Chapter

Targeting Neuroglial Sodium Channels in Neuroinflammatory Diseases

Yu Yao, Xiaoli Wang, Shuzhang Zhang, Zhiping Zhang, Wei Wang, Yudan Zhu, Jiwei Cheng, Guoyi Li and Jie Tao

Abstract

The Hodgkin-Huxley model, at its 66th anniversary, remains a footing stone of neuroscience, which describes how the action potential (AP) is generated. As the core player of AP initiation, voltage-gated sodium channels (VGSCs) are always considered to be required for electrogenesis in excitable cells. Cells which are not traditionally been considered to be excitable, including glial cells, also express VGSCs in physiological as well as pathological conditions. The dysfunction of glial VGSCs is seemingly not related to abnormal excitation of neurons, but of importance in the astrogliosis and M1 polarization of microglia, which could induce refractory neuroinflammatory diseases, such as multiple sclerosis, stroke, epilepsy, and Alzheimer's and Parkinson's diseases. Therefore, in this chapter, we aim to describe the physiological and pathological roles of VGSCs contributing to the activity of glial cells and discuss whether VGSC subtypes could be used as a novel drug target, with an eye toward therapeutic implications for neuroinflammatory diseases.

Keywords: glial VGSCs, neuroinflammatory diseases, astrocytes, microglial cells, oligodendrocytes, gliosis

1. Introduction

So far, the pathogenesis of neuroinflammatory diseases, including multiple sclerosis (MS), epilepsy, Parkinson's disease (PD), Alzheimer's disease (AD), etc., is still unclear, of which therapeutic effects are not satisfactory, bringing great challenges to public health care. The pathological processes of these diseases are often accompanied by the production of neuroinflammation that cause a series of bad effects such as firing pro-inflammatory signaling pathways and even neuron pyroptosis, as well as cell death. The accumulated data show that neuroinflammation is characterized by the activation of glial cells and production of inflammatory mediators in the central nervous system (CNS) and peripheral nervous system (PNS) [1].

Activated glial cells, triggering neuroinflammation, include Schwann cells as well as satellite glial cells in PNS and microglia, astrocytes, and oligodendrocytes in CNS [2]. Glial cells are of importance for critical responses to neurological diseases and injuries, including active tissue remodeling, phagocytosis, etc. [3].

What's more, glial cells often acting as double-edged swords not only evoke the neuronal damage but also promote tissue repair [4]. Under pathological conditions, microglial cells, as one kind of resident immune cells in CNS [5], could secrete a large number of cellular inflammatory factors, increase oxidative stress of neurons, and induce apoptosis or pyroptosis of neurons. At the same time, M2 microglia secrete various neurotrophic factors and anti-inflammatory factors such as IL-4 and IL-3, which play a neuroprotective role in cerebral ischemia and hypoxia [6, 7].

Astrocytes are widely distributed in the nervous system, showing its function by providing nutrition and support to adjoining neurons [8]. Therefore, the excitability of neurons could be regulated by the neurotransmitters secreted from astrocytes. At the same time, it could synthesize and release a variety of immune factors to participate in the neuronal immune response. Oligodendrocytes are the unique neuroglial cells that form the myelin sheath, which is the key structure for neurons to propagate APs. CNS myelin hypoplasia or demyelinating changes are the pathogenic factors of neuroinflammatory diseases [9].

In the mid-twentieth century, after the discovery of AP, Hodgkin and Huxley, who firstly proposed the concept of ion channels, record sodium currents by using voltage clamp technology [10, 11]. Traditionally, glial cells were considered as non-excitatory cells, but with the development of research in this field, sodium channels have been found also to play crucial roles in physiological and pathological function of these cells [12, 13].

The activation of sodium channel is triggered by membrane depolarization, which produces transient sodium current and AP [14]. In neurons, sodium channels are composed of a single α-subunit, which forms ion-selective and voltage-sensitive pores, and one or two auxiliary β-subunits, which seems to affect channel gating and expression [15].

These channels drive electrical generation in neurons (Nav1.1, Nav1.2, Nav1.3, Nav1.6, Nav1.7, and Nav1.8), muscle cells (Nav1.4), and myocardial cells (Nav1.5). The typical role of Nav channels has been widely studied [16]. The dysfunction of sodium channels could result in neurological diseases, including neuropathic pain [17–19], peripheral neuropathy [20], epilepsy [21], and MS [22, 23].

