REVIEW

Normalization of Neuroinflammation: A New Strategy for Treatment of Persistent Pain and Memory/Emotional Deficits in Chronic Pain

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Abstract: Chronic pain, which affects around 1/3 of the world population and is often comorbid with memory deficit and mood depression, is a leading source of suffering and disability. Studies in past decades have shown that hyperexcitability of primary sensory neurons resulting from abnormal expression of ion channels and central sensitization mediated pathological synaptic plasticity, such as long-term potentiation in spinal dorsal horn, underlie the persistent pain. The memory/emotional deficits are associated with impaired synaptic connectivity in hippocampus. Dysregulation of numerous endogenous proteins including receptors and intracellular signaling molecules is involved in the pathological processes. However, increasing knowledge contributes little to clinical treatment. Emerging evidence has demonstrated that the neuroinflammation, characterized by overproduction of pro-inflammatory cytokines and glial activation, is reliably detected in humans and animals with chronic pain, and is sufficient to induce persistent pain and memory/emotional deficits. The abnormal expression of ion channels and pathological synaptic plasticity in spinal dorsal horn and in hippocampus are resulting from neuroinflammation. The neuroinflammation is initiated and maintained by the interactions of circulating monocytes, glial cells and neurons. Obviously, unlike infectious diseases and cancer, which are caused by pathogens or malignant cells, chronic pain is resulting from alterations of cells and molecules which have numerous physiological functions. Therefore, normalization (counterbalance) but not simple inhibition of the neuroinflammation is the right strategy for treating neuronal disorders. Currently, no such agent is available in clinic. While experimental studies have demonstrated that intracellular Mg^{2+} deficiency is a common feature of chronic pain in animal models and supplement Mg^{2+} are capable of normalizing the neuroinflammation, activation of upregulated proteins that promote recovery, such as translocator protein (18k Da) or liver X receptors, has a similar effect. In this article, relevant experimental and clinical evidence is reviewed and discussed.

Keywords: ion channels, synaptic plasticity, cytokine, glial cell, monocytes

Introduction

The prevalence of chronic pain is around 30% in the world population[1–3] and around 2/3 chronic pain patients suffer from memory deficit and mood depression.[4–7] In spite of intensive studies for decades worldwide, clinical treatment of chronic pain is still largely unmet. Understanding the mechanisms underlying the chronic pain and its comorbidities is important scientifically and clinically.

Definition, Classification and a Brief History of Chronic Pain

Pain, a most common chief complaint in clinic,[8] is redefined as an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage in 2020.[9] According to physiological function, pain is classified into nociceptive (physiological) pain and pathological pain. Nociceptive pain is a pain triggered by activation of nociceptors. It serves as an alarm system and protects tissues from further damage, while pathological pain is a consequence of injury and disease, manifested as allodynia (decrease in pain threshold), hyperalgesia (increase in response to noxious stimuli) and spontaneous pain. Clinically, pain is divided into acute pain

5201

and chronic pain. The acute pain is identical to physiological pain. Whereas chronic pain is pain persisting beyond the expected healing period. In clinic practice, the pain that lasts or recurs for longer than 3 months is defined as chronic pain.[10] The short-lasting pathological pain is also protective, such as the pain hypersensitivity induced by skin burning that favors recovery by avoiding touching the injured tissues. In contrast, chronic pain is a leading source of human suffering and disability without any beneficial effect.[11]

