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Review Article

SCN8A (NaV1.6): Gate to Chronic Pain

Wenrui Xie, Judith A. Strong, and Jun-Ming Zhang

ABSTRACT

Aim of review: Sodium channels are large transmembrane proteins with a voltage-gated pore capable of selectively conducting Na+. They are critical determinants of the electrical excitability of sensory neurons and play a key role in pain sensation. Injury and disease affecting peripheral nerves induce neuropathic changes, and increase cellular excitability due to subtype-specific abnormalities in the expression and trafficking of sodium channels. In this review, we present an overview of current research on sodium channel isoform 1.6 (NaV1.6) and the β4 subunit (NaVβ4) in pathological pain conditions.

Methods: We first provide a brief description of the sodium channel isoforms and their roles in neuronal excitability and pain. We then focus on recent findings from our lab regarding the expression and changes of NaV1.6 and NaVβ4 in sensory neurons under physiological and pathological conditions, and how they contribute to the development and maintenance of pathological pain.

Recent findings: Over the last decade, studies on transgenic mice and human mutations have revealed that many sodium channel isoforms such as NaV1.3, NaV1.7, NaV1.8, and NaV1.9 are involved in different aspects of physiological or pathological pain. Although NaV1.6 in conjunction with the modulatory NaVβ4 subunit has the ability to trigger and maintain high-frequency repetitive firing via mediating persistent and resurgent currents, only very recently has its role in pain disorders been recognized.

Summary: The persistent and resurgent currents in sensory neurons are generated predominantly by NaV1.6 in association with NaVβ4. Since spontaneous activity, especially high-frequency repetitive and burst firing in damaged peripheral sensory nerve or neurons, is crucial for the development of neuropathic pain, NaV1.6 and/or NaVβ4 may be new therapeutic targets for managing pathological pain conditions. (Funded by the National Institutes of Health.)

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Neuropathic pain encompasses pain resulting from damage to peripheral nerves, sensory ganglia, and spinal roots, for example by trauma that damages or transects axons, systemic toxins such as chemotherapy drugs, metabolic disorders such as diabetes, or genetic disorders. In neuropathic pain conditions, the intrinsic electrical properties of the sensory neuron membrane are altered, resulting in hyperexcitability or abnormal spontaneous firing, leading to symptoms such as dysesthesias, allodynia, hypersensitivity, and spontaneous pain. Na+ channels play a key role in determining membrane excitability, and make important contributions to neuropathic pain (1). The voltage-gated channels, specialized ion permeable transmembrane proteins, are essential for generating electrical signals in excitable cells. The family of voltage-gated sodium channels (VGSCs), which play fundamental roles in the initiation of action potentials in peripheral sensory fiber terminals and their propagation to the central nervous system, have been long linked to pain disorders (2-4). In mammals, VGSCs are multimeric proteins consisting of a large pore-forming α subunit (NaVα) associated with additional smaller, regulatory β subunits (NaVβ) (5). The α subunits are encoded by nine homologous genes (3, 6-11). Of these nine isoforms, six, including sodium 1.6 (NaV1.6), are highly sensitive to the sodium channel blocker tetrodotoxin (TTX) (5, 12). The β subunits, though not required for Na + permeation, modulate the biophysical properties and trafficking of the channels (3, 7). In dorsal root ganglion (DRG) sensory neurons, robust levels of NaV1.6, NaV1.7, NaV1.8, and NaV1.9 are found in embryonic through adulthood stages. Normally, NaV1.3 is expressed only at embryonic and neonatal stages, but it is re-expressed in adult DRG neurons under certain pathologic pain conditions (13).

Multiple sodium isoforms are expressed in DRG neurons and have distinct voltage-dependence and kinetics, which together regulate neuronal firing patterns (11,12,14,15). NaV1.3 and NaV1.7 are capable of boosting subthreshold stimulation. NaV1.9 can give rise to persistent currents, which will be discussed in detail later. These channels have been considered as threshold channels for firing action potentials and believed to directly contribute to the hyperexcitability of DRG neurons usually observed under pathologic conditions (14). Most of the currents flowing during an action potential are generated through NaV1.3, NaV1.6, or NaV1.8 in the neurons where they are expressed. Among them, cell-specific properties of NaV1.8 expressed primarily in small nociceptive DRG neurons, contribute to differences in repetitive firing properties of different classes of nociceptors (16). Moreover, mutations in SCN9A (which encodes NaV1.7) and SCN11A (which encodes NaV1.9) are directly related to human pain disorders (15). These disorders include congenital indifference to pain due to loss-of-function mutations in SCN9A; many erythromelalgia cases (a disorder characterized by bilateral burning pain of the feet/lower legs and hands, elevated skin temperature of affected areas, and reddened extremities), and hyperexcitability of DRG neurons because of gain-of-function mutations in SCN9A (12, 17, 18); and episodic chronic pain and an unusual syndrome of loss-of-pain sensation, and inclination for self-mutilation associated with gastrointestinal motility disturbances and muscle weakness because of gain-of-function mutations in SCN11A (19, 20). In light of these facts, it is not surprising that NaV1.3, NaV1.7, NaV1.8, and NaV1.9 have been the subject of numerous studies attempting to elucidate their roles in pain signaling (5, 12, 15). Evidence from multiple animal models for pathological pain also suggests these channels as potential therapeutic targets for chronic pain. Mice with a knockout of NaV1.7 only in NaV1.8-positive nociceptors lack noxious mechanical sensation and inflammatory pain (21). Mice with a knockout of NaV1.7 in both sensory and sympathetic neurons lose noxious thermal sensation and mechanical hypersensitivity in a surgical model of neuropathic pain (22). Knockdown of NaV1.8 expression via intrathecal antisense oligodeoxynucleotides (23, 24), or siRNA (25) reduces mechanical allodynia and thermal hyperalgesia in peripheral nerve injury models. Selective blockers of NaV1.8 such as A-803467 and ambroxol show potent effects, suppressing various pain symptoms and neuropathic pain (26, 27). NaV1.9 seems to be critical for inflammatory pain rather than nerve injury-induced pain. Transgenic NaV1.9-null mice show decreased hyperalgesia induced by inflam-

