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REVIEW

The Structure and Function of Glial Networks: Beyond the Neuronal Connections

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Abstract Glial cells, consisting of astrocytes, oligodendrocyte lineage cells, and microglia, account for >50% of the total number of cells in the mammalian brain. They play key roles in the modulation of various brain activities under physiological and pathological conditions. Although the typical morphological features and characteristic functions of these cells are well described, the organization of interconnections of the different glial cell populations and their impact on the healthy and diseased brain is not completely understood. Understanding these processes remains a profound challenge. Accumulating evidence suggests that glial cells can form highly complex interconnections with each other. The astroglial network has been well described. Oligodendrocytes and microglia may also contribute to the formation of glial networks under various circumstances. In this review, we discuss the structure and function of glial networks and their pathological relevance to central nervous system diseases. We also highlight opportunities for future research on the glial connectome.

Keywords Glia network · Gap junction · Calcium coupling · CNS diseases

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Introduction

Over the past few decades, accumulating evidence has suggested that glial cells are much more than supporting cells in the brain [1]. Glial cells, including astrocytes, microglia, oligodendrocytes, and neural glial antigen-2 (NG2) glia (also known as oligodendrocyte progenitor cells, OPCs), constitute >50% of the total number of cells in the rodent and human central nervous system (CNS) [2, 3]. These cells are vital for the maintenance of CNS homeostasis and are responsible for the regulation of a variety of physiological and pathological processes. Astrocytes form tripartite synapses with neurons and take part in synaptic pruning, K^+ buffering, and neurotransmitter release [4]. Astrocytes also contribute to the formation of the blood-brain barrier and participate in tissue-specific immune responses to injury *via* astrogliosis. Oligodendrocytes wrap around axons to form myelin, which allows rapid signal transmission and the metabolic support of neurons. Microglia are the guards of the CNS; they rapidly respond to injury and infection by secreting various pre- or anti-inflammatory cytokines [5]. NG2-glia are proliferative cells in the brain parenchyma that can differentiate into mature oligodendrocytes throughout their lifetime [5].

Brain functions have long been believed to be executed *via* neural circuits where information is interpreted as the temporal-spatial transmission of electrophysiological events or action potentials within one neuron and transferred from one neuron to another through rapid and precise neurotransmission. On top of this, neural circuits are further connected to form a large-scale brain network, which is the basis of cognitive function and intelligence [6]. To the same extent, glial cells also form networks both structurally and functionally. They exchange information with each other in various forms [5, 7]. Channels formed by connexins allow glia-glia communication *via* substrate exchange [8]. Moreover, glial

cells communicate with one another *via* secretory vesicles, especially when activated or stimulated [9]. Finally, Ca^{2+} waves may also carry information and influence both glial and neuronal activity [10–12].

Glial networks resemble neural circuits to some extent. The functions of neural circuits rely on synaptic structures formed between pre- and post-synaptic neurons through the release of neurotransmitters. Likewise, glial cells, in particular astrocytes and oligodendrocytes, form specific structures, termed gap junction channels, between each other; these facilitate a relatively stable spatial relationship between the two types of glial cells. In addition, glial cells regulate the state of neighboring neurons by releasing gliotransmitters or other biological substances. However, glial networks also possess distinct characteristics compared to neuronal networks. The links between cells in the glial network are not highly specific but are much more diverse, and this may ensure the redundancy of glial network functions. The signal transduction between glial cells is not as fast as an action potential, but it lasts longer and can have a long-term influence on brain homeostasis [13, 14].

In this review, we discuss the structural basis and function of glial networks focusing on the following two aspects: (1) glial networks in the healthy brain and (2) the pathological relevance of glial networks in the CNS.

Glial Networks in the Healthy Brain

Cell-to-Cell Contact *via* Connexins

Neurons communicate through synapses, whereas glial cells form relatively stable intercellular positional relationships with each other through gap junctions. Connexins (Cxs) are structurally-related transmembrane proteins that are assembled to form gap junctions in vertebrates and are pivotal for maintaining normal brain function [15, 16]. Many tissues and cell types express two or more members of the Cx family. For instance, Cx29, Cx32, Cx36, Cx37, Cx43, and Cx47 are expressed in the mouse brain, mainly on oligodendrocytes, neurons, endothelial cells, and astrocytes [15]. As a mediator of intercellular communications, Cxs first form hetero- or homo-hemichannels and insert into the plasma membrane. Then, with the aid of specific proteins, mainly cadherins, the hemichannels can dock with the hemichannels of adjacent cells to form gap junctions [8]. Since not all channels are the same, they usually share the property of allowing molecules up to 1–1.5 kDa to pass; for example, ions (K^+, Ca^{2+}, Na^+), second messengers, and small metabolites [8, 17].