Mechanistically, chronic pain is mediated by nociceptive pain, neuropathic pain and nociplastic pain.[12] Persistent nociceptive pain is most common form of chronic pain, such as osteoarthritis pain[13] produced by mechanical stimuli due to bone destruction and chemical stimuli by substance P, K^+ and inflammatory mediators released by damaged tissues. Neuropathic pain is redefined as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system.[14] In experimental studies, neuropathic pain is often produced by injury of peripheral nerves in rats and mice, such as chronic constriction of sciatic nerve (CCI),[15] lumbar 5 and/or 6 spinal nerve ligations (L5/L6-SNL)[16] and spared nerve injury (SNI), in which the two terminal branches of the sciatic nerve (tibial and common peroneal nerves) are lesioned, leaving the sural nerve intact.[17] Neuropathic pain can be also produced by selective injury of motor fibers by L5 ventral root transection (L5-VRT),[18] by application of anti-cancer agents, such as paclitaxel[19] and vincristine,[20] and by streptozotocin that induces type 1 diabetes.[21] Nociplastic pain was first put forward in 2016 as the third mechanistic descriptor for chronic pain,[22] based on the clinical observations that in some types of chronic pain, such as fibromyalgia, complex regional pain syndrome type 1 and nonspecific chronic lower back pain, neither obvious nociceptor activation nor neuropathy is evident and, therefore, cannot be described as nociceptive or neuropathic pain. The nociplastic pain is believed to be caused by alteration of nociceptive processing in central nervous system (CNS),[23] such as changes in cerebral activation, synaptic connectivity and even neuronal structures. However, it is worth noting note that compelling experimental and clinical evidence has demonstrated that the functional and structural plastic changes in both peripheral nerves and CNS can be produced by many pathogenic causes of chronic pain, such as activation of nociceptors by intensive noxious stimuli,[24] nerve injury,[25,26] internal biochemical change, including overproduction of proinflammatory cytokines,[27,28] and external chemical stimuli including anti-cancer agents[29,30] and opioids.[31] Thus, the neuroplastic change is probably a common mechanism of chronic pain (Figure 1).

In medical history, pain was considered merely a symptom of other diseases for a long time. At that time, little is known about the difference in mechanisms of physiological pain and pathological pain. In 1974, Wall et al found that the injured sensory fibers generated action potentials spontaneously after sciatic nerve transection.[32] The ongoing activity
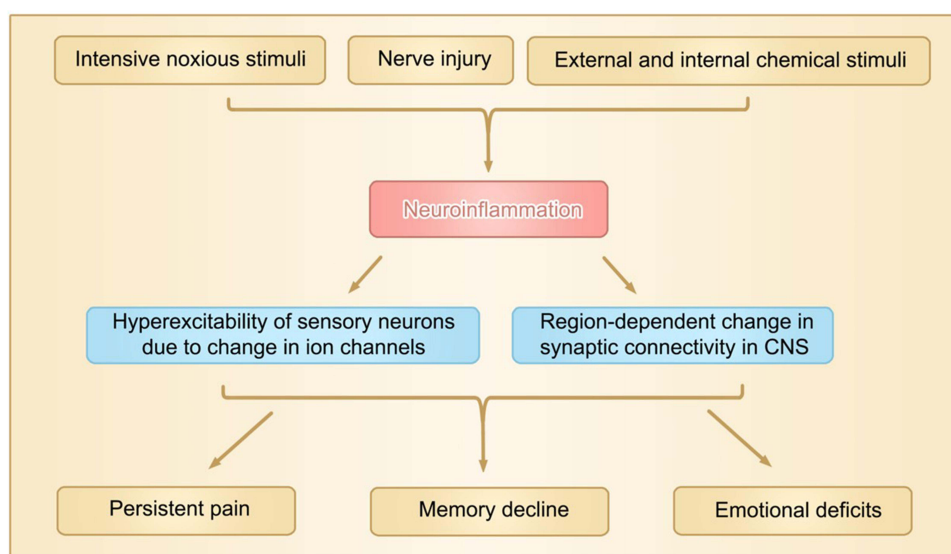

Figure 1 Neuroinflammation produced by various etiological factors plays a central role in functional and structural plastic changes in peripheral and central nervous system, leading to persistent pain, memory decline and emotional deficits. The region-dependent change in synaptic connectivity means differential changes in synaptic connection, such as synaptic connection is enhanced in spinal dorsal horn but reduced in hippocampus in neuropathic pain mice. Over-production of TNF-α is sufficient to induce the change.