mation, but still show pain hypersensitivity after peripheral nerve injury (5, 28-32). On the other hand, controversial reports on the roles of these isoforms in chronic pain are also emerging. Although NaV1.3 expression is induced in adult DRG neurons following peripheral nerve injury (33, 34), NaV1.3 knockout mice exhibit normal responses to inflammatory insults, and their pain hypersensitivity following peripheral nerve injury is not changed (35). Treatment with NaV1.3 antisense oligonucleotides did not alter mechanical pain sensitivity caused by spared nerve injury in rats (36). NaV1.7 is clearly important in normal pain signaling. In spite of this fact, it has been observed that many potent selective antagonists of NaV1.7 are only weakly analgesic (37). Mice with NaV1.7 knockout still develop hypersensitivity and pain following treatment with the chemotherapeutic agent oxaliplatin or in a cancer-induced bone pain model (32). Studies using NaV1.8 or NaV1.7/NaV1.8, or NaV1.9 knockout mice demonstrated that these three channels are not involved in neuropathic pain caused by spinal nerve ligation (32, 38, 39).

NaV1.6

Although NaV1.6 is one of the highly expressed VGSCs in DRG neurons, it has been completely overlooked by the pain field until very recently. SCN8A, the gene encoding NaV1.6, was identified in 1995 by positional cloning of the mouse neurological mutant motor endplate disease (40) and by isolation of a novel sodium channel cDNA in rat brain (41). Its wide expression in every corner of the nervous system is probably one of the main reasons that NaV1.6 is so overlooked in pain pathway studies. Besides DRG neurons, NaV1.6 is highly expressed throughout the brain including in Purkinje cells, motor neurons, pyramidal, and granule neurons, glial cells, and Schwann cells, and is also found in skeletal muscle and cardiac muscle (42-45). The phenotypes of mutation of SCN8A in human and mice also did not suggest any noticeable roles of NaV1.6 in pain pathways. More than ten mutations of SCN8A have been described in human patients who have epileptic encephalopathy or intellectual disability, but not pain disorders (46). Very recently, however, a case report implicating a gain-of-function mutation of NaV1.6 in exacerbating trigeminal neuralgia has been reported (47). Homozygous SCN8A null mice exhibit motor defects including ataxia, dystonia, paralysis and tremor, and do not survive beyond 3 weeks (40, 48-50), but do not exhibit not sensory defects.

NaV1.6 regulates neuronal excitability via three properties: its subcellular localization at the axon initial segment (AIS), the site of initiation of action potentials, and at the nodes of Ranvier; its role in persistent and resurgent current; and the voltage-dependence of its activation (12, 46).

NaV1.6 and the Initiation of Action Potentials

In the central nervous system, the axon initial segment (AIS) is the membrane region of the axon closest to the soma, where the action potentials are initiated. Here, sodium channels are highly concentrated, and electrical signals from the soma and dendrites are integrated (46, 51). In several different CNS neurons, NaV1.6 has been shown to be concentrated the part of the AIS region where action potentials are initiated and where threshold at its lowest (46, 51-57). Knockout of NaV1.6 makes neurons less excitable (52) makes the spike threshold is 8 mV more positive (51).

Action potentials are regenerated and rapidly propagated via the nodes of Ranvier, a specialization of myelinated axons. In the peripheral nervous system, NaV1.6 is predominately enriched at nodes of Ranvier of both sensory and motor axons, and is essential for electrical conduction in both myelinated and unmyelinated axons (58, 59). In mice with loss-of-function mutations in NaV1.6, the maturation of nodes of Ranvier is delayed, and nerve conduction velocity is slowed (59).

NaV1.6 and Repetitive Firing

Both persistent and resurgent currents mediated by NaV1.6 contribute to repetitive firing. Persistent currents are small steady-state sodium currents that persist during a prolonged depolarization instead of inactivating. Persistent currents are involved in action potential initiation at membrane voltages, especially in cells that fire

repetitively (40, 60-66). Resurgent current is voltage- and time-dependent and is a small, transient current elicited during repolarization after the initial action potential (66). This property helps enable neurons to fire repetitively at high frequency. This small transient current flows through sodium channels that reopen in response to hyperpolarization after the decay of the large transient current. Resurgent current has been demonstrated to contribute to spontaneous discharge and multi-peaked action potentials in cerebellar Purkinje cells (67, 68). In SCN8A null mice, reduced repetitive firing, together with decreased persistent and resurgent current, has been consistently observed in several types of CNS neurons (52, 66, 67, 69). On the other hand, mutations that increase NaV1.6 persistent current result in epileptogenesis (70). Overall, the evidence collected from several different lines of SCN8A null and conditional null mice suggests that Nav1.6 is a determining factor for repetitive spiking: rapid spontaneous firing, continuous regular firing during steady depolarization or bursting firing in the central nervous system. As discussed below, it plays a similar role in sensory neurons.

NaV1.6 and Abnormal Spontaneous Activity in Injured or Inflamed Sensory Neurons

Spontaneous activity, especially burst firing or rhythmic repetitive firing, can be observed widely in normal brain. Pharmacology and modeling studies indicate that burst firing in most brain neurons relies on persistent and resurgent sodium currents. Subthreshold oscillations in the θ-frequency range (3-12 Hz), which are in turn caused by the alternating activation of a persistent sodium current and a slow repolarizing potassium current, trigger burst firing. The high-frequency of action potentials observed in a burst requires an after-hyperpolarization to accelerate the removal of sodium channel inactivation. The next spike of the burst, during the subsequent after depolarization, is triggered by the resurgent sodium current (71, 72). As introduced above, NaV1.6 is crucial for the generation of persistent and resurgent currents. Accumulating evidence collected in mice with loss-of-function mutations in SCN8A indicates that NaV1.6 plays dominant roles in burst firing initiation in multiple regions of the brain including cerebellar Purkinje neurons (68, 73), globus pallidus neurons (74), retinal ganglion cells (52), and CA1 pyramidal cells (51).