Astrocyte Network

Functional tests and electrophysiological recordings have demonstrated that astrocytes are extensively coupled to each other by gap junctions in cortical, cerebellar, and hippocampal tissue slices [18]. Astrocytes mainly express three Cx isotypes, namely Cx43, Cx30, and Cx26 [19]. Cx43 is specifically expressed in astrocytes in CNS white matter [19]; despite that, it is the most abundant Cx in the mammalian brain. In the developing nervous system, astrocytes may directly arise from radial glia, from an astrocyte-restricted progenitor population, or from the proliferation of newborn astrocytes [20, 21]. Astrocytes migrate to a specific region in the CNS during development and stay quiescent in adults. These astrocytes are primarily connected by Cx43/Cx43 homo-channels to establish a syncytial system, which enables electrical coupling to rapidly and mutually minimize membrane potential differences among interconnected cells. Thus, syncytial isopotential is a physiological mechanism that efficiently coordinates astrocytic network activity at the network level [17, 22].

It is well known that neurons are circuity- and region-specifically connected. Interestingly, glial cells share some of these features in their connections. For example, in the olfactory glomeruli, thalamus, and anterior hypothalamus, Cx30 prevails, while in the hippocampus, Cx43 is dominant [23]. In contrast, Cx26 expression is restricted to certain sub-cortical regions, including the reticular thalamic and subthalamic nuclei, as well as the hypothalamus and meninges [24]. Regional heterogeneity in gene expression of Cx30, Cx43, and Cx26 may be correlated with their distinct functions. Previous studies have shown that Cx30 deficiency attenuates A2 astrocyte responses and induces severe neurodegeneration [25]. Cx43 mediates adhesion, energy metabolism, and neurodegeneration. In contrast, Cx26 is responsible for neurotoxic signaling [26], demonstrating the complexity of their functionality and regulation.

Furthermore, adult astrocytes arise from clonal divisions of early differentiated astrocytes and these clones may specify domains of distinct classes of astrocytes and perform diverse functions in different brain regions or even in the same brain region [27, 28]. For example, Martin and coworkers found that subpopulations of astrocytes selectively respond to activity in the specific medium spiny neuron subtype in the dorsal striatum [28], indicating that astrocytes can form functionally distinct subpopulations within the same brain region, where they potentially form circuitry-specific connections between each other.

Oligodendrocyte Connection

Oligodendrocytes are differentiated from OPCs and enwrap myelin around axons in response to several intrinsic and extrinsic cues after birth [29]. Myelin sheath gaps, a structure known as the node of Ranvier, enable the increases of both the speed and energy efficiency of action potential propagation and nerve conduction [30].

Oligodendrocytes express various connexin isotypes, such as Cx29, Cx32, and Cx47, which play a crucial role in ensuring effective communications within the oligodendrocyte network internally and with different types of glial cells externally. Oligodendrocytes are electrically and metabolically coupled *via* intercellular gap junctions with other oligodendrocytes (O/O junctions), as well as with astrocytes (O/A junctions) [24, 31]. O/O coupling is facilitated by gap junctions in homotypic configurations with homomeric hemichannels containing Cx32 or Cx47 [32]. Cx32 is mainly expressed in the white matter and is localized in the myelin sheath of large-diameter fibers forming intracellular gap junctions within the myelin sheath. Cx47 is expressed at an early developmental stage in all oligodendrocytes throughout the CNS and is mainly localized on the perikaryal and proximal processes of myelinating cells as well as on OPCs [32]. Cx32 and Cx47 may be localized in the same gap junction plaques on the oligodendrocyte soma. But they do not appear to form heteromeric channels. A deficiency of *Cx32* or *Cx47* in animals only leads to a modest reduction of myelin volume without behavioral defects. In contrast, *Cx32* and *Cx47* double knockout results in severe demyelination, massive apoptotic oligodendrocyte death, and early mortality [33–36].