However, the dysfunction of glial VGSCs is seemingly not related to abnormal excitation of neurons, but of importance in the astrogliosis and M1 polarization of microglia, which could induce refractory neuroinflammatory diseases. Glial sodium channels are closely related to phagocytosis, secretion of cytokines (IL-α, TNF-α), and migration. Then, glial cells are activated after tissue damage or disturbance, accompanied by morphological changes, enhanced migration, phagocytosis, secretion of inflammatory molecules (such as cytokines and nitric oxide), and antigen presentation [24].

Although glial cells do not produce AP under physiological conditions, they can show excitability through ion flux, especially in the form of $[Ca^{2+}]_i$ oscillation. Ca^{2+} kinetics is involved in microglial activation and regulation of many effector functions, including cell migration [25, 26] and release of chemokines/cytokines and nitric oxide [27].

The Na^+/Ca^{2+} exchanger (NCX) operates in a forward mode, transmits Na^+ ions down the concentration gradient to the cell, and then returns to output Ca^{2+}, or if the electrochemical gradient of Na^+ decreases or the cell depolarizes, the operation realizes the reverse mode by outputting Na^+ ions in exchange for Ca^{2+} [28]. Therefore, sodium channel activity has the ability to increase $[Ca^{2+}]_i$ through the reverse mode of NCX.

The research shows that Nav1.6 can generate continuous sodium current [29], drive the reverse operation of NCX, and generate the inlet. Ca^{2+} enters the cytoplasm. In this respect, like all eukaryotic cells, the intracellular free Ca^{2+} level is strictly regulated, which is crucial in the signal transduction pathway of microglia.

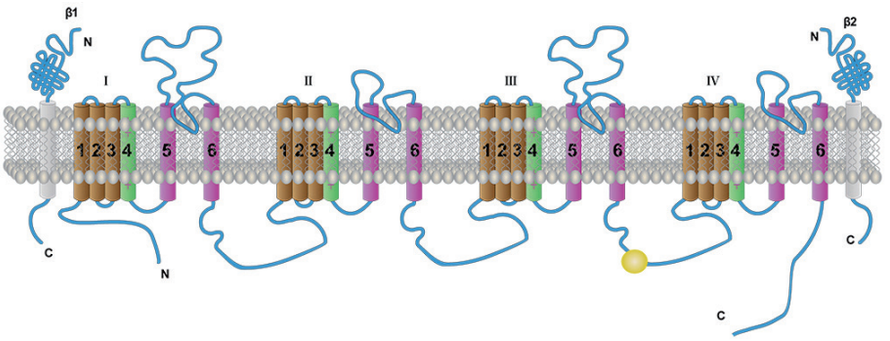

Figure 1.
Structure of Nav channels. Schematic representation of Nav channel subunits. The Nav channel α subunit is often illustrated together with auxiliary subunits β1 and β2; extracellular domains of the β subunits are shown as immunoglobulin-like folds (shown in blue), interacting with the extracellular loops of α subunits. Roman numerals indicate the domains of the α subunit; segments 5 and 6 (shown in purple) are considered as pore-lining segments, and S4 helices (green) make up the voltage sensors. The yellow circle in the intracellular loop of domains III and IV represents the inactivation gating ball, IFM motif.

Studies have shown that Nav1.1 and Nav1.6 were persistently reduced during epileptogenesis [30]. Under the MS pathological condition, the results showed that the removal of Nav1.5 from astrocytes could significantly worsen the clinical outcome of experimental autoimmune encephalomyelitis (EAE) [31] and the sodium channel Nav1.2 is expressed by scar and reactive astrocytes in plaque [32]. Studies have shown that under PD pathological conditions, Nav1.1 [33] in hippocampal astrocytes is significantly increased, and Nav1.6 is highly expressed in activated microglia [34].

There is a close relationship between the structure and the function of sodium channels (**Figure 1**). Therefore, in this chapter, we aim to describe the physiological and pathological roles of VGSCs contributing to the activity of glial cells and discuss whether VGSC subtypes could be used as a novel drug target, with an eye toward therapeutic implications for neuroinflammatory diseases.