In the peripheral nervous system, on the other hand, spontaneous activity is not common, and is only observed in high incidence in animal models of neuropathic and inflammatory pain (75-80) and in humans with chronic pain conditions (81, 82). Spontaneous discharge may arise from all types of sensory neurons with Aβ-, Aδ- and C-fibers. It may originate from the neuroma, the demyelinated regions of the axon, as well as from the somata of sensory neurons (79, 80, 83). The spontaneous activity from injured axons primarily has a high-frequency regular or bursting pattern, while activity from the cell bodies shows more diverse patterns (78, 79, 84). Although abnormal spontaneous discharges are associated with neuropathy-induced pain in humans and in animal models, it is still not fully clear how the aberrant activity contributes to the onset of painful symptoms.

Among various patterns of spontaneous activity observed in damaged peripheral nerve, the bursting pattern is of particular interest. In the sensory system, burst firing has a distinct function in sensory information transmission (72). When depolarized by a single action potential, synapses may have a low probability of transmitter release (85). However, if one or more action potentials follow closely after a first action potential, the resulting accumulation of calcium in the presynaptic terminal causes more transmitter to be released, and to evoke greater postsynaptic responses over the course of high-frequency inputs (86). In this sense, burst firing is more likely to facilitate synaptic plasticity and transmission compared to a single spike or to the same number of spikes evenly timed (72, 87).

Although there was little evidence suggesting that NaV1.6 is involved in regulating pain, its crucial functions in the initiation of action potentials in both peripheral and central nervous systems, and in generation of spontaneous repetitive firing in brain neurons, are well-established. It is likely that the mechanisms underlying spontaneous bursting or repetitive firing are similar

in peripheral sensory neurons: Cummins et al. found that NaV1.6 also mediated resurgent currents in sensory neurons, where the conditional knock down of SCN8A in the DRG almost abolished resurgent current in large and small diameter neurons (12, 88).

We hypothesized that NaV1.6 may be critical for the ongoing ectopic discharge observed in peripheral neuropathy. We previously described a rat model for inflammatory pain, in which a rapid increase in spontaneous high-frequency burst firing in cells with myelinated axons is induced by local inflammation of the DRG, as well as robust, long-lasting mechanical hypersensitivity (89, 90). The burst firing is likely triggered by the underlying subthreshold membrane oscillations as previously described by Amir et al. in injured DRG neurons (91). Based on observations that subthreshold oscillations in central nervous system are caused by persistent sodium currents, we applied riluzole, a drug showing some selectivity for persistent sodium currents, to the inflamed DRG and found that spontaneous burst firing but not irregular firing was fully suppressed by riluzole, suggesting that the mechanisms for initiating burst firing in central nervous system might also be responsible for spontaneous burst firing in DRG neurons (Figure 1). In another low back pain model, induced by chronically compressing the DRG with a metal rod, persistent currents were also found to be increased and responsible for hyperexcitability observed in compressed DRG neurons (92). These studies indirectly suggested that NaV1.6 might play a role in sensory neuron hyperexcitability in these two back pain models, since this isoform had been implicated in repetitive bursting activity in central neurons. Direct evidence for a role of NaV1.6 in regulating neuropathic pain was first reported by Sittle et al. (93), who reported that the chemotherapy drug oxaliplatin-induced cold allodynia in both human subjects and mice by enhancement of NaV1.6-mediated persistent and resurgent currents in myelinated DRG neurons (93).

Our lab provided several lines of critical evidence to demonstrate for the first time that NaV1.6 plays a key role in spontaneous burst firing in inflamed DRG neurons. First, NaV1.6 is expressed in both myelinated and unmyelinated DRG neurons, however, the expression is much more intense in a subset of medium diameter cells, a size class which is more likely to discharge in a bursting pattern under neuropathic or inflammatory conditions (Figure 2) (94). Second, NaV1.6 but not NaV1.7 had a significantly higher expression in normal DRG neurons which are able to fire repetitively in a bursting pattern in response to suprathreshold current injections. Furthermore, we found NaV1.6 was

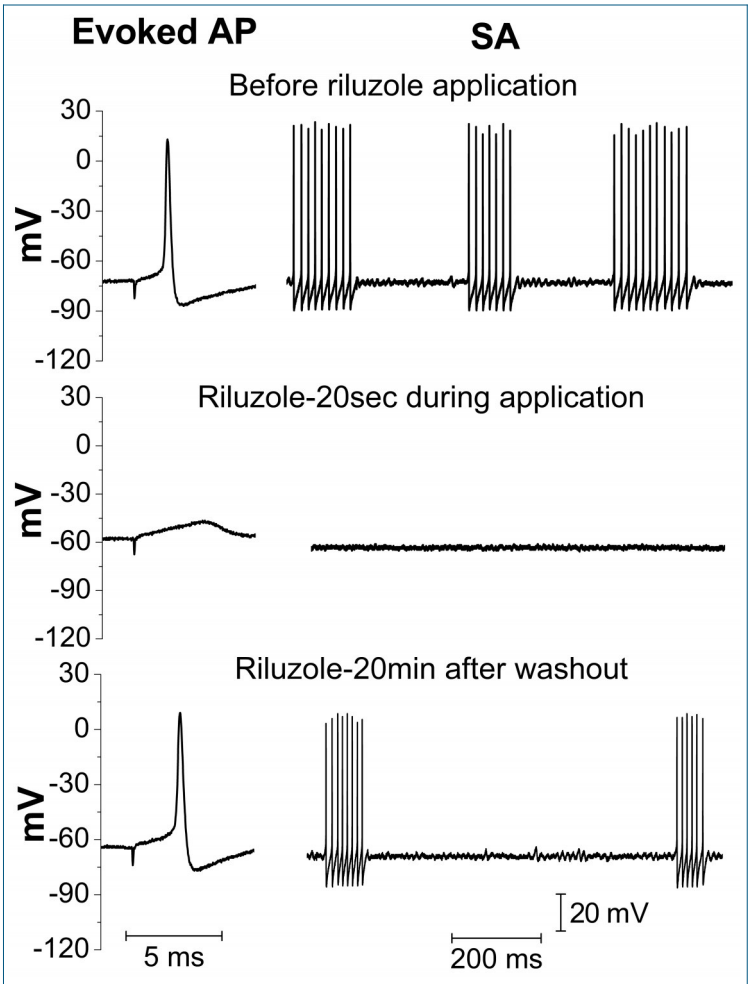

Figure 1. Example of Riluzole Effects on A Spontaneously Burst Firing Aβ Cell from A Locally Inflamed DRG.
In this cell, both spontaneous burst firing and subthreshold oscillations were eliminated, and no action potential could be evoked, after riluzole. Left, expanded view of action potentials observed in response to injection current. Right, recordings of spontaneous activity on a slower time base. Note subthreshold oscillations between the bursts of action potentials. Figure based on reference 89.

