang, F.-R., and Lee, W.-L. (2001) Expression of the group II and III metabotropic glutamate receptors in the hippocampus of patients with mesial temporal lobe epilepsy, *J. Neurocytol.*, **30**, 137-143.
11. Steinhauser, C., and Seifert, G. (2002) Glial membrane channels and receptors in epilepsy: impact for generation and spread of seizure activity, *Eur. J. Pharmacol.*, **447**, 227-237.
12. Nagaraja, R. Y., Becker, A., Reymann, K. G., and Balschun, D. (2005) Repeated administration of group I mGluR antagonists prevents seizure-induced long-term aberrations in hippocampal synaptic plasticity, *Neuropharmacology*, **49**, Suppl. 1, 179-187.
13. Carmignoto, G., and Fellin, T. (2006) Glutamate release from astrocytes as a non-synaptic mechanism for neuronal synchronization in the hippocampus, *J. Physiol. Paris*, **99**, 98-102.
14. Alvarez-Ferradas, C., Morales, J. C., Wellmann, M., Nualart, F., Roncagliolo, M., Fuenzalida, M., and Bonansco, C. (2015) Enhanced astroglial Ca^{2+} signaling increases excitatory synaptic strength in the epileptic brain, *Glia*, **63**, 1507-1521.
15. Fellin, T., Gomez-Gonzalo, M., Gobbo, S., Carmignoto, G., and Haydon, P. G. (2006) Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices, *J. Neurosci.*, **26**, 9312-9322.
16. Navarrete, M., and Araque, A. (2008) Endocannabinoids mediate neuron–astrocyte communication, *Neuron*, **57**, 883-893.
17. Coiret, G., Ster, J., Grewe, B., Wendling, F., Helmchen, F., Gerber, U., and Benquet, P. (2012) Neuron to astrocyte communication via cannabinoid receptors is necessary for sustained epileptiform activity in rat hippocampus, *PLoS One*, **7**, e37320.
18. Mariotti, L., Losi, G., Sessolo, M., Marcon, I., and Carmignoto, G. (2016) The inhibitory neurotransmitter GABA evokes long-lasting Ca^{2+} oscillations in cortical astrocytes, *Glia*, **64**, 363-373.
19. Kogo, N., Dalezios, Y., Capogna, M., Ferraguti, F., Shigemoto, R., and Somogyi, P. (2004) Depression of GABAergic input to identified hippocampal neurons by group III metabotropic glutamate receptors in the rat, *Eur. J. Neurosci.*, **19**, 2727-2740.
20. Somogyi, P., Dalezios, Y., Lujran, R., Roberts, J. D. B., Watanabe, M., and Shigemoto, R. (2003) High level of mGluR7 in the presynaptic active zones of select populations of GABAergic terminals innervating interneurons in the rat hippocampus, *Eur. J. Neurosci.*, **17**, 2503-2520.
21. Lalo, U., Palygin, O., Rasooli-Nejad, S., Andrew, J., Haydon, P. G., and Pankratov, Y. (2014) Exocytosis of ATP from astrocytes modulates phasic and tonic inhibition in the neocortex, *PLoS Biol.*, **12**, e1001747.
22. Ahumada, J., Fernrandez de Sevilla, D., Couve, A., Buno, W., and Fuenzalida, M. (2013) Long-term depression of inhibitory synaptic transmission induced by spike-timing dependent plasticity requires coactivation of endocannabinoid and muscarinic receptors, *Hippocampus*, **23**, 1439-1452.
23. Wetherington, J., Serrano, G., and Dingledine, R. (2008) Astrocytes in the epileptic brain, *Neuron*, **58**, 168-178.
24. Haglund, M. M., and Hochman, D. W. (2005) Furosemide and mannitol suppression of epileptic activity in the human brain, *J. Neurophysiol.*, **94**, 907-918.
25. Maa, E. H., Kahle, K. T., Walcott, B. P., Spitz, M. C., and Staley, K. J. (2011) Diuretics and epilepsy: will the past and present meet? *Epilepsia*, **52**, 1559-1569.