In addition to O/O interaction, O/A gap junction channels are formed through heteromeric hemichannels of Cx47 on oligodendrocytes and Cx43 on astrocytes [37]. Previous studies on astrocyte-oligodendrocyte crosstalk have focused mostly on glial development, the regulation of gap junctions, myelination, and cellular response to CNS injury [38]. It was a popular idea in earlier times that astrocytes and oligodendrocytes share a common precursor [39–41], though recent studies using mice lacking transcriptional factors which determined cell fate disproved that theory and suggested that they have different precursors [42]. Coupling between oligodendrocytes and astrocytes constitutes a more stable glial network than the astrocyte network described before, which promotes K^+ re-distribution and ensures normal axonal activity [32]. Other than K^+ clearance, gap junctions between oligodendrocytes and astrocytes may also serve as metabolic support channels [22].

Neurons have a characteristic of clear orientation. Cell-to-cell communications between glial cells also show directional flow based on unique structures. Earlier work has implied that heterotypic O/A gap junctions exhibit a directional diffusion barrier for the movement of ions and larger negatively-charged molecules from cells expressing Cx47 to those with Cx43 [43]. Ions and small molecules pass unidirectionally from astrocytes into neighboring astrocytes and oligodendrocytes through gap junctions [44]. These studies indicate a potential control hierarchy between interconnected glial cells. It is worthy of note that the directional flow of ions/small molecules has only been demonstrated between astrocytes and oligodendrocytes *in vitro* in one study. Extensive investigations on this topic are required in the future.

Intriguingly, some molecules have been found to be involved in the physical connections between glial cells. For example, in the mouse brain, the PDZ domain-containing protein occludens-1 interacts with several Cxs in astrocytes and oligodendrocytes and helps to anchor signal molecules with the gap junction [45], revealing complex machinery that is a key for stabilizing the spatial relationship between glial cells.

Microglial Connections

It appears that microglia do not form gap junctions with each other or with other types of cells *in vivo* under both physiological and pathological conditions. However, a recent study demonstrated that microglia do have membrane-to-membrane contacts between neighboring microglia. The microglial network has been found to rely on F-actin-based tunneling nanotubes that facilitate the transfer of α-synuclein from overloaded microglia to naïve cells [46]. These findings indicate that even the most dynamic glia are able to link with each other to form a functional intercellular connection. It is worth noting that tunneling nanotubes were first discovered as a new type of transmission in cultured cell systems by Gerdes and co-workers [47]. Tunneling nanotubes can result in the formation of dynamic syncytial cellular networks among different cells in a variety of cell types [48]. The structure is used for intercellular communications by neurons and astrocytes in the CNS [49, 50]. Further research on how tunneling nanotubes are formed between microglia under physiological and pathological conditions and their regulation is required.

Functional Coupling Relies on Calcium Waves

Upon stimulation, neurons generate and conduct action potentials along their axons to affect target cells. Likewise, astrocytes are known to generate Ca^{2+} transients in their processes and sometimes they propagate along the process into the soma, and even between cells [12, 51]. Subsequently, the Ca^{2+} signals regulate the expression of genes involved in the target cells, such as oligodendrocytes and microglia.

Calcium Signaling in Astrocytes

Unlike neurons, astrocytes do not generate action potentials. However, these cells can communicate with each other *via* Ca^{2+} signaling. Ca^{2+} is a major player in astrocytes in encoding and transmitting information; this can regulate the release of gliotransmitters, such as glutamate and ATP, as well as gene expression [52, 53]. There are two types of Ca^{2+} signaling in astrocytes: Ca^{2+} transients and Ca^{2+} waves.

Both propagate in the intracellular milieu. However, Ca^{2+} waves may reflect long-range signaling that can propagate hundreds of micrometers and activate hundreds of cells [12].

Astrocytes intensively express receptors for most neurotransmitters, and respond to them through intracellular Ca^{2+} oscillations, followed by the propagation of intercellular Ca^{2+} waves. The mechanism by which intracellular Ca^{2+} rises is believed to start with phospholipase C activation and IP_3 production. The latter then triggers endoplasmic reticulum-dependent Ca^{2+} release [54].