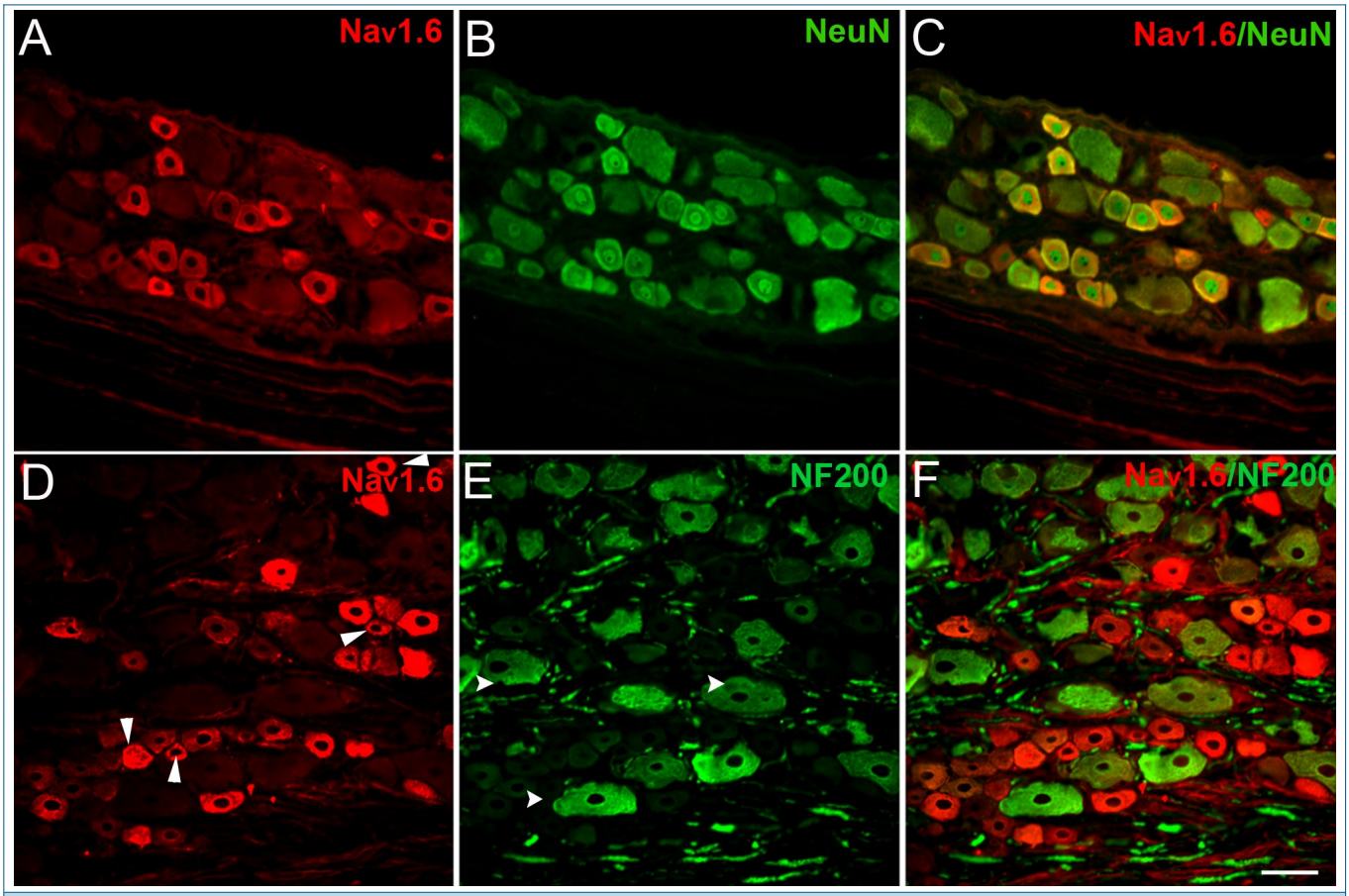

Figure 2. Immunohistochemical Staining of NaV1.6 in Normal Dorsal Root Ganglion Sections Shows NaV1.6 is Expressed in Both Myelinated (NF-200 positive) and Unmyelinated Neurons.
However, the very largest cells are less likely to be Nav1.6 positive. A subset of cells in the medium-diameter range is much more intensely stained. NaV1.6 signal is shown in red. Top: NaV1.6 (A), neuronal marker NeuN (green) (B), merged (C). Bottom: NaV1.6 (D), arrowheads show examples of NaV1.6-positive, NF200-negative cells. NF200, marker for cells with myelinated axons (green) (E), arrowheads show example of NF200-positive, NaV1.6-negative cells. Merged (F). Scale bar = 50 μm. Figure modified from reference 94.

highly expressed in cells exhibiting spontaneous burst firing after DRG inflammation but not in cells that only fired a single spike in response to incremental current injections. Third, when NaV1.6 expression was reduced by siRNA, spontaneous activity was reduced to almost normal levels in inflamed DRG three days after the surgery and siRNA injection (Figure 3). NaV1.6 siRNA also significantly reduced the proportion of myelinated cells capable of repetitive firing (94). Taken together, these results show that NaV1.6 is a key channel for burst firing in inflamed DRG neurons.