2. Neuroglial sodium channels in multiple sclerosis

MS, a chronic inflammatory disease of the CNS, is characterized by demyelination, axonal injury, neuronal loss, and progressive inflammatory responses in the brain and spinal cord [35]. EAE is a classic model of MS, of which the pathological progress is very similar to MS. Under pathological conditions, glial cells (microglia, astrocytes, oligodendrocytes, glial stem cells) can act as regulators, effectors, and even targets of inflammatory response, not only causing tissue damage but also promoting tissue repair [4]. Glial cells are essential for critical responses to neurological diseases and injuries, including active tissue remodeling and phagocytosis [3]. Studies have shown that sodium channels are not only traditionally associated with the generation and transmission of neuronal APs, but also can be expressed in electrically inexcitable cell types including astrocytes [36, 37], oligodendrocyte precursor cells [38–40], Schwann cells [41], microglia [42, 43], and cancer cells [44–46]. The regulation of glial function by sodium channels is of special significance for the response of reactive glial to CNS diseases and insults [14].

2.1 Microglial sodium channels in multiple sclerosis

Microglial cells are the resident immune cells of the CNS. Under physiological conditions, microglial cells are usually highly branched cells with dynamic

processes that can actively monitor the microenvironment of the CNS to protect nerve homeostasis [47]. However, under pathological conditions such as MS, microglia cells could be activated and recruited [48]. Microglial cells undergo significant immunophenotype and cellular and morphological plasticity in response to damage in the activation pathway [49]. The activation of microglial cells is related to the pathological conditions of the CNS. In addition, migration of microglial cells to damaged cells and pathogens plays an important role in microglial-mediated CNS injury and infection [50].

Microglial cells activated in EAE and MS are widely distributed and promote disease processes through a variety of mechanisms, including inducing effector T cell proliferation [51], production of pro-inflammatory cytokines [52], and phagocytosis of myelin. Moreover, studies have found that microglial cells activated in newly formed MS lesions are thought to be the main cell type that triggers the neuroinflammatory cascade after oligodendrocyte apoptosis [53]. Increased intracellular calcium and subsequent stimulation of the signaling cascade have been shown to be central events in the regulation of the function of activated microglial cells [54]. Studies by Matthew et al. have demonstrated that activation of microglial cells as well as macrophages is accompanied by upregulation of sodium channel Nav1.6 in EAE and MS [13]. In both the Nav1.6 blocker model and the Nav1.6 knockout model, the extent of inflammatory infiltration in EAE and the phagocytosis of activated microglia cells were effectively reduced, thus confirming that Nav1.6 was a key contributor to the activation and pathophysiological function of microglial cells [13, 55].

In another important pathological manifestation, migration of microglial cells to lesions of the CNS is a complex and highly coordinated process involving multiple intersecting cellular pathways such as membrane adhesion and retraction, cellular polarization, and receptors transducing external migratory signals [56]. One of the preliminary structural events in chemotaxis is the formation of membrane protrusions and high enrichment in the aggregated F-actin network [57]. In addition, actin-binding protein, calmodulin, and GTP-binding signaling protein Rac also are located at the protrusions [58–60]. The activity of MAP kinase and the reorganization of actin filament also play important roles in cell migration [61]. Importantly, Ca^{2+} signaling seems to have an effect on protrusions and movement [62], as intracellular Ca^{2+} levels can regulate cell migration, and the activity of a variety of migration-related effector molecules including Rac and MAP kinase is modulated by the levels of intracellular Ca^{2+} [63–65]. Studies support the contribution of the sodium channel Nav1.6 in a pathway that controls the extension of lamellipodial protrusion at the initial stage of cell migration [66, 67]. Sodium channels regulate Ca^{2+} transients in ATP-stimulated microglia and play a role in the activation of two key migrating proteins, Rac1 and ERK1/2 [48] (**Figure 2**).

In summary, Nav1.6 sodium channel is involved in the activation and functional regulation of microglia in EAE and MS and has potential value as a therapeutic target.