(ectopic discharge) in the injured afferents is considered to underlie persistent pain (see[33,34] for reviews). In 1983, Woolf provided the first evidence for a central component of persistent pain by showing that the excitability of spinal cord neurons is persistently increased following peripheral injury, and the change is independent of ongoing activity from peripheral nerves.[35] The phenomenon is termed central sensitization. In 1988, Bennett and Xie[15] made the first rat neuropathic pain model, ie, CCI model. The animals exhibit the behavioral signs of neuropathic pain, including allodynia, hyperalgesia and spontaneous pain after surgery. In 1999, Basbaum first proposed that persistent pain should be considered a disease state of the nervous system, not merely a prolonged acute pain symptom of other disease conditions,[36] according to the fact that the neurochemical features of persistent pain are distinct from acute pain revealed by many studies. Twenty years later, chronic pain was systematically classified in 11th revision of the International Classification of Diseases (ICD-11).[37] In the ICD-11, chronic pain with unknown etiology is defined as chronic primary pain. The chronic primary pain is considered a disease of nervous system in its own right, including chronic widespread pain, complex regional pain syndrome, chronic primary headache or orofacial pain, chronic primary visceral pain and chronic musculoskeletal pain. Chronic pain produced by injury or other diseases is classified as chronic secondary pain syndromes, including chronic cancer-related pain, chronic neuropathic pain, chronic secondary visceral pain, chronic posttraumatic and postsurgical pain and chronic secondary musculoskeletal pain. The ICD-11 classification of chronic pain is continuously updated (see http://id.who.int/icd/entity/1581976053).

Increased Excitability of Primary Sensory Neurons and Pathological Synaptic Plasticity in Pain Pathways Underlie Persistent Pain

It has long been proposed that persistent pain is mediated by peripheral sensitization and central sensitization.[35,38] Now, it is well established that the peripheral sensitization, an increased excitability of primary sensory neurons, is resulting from abnormal expression of a variety of ion channels, including voltage-gated sodium (Nav) channels,[39,40] voltage-gated calcium (Cav) channels,[41,42] potassium channels,[43] transient receptor potential (TRP) channels,[44,45] and acid-sensing ion channels (ASICs).[46] The changes of some ion channels in sensory neurons in chronic pain are shown in Figure 2. The hyperexcitability of sensory neurons leads to spontaneous discharges and hypersensitivity to peripheral stimuli. The central sensitization is underlined primarily by long-lasting enhancement of synaptic transmission, called long-term potentiation (LTP), which amplifies signals in pain pathways (see[47,48] for reviews).
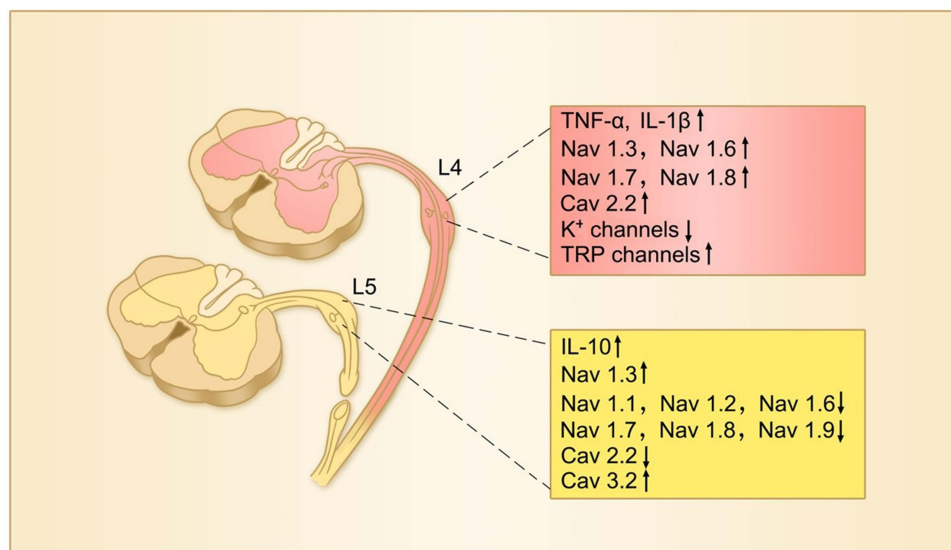
Figure 2 The changes in expression of TNF-α, IL-1β and IL-10 and various ion channels in injured and uninjured (intact) DRG neurons following peripheral nerve injury. In this L5-spinal nerve ligation model of neuropathic pain, L5 or both L5 and L6 spinal nerves are ligated and transected. Therefore, L5 DRG neurons are injured and L4 DRG neurons remain intact.[16]

Mechanisms Underlying the Comorbidity of Persistent Pain and Memory/Emotional Deficits are Under Debate

To explain the comorbidity of persistent pain and memory deficits, it was proposed that persistent pain might impair memory by attracting attention.[49,50] However, human studies have shown that acute pain does not affect memory in healthy individuals[51] and that relief of pain with opioids cannot improve the memory in chronic pain patients.[52] Likewise, depression was also believed to be a consequence of persistent pain.[53,54] It has also been proposed that persistent pain and depression may share common pathological mechanisms but are independent diseases without causal interaction.[55,56]