NaV1.6 and Pathological Pain

Spontaneous activity has been believed to play a critical role in initiating neuropathic pain in response to nerve injury (95, 96). In normal sensory neurons, action potentials and repetitive are only initiated at the spike initiation zone near peripheral terminals, in response to depolarization caused by transduction of the relevant sensory stimulus. However, after nerve injury, ectopic firing can emerge from other sites, including demyelinated areas and the cell soma. Altered sodium

channel properties play key roles in these changes (1). In several preclinical pain models, agents that block or reduce spontaneous activity can block development of the chronic pain. We observed that both riluzole (92) and NaV1.6 knockdown (94) were very effective in reducing mechanical pain behaviors induced by local DRG inflammation, indicating the relevance of NaV1.6-mediated bursting activity to pain induced by this model. We also demonstrated that applying NaV1.6 siRNA to knockdown NaV1.6 locally in the injured DRG in the spinal nerve ligation model (SNL) of neuropathic pain markedly reduced mechanical pain behaviors, as well as subthreshold membrane oscillations and spontaneous activity (especially burst firing) that are induced by this model (97). NaV1.6 knockdown also reduced mechanical pain models in another model of neuropathic pain, in which pain is induced by chronic constriction of the sciatic nerve (97). Recently, through selectively knocking out NaV1.6 in mouse DRG neurons, NaV1.6 specifically expressed in large NaV1.8-negative DRG neurons, that are presumed to be non-nociceptors, NaV1.6 was identified as playing an important role for increased neuronal excitability and mechanical allodynia observed in the spared nerve injury model of neuropathic pain (98).

NaVβ4, An Endogenous Open Channel Blocker for NaV1.6 that Generates Resurgent Currents

Although, in the central nervous system, the ability of a neuron to fire spontaneously in repetitive or bursting patterns usually requires NaV1.6 expression, some neurons that express NaV1.6 do not express resurgent current (12, 66). A mechanism for resurgent sodium current is open channel block, mediated by an endogenous open channel blocking particle. When VGSCs open with depolarization, rather than subsequently inactivating, they can instead be blocked by an open channel blocking particle. Upon repolarization, as the blocker unbinds, the blocked channels reopen and produce resurgent current (99). Unlike the fast inactivation gate, the native blocking particle is not part of NaV1.6 or any other of the pore-forming α subunits. Currently, the protein that has been identified as the endogenous open channel blocker is the auxiliary subunit NaVβ4 (66, 100, 101). NaVβ4 is one of the four Na + channel β subunits, which belong to the immunoglobulin superfamily of cell adhesion molecules having a single transmembrane domain, and which modulate VGSCs' gating, assembly and localization (8, 9, 66, 102-104). Among four Na + channel β subunits only the NaVβ4 subunit has a cytoplasmic tail with a nine-amino-acid domain of positively charged and hydrophobic residues, which is believed to have the necessary properties to act as an endogenous open channel blocker (101). Like NaVβ2, NaVβ4 associates with α subunits covalently, while the other two members of the NaVβ make non-covalent associations (3, 66, 103, 105).

Resurgent current has been found in 20 types of neurons throughout the nervous system (66). Resurgent current plays a key role in allowing some neurons to fire repetitively at high frequency: eighteen types of these neurons are able to fire repetitively and in a bursting pattern; over half of them can fire continuous regular or bursts spontaneously; most of them also express NaVβ4 (66). Knockdown of NaVβ4 expression by siRNA in cultured cerebellar granule cells reduces resurgent current as well as repetitive firing (100). In addition, a synthetic peptide based on the putative blocking particle in NaVβ4 can induce resurgent currents in neurons that lack them, such as CA3 pyramidal cells (101). NaVβ4 is also expressed in DRG sensory neurons where it is likely the endogenous open-channel blocker: NaVβ4 expression was found to be highly correlated with NaV1.6 expression in medium-large diameter DRG neurons (106), the class of neurons in which we also observed the high incidence of spontaneous burst firing in inflamed or injured DRG (94, 97). Knockdown of NaVβ4 expression in DRG neurons with siRNA reduced resurgent current in medium-large diameter sensory neurons. Co-expressing NaVβ4 but not NaVβ2 with recombinant NaV1.6 in sensory neurons increased resurgent current and neuronal excitability (106). However, co-expressing NaVβ4 with NaV1.6, NaV1.1 or NaV1.7 in non-excitable cells such as HEK cells cannot reconstitute resurgent current (107-109), suggesting that other proteins, or modifications to NaVβ4, or

Table 1. The Conclusions about Studies of Pain Regulation by NaV1.6 and NaVβ4 in DRG Neurons.

Conclusions	References
The ability of NaV1.6 to produce a resurgent current in large DRG neurons was demonstrated.	88
NaV1.6 expressed in myelinated DRG neurons and resurgent currents play an important role in regulating chemotherapy-induced pain.	93, 113
NaV1.6 expressed in DRG neurons mediates repetitive firing in myelinated A fiber sensory neurons and contributes to mechanical hypersensitivity and abnormal spontaneous activity in a back pain model caused by DRG inflammation.	94
NaV1.6 expressed in DRG neurons contributes to mechanical hypersensitivity, abnormal sympathetic sprouting and hyperexcitability in medium and large DRG neurons in spinal nerve ligation and chronic constriction injury neuropathic pain models.	97
The resurgent current carried by NaV1.6 in medium-large DRG neurons is regulated by NaVβ4.	106
Resurgent current mediated by NaVβ4 in medium and large DRG neurons contributes to mechanical hypersensitivity induced by DRG inflammation.	111
Through genetic analysis in patients with trigeminal neuralgia, a novel gain-of-function NaV1.6 mutation was revealed and demonstrated to potentiate transient and resurgent sodium currents and increase excitability in trigeminal ganglion neurons.	47
NaV1.6 is required in NaV1.8-negative (non-nociceptor) sensory neurons to mediate neuronal hyperexcitability and mechanical allodynia in the spared nerve injury model of neuropathic pain.	98

DRG, dorsal root ganglion.

some other aspect of the neuronal cellular environment, are also required for this subunit to function as a blocking protein. There are also a few examples of neurons with a resurgent current that do not express NaVβ4 or that do not express NaV1.6 (66).