The Ca^{2+} elevation can be restricted to a single cell, but it can also spread across adjacent non-activated cells as intercellular Ca^{2+} waves (ICWs). Astrocytes derived from cell culture, brain slices, and whole retina preparations are all capable of propagating ICWs [10, 55–57]. Although the velocities by which the Ca^{2+} waves travel vary according to different sample preparations and types of stimuli. Gap junction channel-mediated ICWs were the first pathway identified in astrocytes [58]. Many early studies support the idea that gap junction channels play an important role in the rapid propagation of Ca^{2+} waves, even though the gap junction blockers used may not be highly specific [59, 60]. The extracellular pathway of the spread of intercellular Ca^{2+} waves involves the activation of hemichannels and gliotransmitter release, first described by Osipchuk and Cahalan [61] in non-coupled mast cells. Later, Guthrie and colleagues [62] found that ATP is the extracellular molecule released by activated astrocytes. ATP or glutamate acting on plasma membrane receptors mediates the extracellular mechanism, and most brain regions are more responsive to ATP than glutamate except for the striatum [11, 58].

Whether the association of Ca^{2+} waves with behavioral performance and astrocytic networks can be computed remains a challenge. The computational model of Ca^{2+}-mediated astrocyte function, which was well described in detail by Manninen and Linne [63], provides their thoughts for future studies. One recent study showed that sleep and wakefulness are accompanied by state-dependent changes in astroglial activity [64]. Using miniature microscopy, they found that astroglial Ca^{2+} signals reach a peak when awake and are lowest when asleep. Notably, Ca^{2+} signals are most pronounced in astroglial processes [64]. These data suggest that glial cell networks regulate animal behavior in an enduring and large-scale manner that is different from neural circuits.

Calcium Signaling in Oligodendrocytes

Since communications between astrocytes are partially dependent on Cx-based Ca^{2+} waves, oligodendrocytes communicate with astrocytes through gap junctions as well. Indeed, using laser photo-stimulation and Ca^{2+} imaging in primary cultures, Parys and co-workers demonstrated the presence of bi-directional Ca^{2+} waves from astrocytes to oligodendrocytes that are sensitive to gap junction blockers [65]. Oligodendrocytes do not communicate with each other *via* Ca^{2+} waves. However, they do experience an increase in $[Ca^{2+}]_i$, which is mediated by numerous Ca^{2+} channels on the plasma membrane. Oligodendrocytes express voltage-gated Ca^{2+} channels [ligand-gated Ca^{2+} channels (i.e. AMPARs, NMDARs, and P2X7 channels), and GPCRs (i.e. mGluRs and mAChRs)], Ca^{2+}-sensing receptors, and Ca^{2+} release-activated Ca^{2+} channels, which make oligodendrocytes a perfect effector of glial Ca^{2+} signaling [66–68]. Cx abnormality in both oligodendrocytes and astrocytes induces demyelination [69].

Calcium Signaling in Microglia

Microglia are distributed across the entire brain; they account for 5–12% of the total number of cells in the mouse brain and 0.5–16.6% in the human brain depending on where they are located [2]. Under physiological conditions, microglia are characterized by a ramified morphology with a small soma and long and highly motile processes constantly surveying their territory.

Once activated by pathogen-associated molecular patterns or damage-associated molecular patterns (DAMPs), microglia have elevated intracellular free Ca^{2+} concentrations mediated by ionotropic and metabotropic receptors in the plasma membrane [70, 71]. These elevations, in turn, trigger effector functions of microglia, such as phagocytosis, chemotaxis, and the release of pro- and anti-inflammatory cytokines [72–74]. For example, ATP is a typical DAMP that can activate Ca^{2+}-permeable ligand-gated ion channel P2X receptors [75]. Microglia release ATP in response to glutamatergic AMPAR activation and subsequent PKC activation. This process causes Ca^{2+} release from internal ER stores and acts as positive feedback to allow microglia to rapidly shift states, recruit surrounding cells, and proliferate in response to stimuli [75].

In addition to ligand-activated Ca^{2+} signaling, microglia also undergo spontaneous transient elevations in intracellular Ca^{2+} [70]. Spontaneous Ca^{2+} transients can be recorded in both quiescent and activated microglia *in vivo*, *in situ*, and *in vitro*. Furthermore, these Ca^{2+} transients are not triggered by astrocytic Ca^{2+} waves and the frequency is increased in the absence of neuronal activity. However, in healthy young animals, spontaneous Ca^{2+} transients in cortical microglia measured *in vivo* are relatively rare and their physiological function remains unclear.

Pathological Relevance of Glial Networks in the CNS

Glia in the CNS are activated in response to pathological conditions, such as neurodegenerative diseases, psychiatric

disorders, brain tumors, and pain. Activated glia display a hyper-functional state [76]. The disturbed astrocytic network can exacerbate the progression of CNS disease and clinical symptoms, highlighting its potential for therapeutical intervention.