26. Feng, Z., and Durand, D. M. (2006) Effects of potassium concentration on firing patterns of low-calcium epileptiform activity in anesthetized rat hippocampus: inducing of persistent spike activity, *Epilepsia*, **47**, 727-736.
27. Verkman, A. S. (2002) Physiological importance of aquaporin water channels, *Ann. Med.*, **34**, 192-200.

28. Nagelhus, E. A., Mathiisen, T. M., and Ottersen, O. P. (2004) Aquaporin-4 in the central nervous system: cellular and subcellular distribution and coexpression with Kir4.1, *Neuroscience*, **129**, 905-913.
29. Niermann, H., Amiry-Moghaddam, M., Holthoff, K., Witte, O. W., and Ottersen, O. P. (2001) A novel role of vasopressin in the brain: modulation of activity dependent water flux in the neocortex, *J. Neurosci.*, **21**, 3045-3051.
30. Wolburg, H., Wolburg-Buchholz, K., Fallier-Becker, P., Noell, S., and Mack, A. F. (2011) Structure and functions of aquaporin-4-based orthogonal arrays of particles, *Int. Rev. Cell. Mol. Biol.*, **287**, 1-41.
31. Hsu, M. S., Seldin, M., Lee, D. J., Seifert, G., Steinhauser, C., and Binder, D. K. (2011) Laminar-specific and developmental expression of aquaporin-4 in the mouse hippocampus, *Neuroscience*, **178**, 21-32.
32. Binder, D. K., Nagelhus, E. A., and Ottersen, O. P. (2012) Aquaporin-4 and epilepsy, *Glia*, **60**, 1203-1214.
33. Scharfman, H. E., and Binder, D. R. (2013) Aquaporin-4 water channels and synaptic plasticity in the hippocampus, *Neurochem. Int.*, **63**, 702-711.
34. Chin, J., and Scharfman, H. E. (2013) Shared cognitive and behavioral impairments in epilepsy and Alzheimer's disease and potential underlying mechanisms, *Epilepsy Behav.*, **26**, 343-351.
35. Szu, J. I., and Binder, D. K. (2016) The role of astrocytic aquaporin-4 in synaptic plasticity and learning and memory, *Front. Integrat. Neurosci.*, **10**, Art. 8.
36. Binder, D. K., Yao, X., Sick, T. J., Verkman, A. S., and Manley, G. T. (2006) Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels, *Glia*, **53**, 631-636.
37. Baalman, K., Marin, M. A., Ho, T. S., Godoy, M., Cherian, L., Robertson, C., and Rasband, M. N. (2015) Axon initial segment-associated microglia, *J. Neurosci.*, **35**, 2283-2292.
38. Ahlers, K. E., Karacay, B., Fuller, L., Bonthius, D. J., and Dailey, M. E. (2015) Transient activation of microglia following acute alcohol exposure in developing mouse neocortex is primarily driven by BAX-dependent neurodegeneration, *Glia*, **63**, 1694-1713.
39. Shigemoto-Mogami, Y., Hoshikawa, K., Goldman, J. E., Sekino, Y., and Sato, K. (2014) Microglia enhance neurogenesis and oligodendrogenesis in the early postnatal subventricular zone, *J. Neurosci.*, **34**, 2231-2243.
40. Arno, B., Grassivaro, F., Rossi, C., Bergamaschi, A., Castiglioni, V., Furlan, R., Greter, M., Favaro, R., Comi, G., Becher, B., Martino, G., and Muzio, L. (2014) Neural progenitor cells orchestrate microglia migration and positioning into the developing cortex, *Nat. Commun.*, **5**, 5611.
41. Sipe, G. O., Lowery, R. L., Tremblay, M. E., Kelly, E. A., Lamantia, C. E., and Majewska, A. K. (2016) Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex, *Nat. Commun.*, **7**, 10905.