Together, chronic pain may be resulting from either chronification of acute pain,[57] other diseases or internal molecular changes with unknown cause. In recent years, emerging evidence indicates that neuroinflammation, characterized by over-production of proinflammatory cytokines and glial activation,[58] is a common pathological mechanism of persistent pain, memory decline and mood depression[59,60] (Figure 1). In this article, related evidence is reviewed and a new strategy for treating neuronal disorders, ie, normalization (or counterbalance) but not simply inhibition of neuroinflammation, is proposed.

Cytokine Microenvironment Hypothesis of Chronic Pain

The functions of the nervous system are exerted by the neuronal networks, in which the neurons are connected to each other via synapses. The efficacy of the network depends on the excitability of individual neurons and the strength and pattern of synaptic connections. Now, it is well established that both the excitability of neurons and the synaptic connectivity are not static; rather, they undergo activity- or experience-dependent plastic change in whole life. The numbers of excitatory and inhibitory synapses are also frequently changed, leading to remodulation of neuronal circuits (see[61] for a review). The plastic change means that, once the change occurs, it persists for a period of time without automatic recovery. The plastic change is essential for many physiological and pathological processes in nervous system, such as memory storage[62,63] and chronic pain.[47] A key question is what is the root cause for the activity-dependent plastic change? Before discussing this, let us review a key physiological concept: internal environment homeostasis. The idea was first described by French physiologist Claude Bernard in 1865, and the word "homeostasis" was coined by American physiologist Walter Bradford Cannon in 1926.[64] In this concept, extracellular fluid, in which the cells live, is defined as internal environment. To sustain the functions and survival of the cells, physical and chemical conditions, including temperature, pH, osmolality, the contents of ions, nutriment, oxygen and carbon dioxide etc., in the internal environment should be kept constant. In the last decades, studies from ourselves and others have demonstrated that the overproduction of proinflammatory cytokines plays a key role in persistent pain and accompanied memory/emotional deficits in chronic pain. In 2013, we put forward a cytokine microenvironment hypothesis of chronic pain.[65] The hypothesis has been supported by more and more studies. The updated experimental and clinical evidence is listed below. (1) Overexpression of proinflammatory cytokines is reliably detected in humans and animals with chronic pain. (2) Administration of proinflammatory cytokines is sufficient to induce behavioral signs of persistent pain, memory deficit and depressive behaviors in naive animals. (3) The abnormal expression of numerous ion channels that causes hyperexcitability of sensory neurons is resulting from over-production of inflammatory cytokines. (4) The inflammatory cytokines regulate the synaptic plasticity in a region-dependent manner, ie, enhance and reduce synaptic connectivity in spinal dorsal horn and in hippocampus, respectively, contributing to persistent pain and memory/emotional deficits. Therefore, the inflammatory microenvironment might be a common cause of persistent pain and memory/emotional deficits in chronic pain. In this subsection, the related evidence is reviewed and discussed.

Overexpression of Proinflammatory Cytokines is Reliably Detected in Humans and Animals with Chronic Pain and is Sufficient to Induce Persistent Pain and Memory/Emotional Deficits

Cytokines are proteins, peptides or glycoproteins with a molecular mass between 8 and 30 kDa, including tumor necrosis factor (TNF-α), interleukins, chemokines and many other signaling molecules. Cytokines play key roles in the interactions and communications between cells at nano- to pico-molar concentrations under normal or pathological conditions.[66,67] The production of cytokines is primarily regulated by autocrine and paracrine, ie, the cytokines released by a cell act on itself or on nearby cells by activation of their specific receptors, leading to production of more cytokines, and positive feedback is important in many diseases.[68–70] According to their immune functions, cytokines are divided into two classes: pro-

inflammatory cytokines, including TNF-α, interleukin-1beta (IL-1β), IL-6, and anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta (TGF-β).