2.2 Astroglial sodium channels in multiple sclerosis

Astrocytes participate in ionic homeostasis, neuronal metabolic support, and the formation and maintenance of the blood–brain barrier in the normal CNS and react to form glial scars when injured [8]. With the deepening understanding of the importance of astrocytes in CNS pathology, it has been proven that astrocytes play a key immunoregulatory role in damaged CNS [68–70]. Though astrocytes have traditionally been considered to be electrically unexcitable, studies have

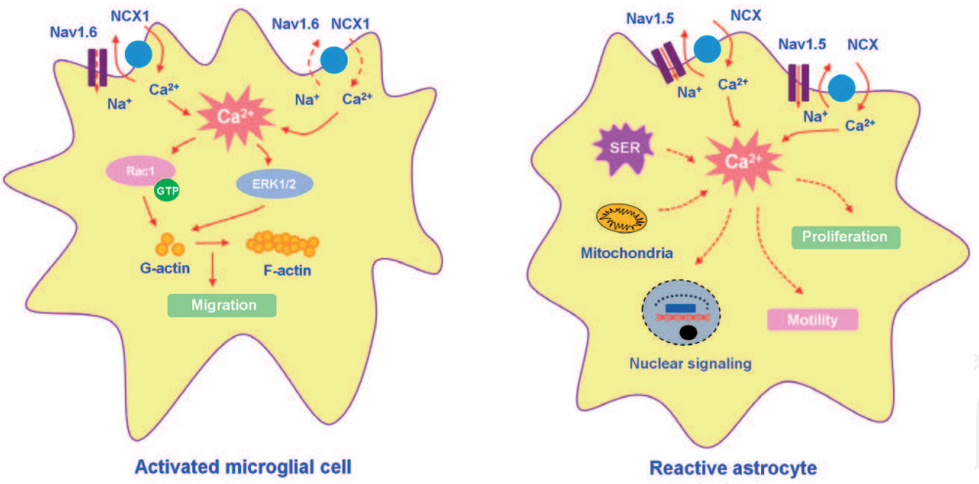

Figure 2.
Neuroglial Nav channels evoking Ca^{2+} signaling pathway. Schematic of putative cell signaling of Nav channel contribution to intracellular Ca^{2+} levels and downstream pathways. Depolarization of neuroglial membrane leads to activation of VGSCs (Nav) allowing influx of Na^+. Increased $[Na^+]_i$ causes reverse operation of NCX, which contribute to the level of Ca^{2+}. The Ca^{2+} signaling initiates downstream effects on neuroglial cell functions.

demonstrated that these cells express VGSCs [71, 72], including the subtype Nav1.5 [73, 74]. It is worth noting that the expression of astrocyte sodium channels in rodents is not a static process, but a dynamic process that changes with the age of astrocytes, exposure to extracellular factors, and damage [75, 76]. The voltage-gated sodium channel Nav1.5 is less expressed in astrocytes in non-pathological human brain but shows a strong upregulation of Nav1.5 in both acute and chronic MS lesions [71].

Different from excitable neurons, astrocytes exhibit their excitability by mainly in the form of $[Ca^{2+}]_i$ oscillations. Cytoplasmic Ca^{2+} levels in astrocytes come from multiple regions, including the endoplasmic reticulum [77], mitochondrial sodium-calcium exchange [78], and extracellular space [79]. The $[Ca^{2+}]_i$ flux of astrocytes not only regulates neuronal synaptic transmission, but is also important for many steady-state cell functions, including migration and proliferation, of astrocytes [78, 80, 81]. An important mechanism by which $[Ca^{2+}]_i$ is regulated in astrocytes is the reverse (Ca^{2+} import) activity of the NCX [82]. The positive pattern of NCX is to transport Na^+ to the cell and then return Ca^{2+} to the cell [28]. When the Na^+ electrochemical gradient is reduced or the cell depolarizes, NCX outputs Na + in exchange for Ca^{2+} by running in reverse mode [82, 83]. Therefore, sodium channel activity has the ability to increase $[Ca^{2+}]_i$ through the reverse pattern of NCX [82]. Interestingly, mechanical strain injury increases intracellular Na^+, causing NCX to operate in a reverse mode in cortical astrocytes, increasing the level of $[Ca^{2+}]_i$ [84].

Recent studies have shown that Nav1.5 plays an important role in in vitro models of glial injury by triggering the reverse mode operation of the NCX [71, 74]. Laura et al. confirmed that in the conditional knockout Nav1.5 model in astrocytes, the absence of Nav1.5 leads to a significant deterioration in the clinical outcome of EAE and an increase in inflammatory infiltration [31]. While the previous studies of MS in vivo models have shown that a variety of voltage-gated sodium channel blockers, including phenytoin [85], lamotrigine [86], carbamazepine [85], and safinamide and flecainide (Nav1.5 blocker) [87], could improve the clinical status and axonal damage, this suggests that Nav1.5 and NCX may be potential targets for the treatment of MS by acting on $[Ca^{2+}]_i$ to regulate astrocyte proliferation (**Figure 2**).