Resurgent sodium current produced by NaV1.6 in DRG neurons also could be differentially regulated by isoforms of fibroblast growth factor homologous factor 2 (FHF2). A recent study done by Barbosa et. al, suggested that FHF2A reduced resurgent current and enhanced NaV1.6 occupancy of inactivated states and delayed its recovery, whereas FHF2B increased resurgent currents instead of enhancing inactivation. They also observed a downregulation of FHFA isoforms and upregulation of FHF2B in inflamed DRG neurons and found that pain behaviors and increased spontaneous activity in DRG neurons induced by inflammation of the DRG were reduced by FHFA peptide (110).

NaVβ4 Expressed in DRG Neurons Contributes to Pain from DRG Inflammation

The observations that NaVβ4 is the open-channel blocker for NaV1.6 generating resurgent currents in DRG neurons, and that knockdown of NaV1.6 strongly reduced spontaneous firing of large-medium diameter DRG neurons as well as mechanical hypersensitivity induced by local inflammation of the DRG, suggested that NaVβ4 in DRG sensory neurons may be involved in regulating pain. We found that tetrodotoxin-sensitive resurgent currents are increased by local inflammation of DRG. This increase may partially result from upregulated NaVβ4 expression in inflamed DRG. Knockdown of NaVβ4 expression in inflamed DRG neurons by siRNA significantly reduced both persistent and resurgent currents in medium-diameter neurons (Figure 4). In addition, marked neuronal hyperexcitability and spontaneous activity in inflamed DRG neurons are also reduced by NaVβ4 siRNA. The reduction was similar to that observed when Nav1.6 expression was decreased in inflamed DRG. Thus, it should not be surprising that knockdown of NaVβ4 also almost completely prevented the development of mechanical hypersensitivity after DRG inflammation. Interestingly, knockdown of NaVβ4 also reduced expression of NaV1.6 in both inflamed and normal DRG, whereas the TTX-sensitive transient current am-

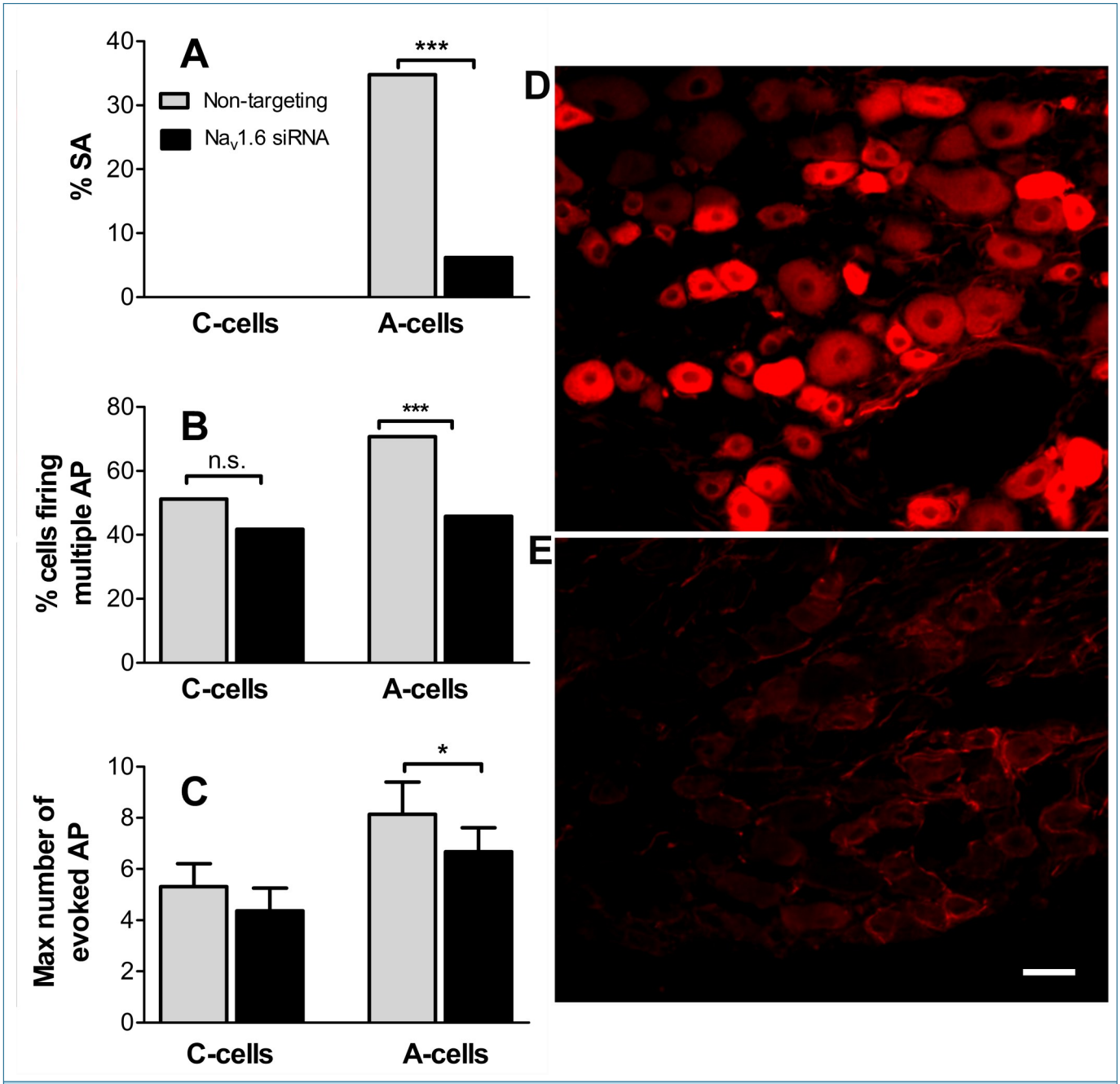

Figure 3. Electrophysiological Effects of Nav1.6 Knockdown in Inflamed DRG Measured on Postoperative day (POD) 3.