The increase in proinflammatory cytokines is reliably detected in human patients with many forms of chronic pain. A recent systematic review indicates that plasma TNF-α is increased in patients with chronic non-specific lower back pain, which is one of the greatest contributors to suffering and disability in the world.[71] Plasma TNF-α is also enhanced in patients with fibromyalgia, a syndrome characterized by widespread chronic pain,[72] and with rheumatoid arthritis.[73] TNF-α is increased while Il-10 is decreased in plasma of women with migraine.[74]

Cytokines are originally identified in peripheral immune cells, such as lymphocytes and monocytes,[67] while emerging experimental evidence has shown that both TNF-α[28,75] and IL-1β[59,76] are expressed in nervous system and are persistently upregulated in dorsal root ganglion (DRG), spinal dorsal horn, hippocampus and many other brain regions in neuropathic pain condition, upregulation of TNF-α or IL-1β in sensory neurons is detected a few hours or even tens of minutes after nerve injury.[59,77] It has been shown that knockout of either TNF-α or IL-1β only partially prevents the mechanical allodynia induced by peripheral nerve injury, while knockout of both of them completely abolishes the behavioral sign of neuropathic pain.[78] Thus, both TNF-α and IL-1β may be needed for full expression of neuropathic pain. Upregulation of TNF-α in DRG neurons is also reported in vincristine- and bortezomib-induced chemical neuropathy,[29,79] diabetic neuropathy[80] and menopausal-induced chronic pain,[27] while blockage of TNF-α synthesis or genetic deletion of TNF receptor 1 (TNFR1) substantially prevents neuropathic pain.[75,81] Importantly, local application of TNF-α[70,82] or IL-1β[83] onto sciatic nerve at physiological concentrations in naive rats reliably induces the behavioral signs of neuropathic pain. Thus, overproduction of the inflammatory cytokines is not only necessary but also sufficient to induce neuropathic pain. Furthermore, pro-inflammatory cytokines are also critically involved in memory deficits (see[84,85] for reviews) and major depression (see[86,87] for reviews) in many diseases other than chronic pain. Likewise, the working memory and short-term memory impairments produced by SNI are prevented by genetic deletion of TNFR1 and mimicked by intracerebroventricular or intrahippocampal injection of TNF-α.[28] SNI induced-depression-like behavior and upregulation of IL-1β mRNA in the frontal cortex are ameliorated by intracerebroventricular administration of IL-1 receptor antagonist.[88] The SNI-induced persistent pain, memory decline and depression-like behaviors are mimicked in naive rats by repetitive intravenous injection of recombinant rat IL-1β at a pathological concentration, determined in SNI rats.[59] Thus, upregulation of the proinflammatory cytokines is sufficient to induce memory/emotional deficits, too.

Role of Ion Channels in Sensory Neurons for Persistent Pain and Their Regulation by Cytokines

In peripheral nerves, nociceptive signals (action potentials) are initiated and conducted by myelinated Aδ- and unmyelinated C-fibers; their cell bodies are located in DRG and trigeminal ganglia. The peripheral terminals (nociceptors) of the sensory neurons are distributed in whole body and their central terminals make synapses with the second order neurons in spinal dorsal horn or in trigeminal subnucleus caudalis. The ion channels are essential for nociceptor transduction (conversion of peripheral noxious stimuli into electrical signals), generation and conduction of action potentials, and neurotransmitter release in central terminals. The sensory neurons that give rise to myelinated A-fibers are neurofilament-200 (NF-200)⁺, while the neurons that give rise to unmyelinated C-fibers are either calcitonin gene-related peptide (CGRP)⁺, called peptidergic C-fibers, or isolectin B4 (IB4)⁺, called non-peptidergic C-fibers. The ion channels are unevenly distributed in different types of sensory neurons. Emerging evidence shows that the ion channels in sensory neurons are regulated by cytokines. The roles of various ion channels in persistent pain and their regulation by cytokines are discussed in this subsection (Table 1).