2.3 Oligodentroglial sodium channels in multiple sclerosis

During the development of CNS, oligodendrocytes (NG2 cells) originating in different regions of the brain migrate to their destinations to participate in the development [88, 89]. The directed migration of these glial progenitor cells is critical not only for the formation of myelin in the developing brain, but also for the repair of myelin after injury [90, 91]. Although NG2 cells express VGSC, they only trigger transient depolarization and fail to produce typical APs [92, 93]. Studies have found that intracellular Na^+ and Ca^{2+} levels, membrane depolarization, and migration ability of NG2 cells increased after the application of GABA [93]. While in the siRNA knockdown or blocking sodium channel model, the increasing tendency of $[Na^+]_i$ and $[Ca^{2+}]_i$ was significantly reduced, and cell migration was suppressed [93]. Moreover, a similar reduced $[Ca^{2+}]_i$ and decreased cell migration were also shown in the NCX siRNA knockdown and blocker models [93].

In general, GABA induced the depolarization of the NG2 cells and activated the sustained Na^+ current, which reverted the activity of type I Na^+/Ca^{2+} exchangers (NCX1) to evoke the increase of $[Ca^{2+}]_i$ [93]. Significantly, further evidence suggests that this unique pathway is associated with NG2 cell migration [93, 94]. Therefore, the important role of non-inactivated Na^+ channels and NCXs in the development and function of NG2 glial cells in the brain suggests its potential values in myelin repair.

3. Neuroglial sodium channels in epilepsy

Epilepsy is a kind of chronic brain dysfunction syndrome caused by abnormal firing of neurons, which has been listed as one of the five major neuropsychiatric diseases by the World Health Organization (WHO). At present, the number of epilepsy patients has reached 65 million worldwide [95], and especially the developing countries account for four fifths of this number [96], bringing serious economic burden to the patients' families and their country.

The previous studies on epilepsy were focused on clarifying the mechanisms of neuron dysfunction as well as neural network. In recent years, the researches on glial cells regulating neuron activity have surfaced with increasing frequency, which provide sufficient evidence for the involvement of glial cells in inducing epilepsy [95]. Glial hyperplasia is an important hallmark in the course of epilepsy; it refers to a spectrum of physicochemical and physiological changes in glial cells, particularly in astrocytes and microglia. The activation of astrocytes after epileptic seizure may be beneficial to the recovery of extracellular homeostasis [97]. However, more and more evidence proved that reactive glial cells could induce neuroinflammation by releasing the cytokines, chemokines, and other molecules, which is likely to cause neuron death, tissue damage, and microglial hyperplasia [98]. Unrestrained reactive gliosis might also cause hippocampal sclerosis, disturbing the normal physiological regulation function and promoting the epileptic seizure [99].

Glial cells express several types of ion channels. The Cl^-, K^+, H^+, and Ca^{2+} channels have been found to express in microglia, and the Kir [100] and Na^+ channels are highly expressed in astrocytes, which have been implicated in multiple functions of these cells [101]. These channels of different subtypes can be involved in regulating the membrane potential, migration, phagocytosis, intracellular ion concentration, and secretion of various cytokines and chemokines in glial cells [102].

Genetic studies have shown that the mutations associated with epilepsy mainly occur in genes encoding sodium channels [103]. VGSCs are a class of voltage-dependent ion channels that are highly expressed not only in excitable cells [104],

but also in non-excited cells, such as astrocytes, oligodendrocytes, microglial cells, etc. [105]. Recently, it has been found that sodium channel plays an important role in the activation of glial cells and may become a new target for antiepileptic drugs.

3.1 Astroglial sodium channels in epilepsy

Neuronal excitability is closely related to the movement of sodium or potassium across the extracellular space (ECS). Because of the narrow volume of this space, the extremely small fluxes could also evoke significant changes in anion concentration [95]. Normally, a single AP can increase the extracellular K^+ concentration by nearly 1 mM. However, at the epileptic seizure period, the continuous neuron firing could raise the potassium concentration from the normal level ~3 mM to 12 mM [106]. In normal brain, neurons rapidly regulate K^+ concentration to 3 mM through Na^+/K^+ ATPase, and Na^+ activates Na^+/K^+ ATPase activity through VGSCs in astrocytes, providing an important feedback pathway for regulating K^+ level in extracellular space and maintaining stability of the central nervous system [106].