42. Eyo, U. B., Murugan, M., and Wu, L.-J. (2016) Microglia–neuron communication in epilepsy, *Glia*, in press, doi: 10.1002/glia.23006.
43. Boer, K., Spliet, W. G., Van Rijen, P. C., Redeker, S., Troost, D., and Aronica, E. (2006) Evidence of activated microglia in focal cortical dysplasia, *J. Neuroimmunol.*, **173**, 188-195.
44. Avignone, E., Lepleux, M., Angibaud, J., and Nagerl, U. V. (2015) Altered morphological dynamics of activated microglia after induction of status epilepticus, *J. Neuroinflamm.*, **12**, 202.
45. Eyo, U. B., Peng, J., Swiatkowski, P., Mukherjee, A., Bispo, A., and Wu, L. J. (2014) Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus, *J. Neurosci.*, **34**, 10528-10540.
46. Jung, K. H., Chu, K., Lee, S. T., Kim, J. H., Kang, K. M., Song, E. C., Kim, S. J., Park, H. K., Kim, M., Lee, S. K., and Roh, J. K. (2009) Region-specific plasticity in the epileptic rat brain: a hippocampal and extrahippocampal analysis, *Epilepsia*, **50**, 537-549.
47. Eyo, U. B., Gu, N., De, S., Dong, H., Richardson, J. R., and Wu, L. J. (2015) Modulation of microglial process convergence toward neuronal dendrites by extracellular calcium, *J. Neurosci.*, **35**, 2417-2422.
48. Hickman, S. E., Kingery, N. D., Ohsumi, T. K., Borowsky, M. L., Wang L. C., Means, T. K., and El Khoury, J. (2013) The microglial sensome revealed by direct RNA sequencing, *Nat. Neurosci.*, **16**, 1896-1905.
49. Elmore, M. R., Najafi, A. R., Koike, M. A., Dagher, N. N., Spangenberg, E. E., Rice, R. A., Kitazawa, M., Matusow, B., Nguyen, H., West, B. L., and Green, K. N. (2014) Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain, *Neuron*, **82**, 380-397.
50. Ali, I., Chugh, D., and Ekdahl, C. T. (2015) Role of fractalkine-CX3CR1 pathway in seizure-induced microglial activation, neurodegeneration, and neuroblast production in the adult rat brain, *Neurobiol. Dis.*, **74**, 194-203.
51. Banerjee, M., Sasse, V. A., Wang, Y., Maulik, M., and Kar, S. (2015) Increased levels and activity of cathepsins B and D in kainate-induced toxicity, *Neuroscience*, **284**, 360-373.
52. Lauro, C., Catalano, M., Trettel, F., and Limatola, C. (2015) Fractalkine in the nervous system: neuroprotective or neurotoxic molecule? *Ann. N. Y. Acad. Sci.*, **1351**, 141-148.
53. Hoshiko, M., Arnoux, I., Avignone, E., Yamamoto, N., and Audinat, E. (2012) Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex, *J. Neurosci.*, **32**, 15106-15111.
54. Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T. A., Guiducci, E., Dumas, L., Ragozzino, D., and Gross, C. T. (2011) Synaptic pruning by microglia is necessary for normal brain development, *Science*, **333**, 1456-1458.
55. Rogers, J. T., Morganti, J. M., Bachstetter, A. D., Hudson, C. E., Peters, M. M., Grimmig, B. A., Weeber, E. J., Bickford, P. C., and Gemma, C. (2011) CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity, *J. Neurosci.*, **31**, 16241-16250.
56. Roumier, A., Bechade, C., Poncer, J. C., Smalla, K. H., Tomasello, E., Vivier, E., Gundelfinger, E. D., Triller, A., and Bessis, A. (2004) Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse, *J. Neurosci.*, **24**, 11421-11428.
57. Hayashi, Y., Ishibashi, H., Hashimoto, K., and Nakanishi, H. (2006) Potentiation of the NMDA receptor-mediated

responses through the activation of the glycine site by microglia secreting soluble factors, *Glia*, **53**, 660-668.