Neurodegenerative Diseases

Neurodegeneration is a chronic process with gradual loss of the structure and function of neurons, which are essential for mobility, coordination, strength, sensation, and cognition. Neurodegenerative diseases, including amyotrophic lateral sclerosis, Parkinson's disease (PD), Alzheimer's disease (AD), and Huntington's disease, occur as a result of neurodegenerative processes [77–79]. The deregulated glial network has been implicated in these diseases. Targeting dysfunctional glial networks, instead of the direct intervention in neuronal activity, could be a new therapeutic strategy against neurodegenerative diseases [80].

As previously described, glial network scaffolds and Ca^{2+} waves mediated by gap junctions have multiple functions in regulating glial and neuronal activity and cell-to-cell communication. For example, *Cx30*-deficiency attenuates A2 astrocyte responses and induces severe neurodegeneration in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride-induced mouse model of PD [25]. Moreover, analysis of GJA1 (Cx43) expression across 29 transcriptomic and proteomic datasets from post-mortem AD and normal control brains has revealed that GJA1 is strongly associated with AD pathology and cognitive decline [81]. Astrocytes lacking GJA1 exhibit reduced ApoE levels and impaired A β phagocytosis [81], suggesting the importance of *GJA1* in AD pathogenesis. At the network level, A $\beta_{25\text{-}35}$ has been reported to increase the intracellular pool of Cx43 but impairs the assembly of functional gap junctions between astrocytes [82]. This study provided a novel insight into the intercellular communication between astrocytes in AD and provided a new therapeutic strategy for AD intervention.

Besides a deficiency of gap junctions, they also mediate the systemic pathology of neurodegeneration. It has been reported that gap junctions can contribute to β oscillations in the basal ganglia of parkinsonian animal models and PD patients, and administration of a gap-junction inhibitor attenuates β oscillations and improves left forepaw akinesia [83].

Although currently there is no direct evidence showing connections between microglia, a recent study has revealed that, in the context of PD, microglia establish an "on-demand" functional network allowing the transfer of the burden of aggregated α-synuclein to neighboring microglia. Moreover, in addition to the a-synuclein burden, microglia also share mitochondria through tunneling nanotubes consisting of F-actin [46]. This process is crucial for PD pathogenesis, as its disruption leads to increased inflammatory profiles and cell death in PD [46].

Psychiatric Diseases

Astrocytic Cx dysfunction is closely associated with depressive-like behavior. In a mouse model of social defeat stress, the frequencies of sEPSCs in the medial prefrontal cortex (mPFC) and hippocampus were significantly reduced along with a decrease in astrocytic Cx30 and Cx43. And this reduction only occurred in the mPFC and hippocampus but not in the amygdala and ventral tegmental area, indicating that the region-specific function of the astrocyte network is pivotal [84].

Glioblastoma

Glioblastoma is an aggressive type of cancer that begins in astrocytes in the brain or spinal cord. Glioma cells are reported to form gap junctions with surrounding astrocytes and microglia *in vitro*. The hetero-cellular gap junctions between glioma cells and astrocytes/microglia are increased after a longer incubation period with a higher number of glioma cells, supporting glioma invasion, adhesion, and migration [59, 85].

Pain

Unlike many other neurological diseases, neuropathic pain has a rapid onset. As mentioned previously, the gap junction communication between astrocytes is mediated by Cx43. Nerve injury induces the expression of Cx43 in astrocytes and switches the function of Cx43 from forming gap junction to paracrine modulation, which causes increases in the release of glutamate, ATP, and chemokines [86, 87]. Peripheral nerve injury is characterized by mechanical allodynia, a pain evoked by normally innocuous stimulation, such as light touch. This causes remarkable microgliosis in the spinal cord. Microgliosis in response to nerve injury is elevated by ATP and the ATP receptor subtype P2X4 is upregulated [88]. Tsuda *et al*. demonstrated that spinal injection of ATP-activated microglia was sufficient to evoke rapid mechanical allodynia within one hour of injection [89]. Together, these results suggest that activation of microglia and astrocyte induces neuropathic pain *in vivo*.