Thus far more and more reports have mentioned that sodium channel subtypes are widely distributed in most CNS glial cells [72, 107], including the TTX-S sodium channel Nav1.3 and Nav1.6 as well as TTX-R sodium channel Nav1.5 [14]. In the post-status epilepticus (SE) model induced by kainic acid (KA) intrahippocampal injection, the expression of Nav1.6 in ipsilateral hippocampal peaked at 21 days in astrocytes. On the contrary, there was no change in the expression of astrocyte Nav1.6 in the PTZ-induced epileptic seizure models, indicating that astrocyte Nav1.6 played a crucial role in promoting the epileptic process, but not in seizure period [108].

It is known to all that the voltage-gated sodium channel is composed of one α subunit and two auxiliary β subunits [109]. Co-expressed with α subunits, β subunits could significantly increase the current density of sodium channels, which is partly due to the enhancement effects of β subunits on the expression of sodium channels [110]. In the chronic epilepsy model induced by electrical stimulation, sodium channel β1 subunits are colocalized with the reactive astrocytes, and the number of positive cells significantly enhanced a week after SE, so it is speculated that the β1 subunits could interact with extracellular matrix, promoting the network of intercellular synapses in the process of epilepsy [111].

3.2 Microglial sodium channels in epilepsy

Microglia, as resident cells in the CNS, provide continuous immunosurveillance for the brain as well as the spinal cord [47]. When the body is invaded by exogenous substances, microglial cells respond to ATP or other cell signals, activated rapidly so as to provide immune defense. However, microglia cells can produce inflammatory factors in pathological conditions, causing damage to the body [49]. Microglial cells always express a large number of ion channels and surface receptors, which can induce relevant signaling pathways to convert extracellular changes into intracellular responses.

VGSCs are also distributed in microglial cells. Studies have shown that microglia not only express the TTX sensitivity sodium channels (Nav1.6 [13] and Nav1.1 [14]), but also express the Nav1.5 [14] (a TTX-resistant sodium channel) [14]. The blockade sodium channels of TTX and phenytoin could significantly weaken a variety of functions of activated microglia cells [12], such as the release of inflammatory cytokines [12, 102]. After a week of spontaneous epilepsy induced by electrical stimulation, the sodium channel subtypes were found to be highly expressed in microglial cells [112]. The Nax channel encoded by the SCN7A gene was observed

to be significantly increased during the onset and development of epilepsy, and especially the high expression of Nax was detected in hippocampal sclerosis tissues from drug-resistant patients [113].

Ocasepine, as a clinical drug that targeted on VGSCs, is often used to suppress the epileptic seizure clinically. This drug has been found that it could significantly reduce the number of activated astrocytes as well as microglial cells in the hippocampus CA1 region in cerebral ischemia model, which also reduce the neuron death in the hippocampus caused by ischemia [14]. It implies that sodium channels are involved in microglia activation, which could also promote the progression of neuroinflammatory disease, such as epileptogenesis.

4. Neuroglial sodium channels in neurodegenerative diseases

4.1 Neuroglial sodium channels in Alzheimer's disease

AD is the most common progressive neurodegenerative disease, which is characterized by dystrophic neurites, neurofibrillary tangles, brain atrophy amyloid plaques, and loss of neurons and synapses [114]. In addition, AD is the cause of dementia and seriously affects the quality of life of the elderly [115]. Accumulated data show that the genetic mechanism of AD is mainly the accumulation of Aβ peptides and their aggregation in and deposition in amyloid plaques [116, 117]. The human genetics of familial AD also suggested that excessive production of amyloidogenic Aβ is a cause of early-onset AD; mutations in amyloid precursor protein (APP) or in its processing enzyme result in increased β-site cleavage of APP or favored production of longer, aggregation-prone variants of Aβ peptide [118]. In recent years, however, many studies found that microglia play an important role in the pathogenesis of AD. The reactive gliosis of AD histopathology revealed the abnormal morphology and proliferation of microglia [119, 120]. Several reports have linked microglia dysfunctions to AD, by showing microglial motility impairment in AD mice models [121]. Recently, it was recognized that microglia express voltage-gated ion channels, including Nav1.1, Nav1.5, and Nav1.6 [122, 123]. Furthermore, pharmacological block of the sodium channels has been attempted as a symptomatic treatment of epileptic features often associated with AD, as well as a relief to detrimental behavioral and psychological symptoms of dementia [124]. An interesting debate is if sodium channel activators could just be enough to compensate microglial dysfunctions to altered physiological properties of dysfunctional neuronal networks in AD patients [125].