Voltage-Gated Sodium Channels

At least nine subtypes of sodium channels (Na1.1 to Nav1.9) have been identified, and most of them (except for Nav1.4 and Nav1.5) are expressed in DRG neurons. Nav1.8 and Nav1.9 are tetrodotoxin (TTX)-resistant and others are TTX-sensitive. Nav1.7, Nav1.8 and Nav1.9 are preferentially expressed in the afferent sensory neurons, including DRG and trigeminal ganglia (see[89] for a review). To date, mutation of Nav1.7, Nav1.8 and Nav1.9 has been reported to be involved in human chronic pain. Loss-of-function mutation of $Na_v1.7$ leads to complete inability to sense pain,[90] while gain-of-function of

Table 1 The Roles of Ion Channels in Sensory Neurons for Persistent Pain and Their Regulation by Cytokines

Channels	Animal Models	Human Patients	Cytokine Regulation
Nav1.3	Involved in NP.[112–115]	Not determined.	Up- and downregulated by TNF-α[106] and IL-10.[138]
Nav1.6	Involved in diabetic NP.[116,117]	Not determined.	Up- and downregulated by TNF-α and IL-10.[141]
Nav1.7	Involved in nociceptive pain and NP.[100–104]	Loss-of-function causes analgesia,[90] gain-of-function to paroxysmal extreme pain disorder.[91] Meanwhile, its blockers are ineffective for NP and acute pain.[118–120]	Up- and downregulated by TNF-α[133] and IL-10.[142]
Nav1.8	Involved in nociceptive pain and NP.[107–110]	Gain-of-function leads to painful neuropathy.[92–95]	Up- and downregulated by TNF-α[106] and IL-10.[141]
Nav1.9	Involved in inflammatory pain and NP.[111]	Gain-of-function leads to painful neuropathy.[92–95]	No data is available.
Cav 3.2	Involved in both nociceptive pain and NP.[42]	Not determined.	Upregulated by IL-6.[160]
Cav 2.2	Upregulation in intact DRG neurons leads to NP.[41]	Cav 2.2 modulators are-first line drugs for NP. Its blocker ziconotide is used to treat severe chronic pain.[158]	Up- and downregulated by IL-1β and IL-10.[41]
M-channel (Kv7.2, Kv7.3)	Reduction of the channel increases neuronal excitability.[161]	M-channel opener Flupirtine was used as an analgesic and withdrawn in 2018 for liver toxicity.[162]	Both TNF-α and IL-β downregulate K$^+$ channels in DRG neurons.[163–165]
TRPV1 TRPA1	Upregulation contributes to NP.[169–172]	Inhibitors failed in clinical trials due to thermoregulatory function and other adverse side effects.[45]	TNF-α upregulates TRPV1[174–177] and TRPA1.[178–181] IL-β upregulates TRPV1.[182–185]
ASIC1b ASIC3	Involved in inflammatory pain and NP.[188–190]	Not determined.	TNF-α enhances ASIC currents.[191,192] IL-1β upregulates ASIC3.[193–195]

Note: The channel subtypes whose roles have been determined in persistent pain are included in this table.
Abbreviation: NP, neuropathic pain.

Nav1.7 results in paroxysmal extreme pain disorder.[91] Gain-of-function mutation of Nav1.8 and Nav1.9 leads to painful peripheral neuropathy[92,93] and to painful neuropathy[94] or familial episodic pain,[95] respectively.

In rats, Nav1.7 is mainly expressed in nociceptive neurons that give rise to Aδ-fibers and C-fibers. Nav1.7 is detected in every part of DRG neurons, including cell body, peripheral axons in the sciatic nerve and peripheral terminals in skin and central terminals in superficial spinal dorsal horn.[96] Nav1.7 is also expressed in sympathetic ganglion neurons[97,98] and in spinal dorsal horn neurons.[99] In rodents, deletion of Nav1.7 in DRG neurons attenuates nociceptive pain and the persistent pain induced by nerve injury, inflammation and skin burning.[100–103] Blockage of Nav1.7 significantly alleviates neuropathic pain induced by the anti-cancer agent paclitaxel.[104]

Nav1.8 is expressed in all types of DRG neurons,[105,106] and is involved in both nociceptive pain and neuropathic pain. In Nav1.8-null mutation mice, response to cold, mechanical stimuli, and thermal hyperalgesia are reduced.[107] In rodents, selective knockdown of Nav1.8 with specific antisense oligodeoxynucleotides[108] or blockage of Nav1.8 with different kinds of specific antagonists reverses neuropathic pain.[109,110]

Nav1.9 is also expressed in the soma, peripheral and central terminals of all types of DRG neurons and is involved in inflammatory pain and neuropathic pain in animal models (see[111] for a review).