Challenges in Glial Network Research

Glial Heterogeneity

It is now well established that astrocytes are not a physiologically homogenous population; they differ between brain

regions and even within the same brain region. For example, Martín and co-workers found that striatal subpopulations of astrocytes release glutamate that selectively activates NMDRs in homotypic, but not heterotypic, medium spiny neurons [28]. Their finding raises the possibility that cell-specific astrocytic mini-networks regulate information flow in certain brain regions, providing a new perspective on circuit assembly and dynamics in the brain. Moreover, in the past few decades, reactive astrocytes have been identified in nearly all diseased conditions, such as CNS injury, neurodegeneration, and brain tumors. Two different types of reactive astrocytes, termed A1 and A2, may be induced by neuroinflammation. Each type displays distinct responses and properties in different injuries [27, 90, 91]. Furthermore, the soma and processes of astrocytes may have different responses to the same stimuli [92]. For example, the Ca^{2+} signaling pathways we know so far are mainly derived from studies on astrocyte somas. In contrast, our understanding of Ca^{2+} signaling in the processes remains rather limited. Altogether, these features of astrocytes further highlight the complexity of astrocyte networks in the brain.

Like astrocytes, microglial heterogeneity has also been investigated [93]. In healthy mouse and human brains, unbiased clustering has revealed the presence of nearly 10 subclusters of microglia [94]. The current understanding of cell markers and the biological functions of these subclasses of microglia in the healthy brain remains limited. Further, in disease states microglia are classified into several different subtypes, such as M1 and M2 subtypes, DAMs (disease-associated microglia), plaque-associated microglia, dark microglia, and human Alzheimer's microglia [95] Both healthy and disease-associated microglial subclusters could exist in the CNS at the same time. How these different microglial subtypes influence each other and interact with other glia in response to different stimuli is still a very challenging question.

In addition to temporal and spatial heterogeneity, species heterogeneity is also a very important aspect. Morphological analysis of astrocytes in the rodent and human neocortex shows marked differences between the two species. In contrast, the functional properties of astrocytes and NG2 glial cells in these species are strikingly similar [96]. The difference in glial biology between experimental animal models and humans may substantially impact our data interpretation.

The Glial Network is not Quiescent but Highly Dynamic

Unlike neurons, glial cells, in particular microglia, often change their morphology to a more mobile type in response to stimuli. Using two-photon imaging microscopy, researchers have revealed that microglial processes make brief, repetitive contacts with synapses at a frequency of about once per hour in the somatosensory and visual cortex [97]. And this microglial behavior is modulated by neuronal activity. For instance, light deprivation reduces motility while re-exposure to light reverses it. Depending on the timing and type of the disease, microglia can be multifaceted, such as the cause, contributor, bystander, protector, or consequence of neuronal dysfunction [98, 99]. This comes up with a new question of how the glial network changes its characteristics during disease progression.

The Glial Network is much more Complex than Glia Themselves.

Given that Cx-based gap junctions serve as links connecting oligodendrocytes (O/O junctions), as well as with astrocytes (O/A junctions), it is reasonable to suspect that each glial networks are not entirely independent of each other. Instead, they may function collaboratively, depending on the context and the specific needs. Interestingly, different types of glial cells influence each other. For instance, astrocytic gap junctional communication and Cx43 expression are inhibited by microglia-derived IL-1β and tumor necrosis factor-α (TNF-α) [100]. Conversely, activated astrocytes facilitate microglial activation *via* ATP, which acts on purinergic receptors in microglia. It is plausible that astrocytes control the activation of microglia not only in the local region but also in a broader area since during the propagation of Ca^{2+} waves in the astrocyte network, distant microglia can be activated in response to ATP released by astrocytes [101]. On the other hand, under certain circumstances, activated astrocytes can also inhibit microglia by suppressing the production of pro-inflammatory mediators, such as nitric oxide, reactive oxygen species, and TNF-α [102]. These findings add new layers of complexity to glial networks.

Perspective: Mapping the Glial Connectome

Glia cells are wired together through gap junctions or tunneling nanotubes, demonstrating a potential for glial cells to function as a whole at the system level in the modulation of neuronal activity. It remains to be determined how glial cells are connected and how their connections are functionally regulated under various conditions. Therefore, it is important to investigate the glial connectome focusing on the structural and/or functional connections between a variety of types of glial cells, in addition to neuronal-level connectomes to which much attention is currently being paid.