4.2 Neuroglial sodium channels in Parkinson's disease

PD is the second most common age-related disabling neurodegenerative disorder, estimated to affect over 10 million people worldwide, which PD presents clinically as bradykinesia, muscular rigidity, arresting tremor, and postural stability [126–128]. In addition, PD is characterized by dopamine depletion and the loss of dopaminergic (DA) neurons with accompanying neuroinflammation. The potential causes of PD remain uncertain, but recent studies suggest neuroinflammation and microglia activation play important roles in PD pathogenesis [129, 130]. However, persistent activation of microglia can mediate neuronal death and neurodegeneration by increasing the secretion of inflammatory molecules and cytokines, including tumor necrosis factor alpha (TNF-α) and reactive oxygen species (ROS) [131, 132]. Microglia express a number of ion channels, including sodium channels that regulate various aspects of inflammatory process, providing a potential target for intervention [14, 133]. Several studies demonstrated that VGSC can regulate a

number of cellular functions such as morphological transformation, migration, and phagocytosis of microglia [12, 35]. This also indicates the well potential immunomodulatory properties of VGSC. 6-Hydroxydopamine (6-OHDA)-induced PD rat model found that the expressions of Nav1.1, Nav1.3, and Nav1.6 in the hippocampus were dynamically increased at different time points after dopamine depletion. Furthermore, cognitive deficits were effectively improved by phenytoin (sodium channel blocker) that has inhibitory effects on VGSCs in the brain [33]. Other study suggested that zonisamide, targeting VGSCs, may reduce neuroinflammation through the downregulation of microglial Nav 1.6 [134]. Those studies may contribute to its reported neuroprotective role in preclinical models of PD.

5. Application of neuroimaging in studying neuroglial sodium channels

Electrophysiological patch clamp is a classic technique for traditionally recording neuronal sodium channels. Neuroglial cells are non-excitable cells, of which the cell membrane depolarization is not obvious. The bioproperties of neuroglial sodium channels evoked by high voltage in patch clamp recordings might be different from the actual situation in vivo. Therefore, this method has certain limitations in the process of studying neuroglial cells. With the development of ion probes, especially the recent discovery of visible light sodium ion probes as well as calcium ion probes, which could provide more intuitive results (such as higher resolution, better observation) for the functional study of glial cell Nav channels and Nav-NCX complexes [14]. The combined use of ion probes and patch clamping will make the experimental results both more abundant and accurate.

In addition, the invention of the miniature two-photon microscope in 2017 [135] provided a powerful means for the functional research of nerve cells as well as glial cells. During studying brain activities and the development of neuroinflammatory diseases, glial cells could be labeled by GCaMP6 or dTomato to explore the function of the glial Nav-NCX complex. Two-photon imaging combined with EEG could not only explain the relationship between glial cell activity and EEG frequency more effectively, but also elucidate the important role of glial cells in neurological disorders, such as epilepsy. By using the miniature two-photon microscope, the conditional knockout mice could be applied to study the role of glial sodium channels in regulating the function of glial cells or the interaction between glial cells and neurons, including pruning of neuron synapses by microglial cells or regulating the neuroexcitability in vivo.

6. Conclusion

In view of the central role of glia in CNS health and disease, it is necessary to further understand the physiological correlation of glial sodium channels and characterize the molecular pathways that control the function of sodium channels in these cells. There has been much work performed in cell culture, but further in vivo studies are of crucial importance for determination of the therapeutic implications of targeting glial sodium channels in neurological disorders. Therefore, novel neuroimaging techniques are of importance for studying the roles of neuroglial sodium channels in neuroinflammatory diseases. Meanwhile, with the heightened focus on developing sodium channel specific blockers, it is increasingly relevant to assess the roles of individual sodium channel isoforms in neuroglia cells (e.g., Nav1.6 in microglial cells, Nav1.5 in astrocytes). Further understanding of the signaling cascade linking sodium channel activity to glial effector function will facilitate the development of specific therapeutic targets for neurological diseases.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

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