58. Rodgers, K. M., Hutchinson, M. R., Northcutt, A., Maier, S. F., Watkins, L. R., and Barth, D. S. (2009) The cortical innate immune response increases local neuronal excitability leading to seizures, *Brain*, **132**, 2478-2486.
59. Zhang, J., Malik, A., Choi, H. B., Ko, R. W., Dissing-Olesen, L., and MacVicar, B. A. (2014) Microglial CR3 activation triggers long-term synaptic depression in the hippocampus via NADPH oxidase, *Neuron*, **82**, 195-207.
60. Li, Y., Du, X. F., Liu, C. S., Wen, Z. L., and Du, J. L. (2012) Reciprocal regulation between resting microglial dynamics and neuronal activity *in vivo*, *Dev. Cell*, **23**, 1189-1202.
61. Dityatev, A., and Fellin, T. (2008) Extracellular matrix in plasticity and epileptogenesis, *Neuron Glia Biol.*, **4**, 235-247.
62. Novak, U., and Kaye, A. H. (2000) Extracellular matrix and the brain: components and function, *J. Clin. Neurosci.*, **7**, 280-290.
63. Dansie, L. E., and Ethell, I. M. (2011) Casting a net on dendritic spines: the extracellular matrix and its receptors, *Dev. Neurobiol.*, **71**, 956-981.
64. Mataga, N., Mizuguchi, Y., and Hensch, T. K. (2004) Experience-dependent pruning of dendritic spines in visual cortex by tissue plasminogen activator, *Neuron*, **44**, 1031-1041.
65. Wintergerst, E. S., Faissner, A., and Celio, M. R. (1996) The proteoglycan DSD-1-PG occurs in perineuronal nets around parvalbumin-immunoreactive interneurons of the rat cerebral cortex, *Int. J. Dev. Neurosci.*, **14**, 249-255.
66. Wang, D., and Fawcett, J. (2012) The perineuronal net and the control of CNS plasticity, *Cell Tissue Res.*, **349**, 147-160.
67. Pyka, M., Wetzel, C., Aguado, A., Geissler, M., Hatt, H., and Faissner, A. (2011) Chondroitin sulfate proteoglycans regulate astrocyte-dependent synaptogenesis and modulate synaptic activity in primary embryonic hippocampal neurons, *Eur. J. Neurosci.*, **33**, 2187-2202.
68. Bradbury, E. J., Moon, L. D., Popat, R. J., King, V. R., Bennett, G. S., Patel, P. N., Fawcett, J. W., and McMahon, S. B. (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury, *Nature*, **416**, 636-640.
69. Hensch, T. K. (2005) Critical period plasticity in local cortical circuits, *Nat. Rev. Neurosci.*, **6**, 877-888.
70. Carulli, D., Pizzorusso, T., Kwok, J. C., Putignano, E., Poli, A., Forostyak, S., Andrews, M. R., Deepa, S. S., Glant, T. T., and Fawcett, J. W. (2010) Animals lacking link protein have attenuated perineuronal nets and persistent plasticity, *Brain*, **133**, 2331-2347.
71. Gogolla, N., Caroni, P., Luthi, A., and Herry, C. (2009) Perineuronal nets protect fear memories from erasure, *Science*, **325**, 1258-1261.
72. Suzuki, F., Makiura, Y., Guilhem, D., Sorensen, J. C., and Onteniente, B. (1997) Correlated axonal sprouting and dendritic spine formation during kainate-induced neuronal morphogenesis in the dentate gyrus of adult mice, *Exp. Neurol.*, **145**, 203-213.
73. McRae, P. A., and Porter, B. E. (2012) The perineuronal net component of the extracellular matrix in plasticity and epilepsy, *Neurochem. Int.*, **61**, 963-972.
74. Garwood, J., Rigato, F., Heck, N., and Faissner, A. (2001) Tenascin glycoproteins and the complementary ligand DSD-1-PG/phosphacan-structuring the neural extracellular matrix during development and repair, *Restor. Neurol. Neurosci.*, **19**, 51-64.