We hypothesize that astrocytic Ca^{2+} is at the core of glia-to-glia interactions. Astrocytes receive stimulatory inputs from neurons or neighboring astrocytes, which leads to the generation and propagation of Ca^{2+} waves. The astrocytic Ca^{2+} wave likely encodes information about glial activity

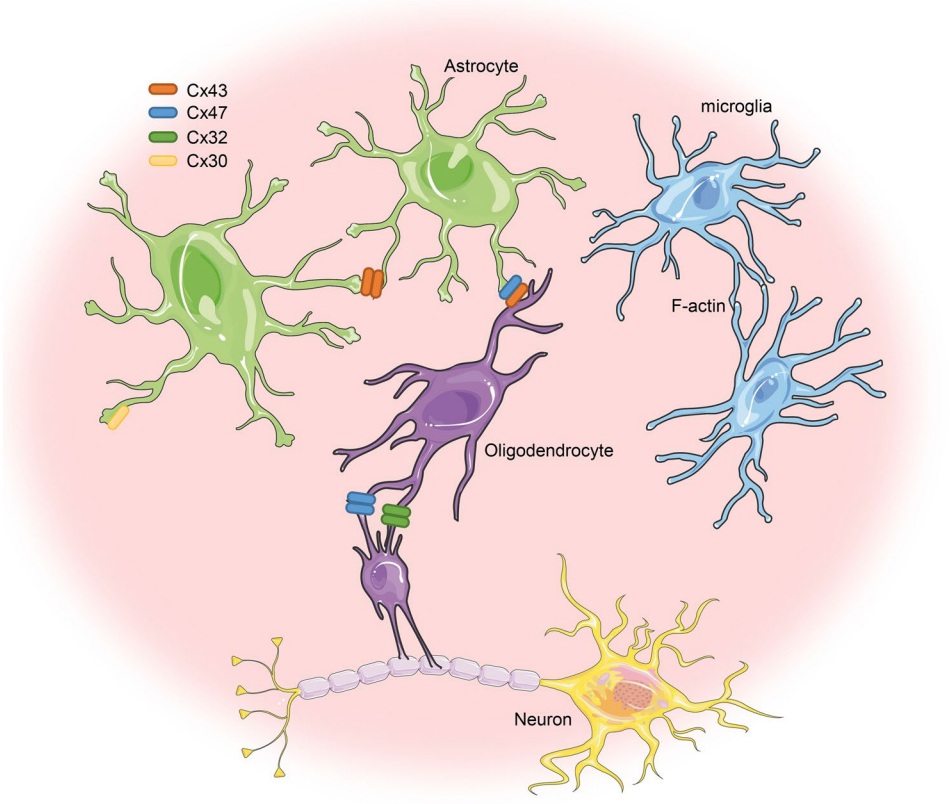

Fig. 1 Astrocytes and oligodendrocytes are connected mainly through connexin-based gap junction channels. Astrocytes form A/A gap junction through Cx43 and hemichannels through Cx30. Astrocytes form A/O gap junction through Cx43 on astrocytes and Cx47 on oligodendrocytes. Oligodendrocytes form O/O gap junctions through Cx47 and Cx32. Microglia wire together through F-actin-containing membrane-membrane connections.

and synaptic transmission, and it could be shaped and altered to some extent by neuronal activity during its propagation. Ca^{2+} signals also reach oligodendrocytes and microglia but have much more enduring effects on diverse biological processes. Given its quantifiable and propagative nature, astrocytic Ca^{2+} activity may represent an embodiment of higher-level brain functions. Decoding glial Ca^{2+} signaling may be an important step for understanding glial communication and the regulation of brain activity as a whole in the future(Fig. 1; Table 1).

Table 1 Glial network in CNS diseases.

	Glia-glia connections	Glial Ca^{2+} signaling
Alzheimer's disease	GJA1 is strongly associated with AD amyloid and tau pathologies and a decline in cognitive function	β-amyloid peptide stimulates L-type voltage-gated Ca^{2+} channels.
Parkinson's disease	Cx30 deficiency induces severe neurodegeneration in a PD animal model	A53T mutation in α-synuclein upregulates and hyperactivates N-type voltage-gated Ca^{2+} channels
Brain injury and Neuroinflammation	Microglia may be capable of forming homo-cellular syncytia mediated by gap junctions in the context of CNS bacterial infections	LPS challenge significantly elevates the baseline of spontaneous microglial Ca^{2+} activity.
Brain tumor	Glioma cells form gap junctions with surrounding astrocytes and microglia *in vitro*	
Pain	Cx43 is up-regulated in astrocytes and switches function from forming gap junctions to paracrine modulation	

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