Nav1.6 siRNA or nontargeting siRNA was injected into the DRG at the time of DRG inflammation (POD 0). (A) The incidence of spontaneous activity (SA) measured was significantly reduced in A (myelinated) cells from Nav1.6 siRNA treated animals. Incidence was zero in both C (unmyelinated) cell groups. (B) Percent of cells capable of firing > 2 action potentials (AP) in response to suprathreshold current injection (includes all SA cells). (C) Maximum number of action potentials fired during 270-ms suprathreshold current injections (non-SA cells only). (D, E) Examples of DRG sections from nontargeting (D) or NaV1.6 siRNA (E)-treated inflamed DRG on POD 3 stained for NaV1.6. Scale bar = 50 μm. *, P = 0.01 to <0.05; ***, P < 0.001, Fisher's exact test or Mann-Whitney test; N = 181-193 A cells/group and 39-43 C cells per group. The figures are modified from reference 94.

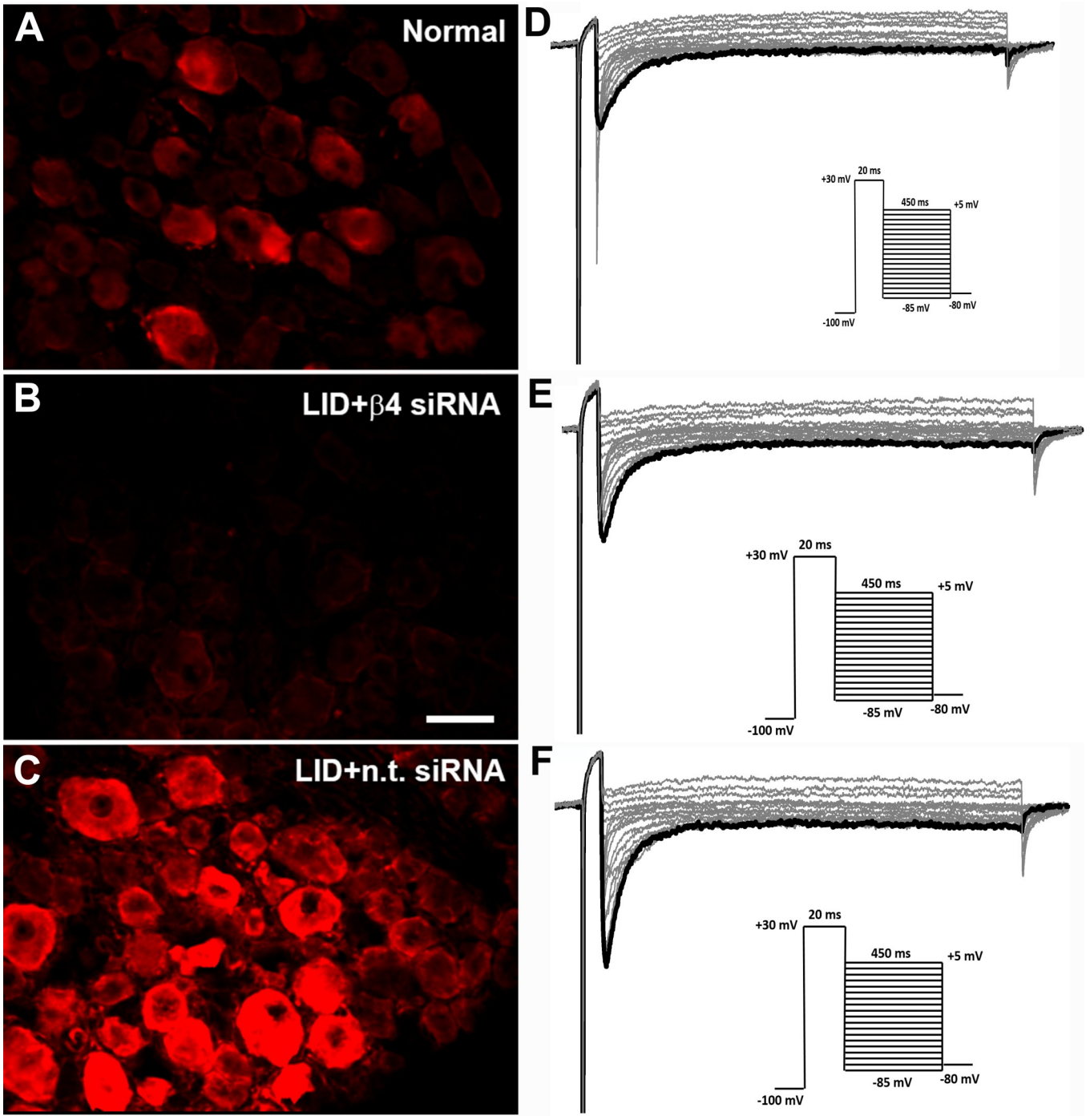

Figure 4. Both NaVβ4 Expression and Tetrodotoxin (TTX) -Sensitive Resurgent and Transient Currents in DRG Neurons are Increased by DRG Inflammation, and Reduced by Knockdown of NaVβ4 by siRNA.
Sample sections stained for NaVβ4 are shown from normal DRG (A) and DRG 4 days after DRG inflammation (LID) and injection of either nontargeting (n.t.) (B) or NaVβ4 (C) siRNA. Scale bar = 50 μm. Na+ currents were recorded in acutely cultured medium diameter DRG neurons from control DRG or from DRG 3 days after DRG inflammation (LID) and injection of nontargeting (n.t.) or NaVβ4 siRNA. Data are from medium diameter cells that only expressed TTX-sensitive Na+ current. Sample traces are shown of resurgent current recordings from cells cultured from sham control (D), and n.t. siRNA (E) and NaVβ4 siRNA (F) injected inflamed DRG. The much large transient current during the +30 mV prepulse (inset: voltage protocol) is off-scale. Figures and traces are modified from reference 111.