75. Faissner, A., Heck, N., Dobbertin, A., and Garwood, J. (2006) DSD-1-proteoglycan/phosphacan and receptor protein tyrosine phosphatase-beta isoforms during development and regeneration of neural tissues, *Adv. Exp. Med. Biol.*, **557**, 25-53.
76. Okamoto, M., Sakiyama, J., Mori, S., Kurazono, S., Usui, S., Hasegawa, M., and Oohira, A. (2003) Kainic acid-induced convulsions cause prolonged changes in the chondroitin sulfate proteoglycans neurocan and phosphacan in the limbic structures, *Exp. Neurol.*, **184**, 179-195.
77. Heck, N., Garwood, J., Loeffler, J. P., Larmet, Y., and Faissner, A. (2004) Differential upregulation of extracellular matrix molecules associated with the appearance of granule cell dispersion and mossy fiber sprouting during epileptogenesis in a murine model of temporal lobe epilepsy, *Neuroscience*, **129**, 309-324.
78. Kurazono, S., Okamoto, M., Sakiyama, J., Mori, S., Nakata, Y., Fukuoka, J., Amano, S., Oohira, A., and Matsui, H. (2001) Expression of brain specific chondroitin sulfate proteoglycans, neurocan and phosphacan, in the developing and adult hippocampus of Ihara's epileptic rats, *Brain Res.*, **898**, 36-48.
79. McRae, P. A., Baranov, E., Rogers, S. L., and Porter, B. E. (2012) Persistent decrease in multiple components of the perineuronal net following status epilepticus, *Eur. J. Neurosci.*, **36**, 3471-3482.
80. Rankin-Gee, E. K., McRae, P. A., Baranov, E., Rogers, S., Wandrey, L., and Porter, B. E. (2015) Perineuronal net degradation in epilepsy, *Epilepsia*, **56**, 1124-1133.
81. Yuan, W., Matthews, R. T., Sandy, J. D., and Gottschall, P. E. (2002) Association between protease-specific proteolytic cleavage of brevican and synaptic loss in the dentate gyrus of kainate-treated rats, *Neuroscience*, **114**, 1091-1101.
82. Yepes, M., Sandkvist, M., Coleman, T. A., Moore, E., Wu, J. Y., Mitola, D., Bugge, T. H., and Lawrence, D. A. (2002) Regulation of seizure spreading by neuroserpin and tissue-type plasminogen activator is plasminogen-independent, *J. Clin. Invest.*, **109**, 1571-1578.
83. Achuta, V. S., Rezov, V., Uutela, M., Louhivuori, V., Louhivuori, L., and Castren, M. L. (2014) Tissue plasminogen activator contributes to alterations of neuronal migration and activity-dependent responses in fragile X mice, *J. Neurosci.*, **34**, 1916-1923.
84. Kurshan, P. T., Phan, A. Q., Wang, G. J., Crane, M. M., Lu, H., and Shen, K. (2014) Regulation of synaptic extracellular matrix composition is critical for proper synapse morphology, *J. Neurosci.*, **34**, 12678-12689.
85. Takacs, E., Nyilas, R., Szepesi, Z., Baracskay, P., Karlsen, B., Rosvold, T., Bjorkum, A. A., Czurko, A., Kovacs, Z., Kekesi, A. K., and Juhasz, G. (2010) Matrix metalloproteinase-9 activity increased by two different types of epileptic seizures that do not induce neuronal death: a possible role in homeostatic synaptic plasticity, *Neurochem. Int.*, **56**, 799-809.
86. Pitkanen, A., Ndode-Ekane, X. E., Lukasiuk, K., Wilczynski, G. M., Dityatev, A., Walker, M. C., Chabrol, E., Dedeurwaerdere, S., Vazquez, N., and Powell, E. M. (2014) Neural ECM and epilepsy, *Prog. Brain Res.*, **214**, 229-262.

87. Iyer, A. M., Zurolo, E., Boer, K., Baayen, J. C., Giangaspero, F., Arcella, A., Di Gennaro, G. C., Esposito, V., Spliet, W. G., Van Rijen, P. C., Troost, D., Gorter, J. A., and Aronica, E. (2010) Tissue plasminogen activator and urokinase plasminogen activator in human epileptogenic pathologies, *Neuroscience*, **167**, 929-945.
88. Lahtinen, L., Huusko, N., Myohanen, H., Lehtivarjo, A. K., Pellinen, R., Turunen, M. P., Yla-Herttuala, S., Pirinen, E., and Pitkanen, A. (2009) Expression of urokinase-type plasminogen activator receptor is increased during epileptogenesis in the rat hippocampus, *Neuroscience*, **163**, 316-328.
89. Kopjas, N. N., Jones, R. T., Bany, B., and Patrylo, P. R. (2006) Reeler mutant mice exhibit seizures during recovery from isoflurane-induced anesthesia, *Epilepsy Res.*, **69**, 87-91.
90. Slaker, M., Chuchill, L., Todd, R. P., Blacktop, J. M., Zuloaga, D. G., Raber, J., Darling, R. A., Brown, T. E., and Sorg, B. A. (2015) Removal of perineuronal nets in the medial prefrontal cortex impairs the acquisition and reconsolidation of a cocaine-induced conditioned place preference memory, *J. Neurosci.*, **35**, 4190-4202.
91. Geissler, M., Gottschling, C., Aguado, A., Rauch, U., Wetzel, C. H., Hatt, H., and Faissner, A. (2013) Primary hippocampal neurons, which lack four crucial extracellular matrix molecules, display abnormalities of synaptic structure and function and severe deficits in perineuronal net formation, *J. Neurosci.*, **33**, 7742-7755.
92. Schousboe, A., Madsen, K. K., Barker-Haliski, M. L., and White, H. S. (2014) The GABA synapse as a target for antiepileptic drugs: a historical overview focused on GABA transporters, *Neurochem. Res.*, **39**, 1980-1987.
93. Fries, P., Nikolic, D., and Singer, W. (2007) The gamma cycle, *Trends Neurosci.*, **30**, 309-316.
94. Ma, Y., and Prince, D. A. (2012) Functional alterations in GABAergic fast-spiking interneurons in chronically injured epileptogenic neocortex, *Neurobiol. Dis.*, **47**, 102-113.
95. Sessolo, M., Marcon, I., Bovetti, S., Losi, G., Cammarota, M., Ratto, G. M., Fellin, T., and Carmignoto, G. (2015) Parvalbumin-positive inhibitory interneurons oppose propagation but favor generation of focal epileptiform activity, *J. Neurosci.*, **35**, 9544-9557.
96. Shiri, Z., Manseau, F., Levesque, M., Williams, S., and Avoli, M. (2015) Interneuron activity leads to initiation of low-voltage fast-onset seizures, *Ann. Neurol.*, **77**, 541-546.
97. Saghatelyan, A. K., Nikonenko, A. G., Sun, M., Rolf, B., Putthoff, P., Kutsche, M., Bartsch, U., Dityatev, A., and Schachner, M. (2004) Reduced GABAergic transmission and number of hippocampal perisomatic inhibitory synapses in juvenile mice deficient in the neural cell adhesion molecule L1, *Mol. Cell. Neurosci.*, **26**, 191-203.
98. Hoffmann, K., Sivukhina, E., Potschka, H., Schachner, M., Loscher, W., and Dityatev, A. (2009) Retarded kindling progression in mice deficient in the extracellular matrix glycoprotein tenascin-R, *Epilepsia*, **50**, 859-869.
99. Kim, S. Y., Porter, B. E., Friedman, A., and Kaufer, D. (2016) A potential role for glia-derived extracellular matrix remodeling in postinjury epilepsy, *J. Neurosci. Res.*, **94**, 794-803.