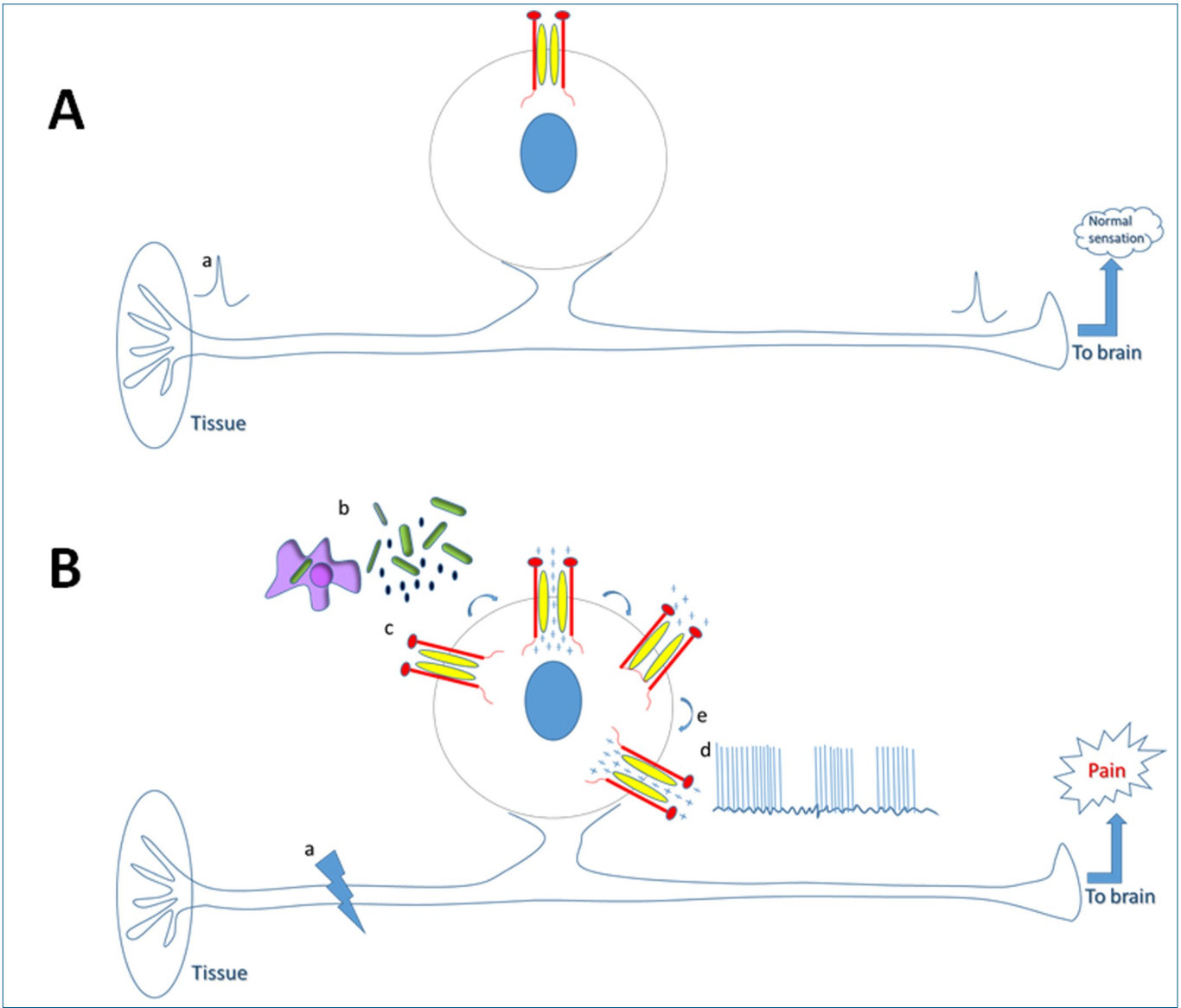

Figure 5. Schematic of Resurgent Current in Pain Regulation under Pathological Condition.
A. Action potential (a) is generated at the receptive field when tissue is stimulated and conducted through primary afferent into the spinal cord and brain to form normal sensation. B. As periphery nerve is damaged (a) or inflamed (b), spontaneous activity can be generated in DRG neuron because of activation of sodium channels (c). Co-expression of NaV1.6 (yellow) and NaVβ4 (red) enables a medium or large DRG neuron to generation burst or repetitive spontaneous activity (d) through resurgent current, which is produced as blocked channels reopen as blocker unbind (e), and contribute to pain hypersensitivity.

plitude was not affected (111). Although there is evidence suggesting that NaVβ subunits can regulate localization and trafficking of the α subunits, mechanisms underlying NaVβ4 effects on Nav1.6 trafficking and expression require further study.

Summary

The importance of NaV1.6 and NaVβ4 in pain regulation has only been recognized in recent years. In Table 1, we summarize some recent

studies on this topic. Persistent and resurgent currents in DRG neurons play key roles in triggering spontaneous repetitive and burst firing in injured DRG neurons. As in the central nervous system, the persistent and resurgent currents in DRG neurons are generated predominantly by NaV1.6 in association with NaVβ4. Since spontaneous activity, especially high-frequency repetitive and burst firing in damaged peripheral sensory nerve or neurons, is crucial for the development of neuropathic pain, NaV1.6 and/or NaVβ 4 may be new therapeutic targets for managing pathological pain conditions. Based on current knowledge the roles of NaV1.6 and NaVβ4 in regulating pain are summarized in figure 5. As discussed above and in reference 58, Nav1.6 is widely distributed throughout the peripheral nervous system, including all nodes of Ranvier in peripheral motor and sensory axons, and throughout the central nervous system, in different cellular locations including nodes of Ranvier, unmyelinated axons, axon initial segments, dendrites, and pre- and postsynaptically. This has understandably limited enthusiasm for developing even the highly selective NaV1.6 blockers for clinical use, which will presumably be limited by the potential side effects. However, local application of NaV1.6 blockers or targeting the persistent and resurgent current, which often rely on NaVβ4 could be a more promising strategy. Spontaneous activity is more sensitive to some agents (for example, local anesthetics) than conducted action potentials are (112); focusing on the properties of NaV1.6 and NaVβ4 that mediate persistent and resurgent currents might improve the development of such pharmaceutical reagents for use in pain conditions.

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