Nav1.3 is hardly detected in DRG neurons of adult rodents, but quickly re-expressed in injured and intact sensory neurons after SNI.[112] Knockdown of Nav1.3 in L4 DRG by injection of virus-mediated shRNA directed against Nav1.3 attenuates SNI-induced neuropathic pain in rats.[113] The same manipulation also alleviates tactile allodynia in

streptozotocin-induced diabetic models of neuropathic pain.[114] Nav1.3 is also re-expressed in dorsal horn neurons in rat CCI model. Intrathecal antisense oligodeoxynucleotides targeting Nav1.3 reduces both hypersensitivity of dorsal horn neurons and pain-related behavior.[115] Thus, the re-expression of Nav1.3 DRG neurons may contribute to neuropathic pain. Nav.16 is upregulated in diabetic peripheral neuropathy rats.[116] Nav1.6 activation in skin and gut leads to increased response to mechanical stimuli and mechanical allodynia but not thermal allodynia.[117] The roles of Nav1.3 and Nav 1.6 in human chronic pain have not been determined.

Regardless of importance of Navs in nociceptive and neuropathic pain in humans and in rodents discussed above, to date no specific Nav subtype blocker is used in clinic for pain relief. In clinical trials, specific Nav1.7 blockers are ineffective for painful diabetic peripheral neuropathy,[118] trigeminal neuralgia[119] and acute pain induced by noxious stimuli in healthy subjects.[120] As the nociceptive process, from nociceptor transduction to synaptic transmission in superficial spinal dorsal horn, is not dependent on a single Nav subtype, it was proposed that various Nav types, such as Nav1.7 and Nav1.8, should be blocked spontaneously to achieve effective analgesia (see[40] for a review).

Because of the importance of Navs in the genesis and propagation of action potentials, it was hypothesized that upregulation of the sodium channels in injured sensory neurons might contribute to neuropathic pain. However, animal studies in rodents from different groups have revealed that, apart from Nav1.3, all other Nav subtypes, including Nav 1.1, Nav1.2, Na1.6, Nav1.7-Nav1.9, are downregulated in the injured DRG neurons in SNI and L5-SNL models of neuropathic pain (see[121] for a review). Apparently, the experimental data seem contradictory to the clinical observation that sodium channel blockers (such as lidocaine) can effectively inhibit a variety of neuropathic pain syndromes in patients[122] and to the long-standing general belief that the ectopic discharges in injured sensory neurons directly contribute to neuropathic pain.[123–125]

A previous work showed that the behavioral sign of neuropathic pain was not dependent on inputs from injured nerve fibers but on those from intact ones by showing that mechanical hyperalgesia induced by lesion of L5 spinal nerve was reversed by L4 dorsal rhizotomy but not by L5 dorsal rhizotomy.[126] The data suggest that the intact DRG neurons may be responsible for abnormal pain behavior. Consistently, it has been shown that the intact DRG neurons also discharged spontaneously after nerve injury.[127,128] Furthermore, selective injury of motor neurons, leaving the sensory neuron intact, by L5-VRT produces persistent mechanical allodynia and thermal hyperalgesia in bilateral hind-paws,[18,75,106,129] while selective injury of sensory neurons by L5 dorsal root transection fails to induce the behavioral signs of neuropathic pain.[129–134] The data suggest that damage of sensory neurons is neither necessary nor sufficient to induce neuropathic pain. And then, how could damage of motor fibers lead to neuropathic pain? We found that selective injury to motor neurons by L5-VRT upregulated TNF-α in the neurons of DRG and spinal dorsal horn. Blockage of TNF-α synthesis or genetic deletion of TNFR1 substantially prevents neuropathic pain.[75,81] Furthermore, L5-VRT also persistently upregulates Nav1.3 and Nav1.8 at both mRNA and protein levels in the neurons of L4 and L5 DRGs. Importantly, the upregulation of sodium channels is substantially prevented by inhibition of TNF-α synthesis or genetic deletion of TNFR1, and is mimicked by peri-sciatic application of TNF-α.[106] In cultured DRG neurons, TNF-α at 10–1000 pg/mL dose-dependently upregulates Nav1.3, Nav1.8[106] and Nav1.7,[135] which is in parallel with the increase in Na$^+$ currents and the excitability of DRG neurons.[81] IL-1β also increases the excitability of DRG and trigeminal sensory neurons by enhancing sodium current.[136–139] Whereas, anti-inflammatory cytokine IL-10, which is effective to suppress neuropathic pain,[140] downregulates Nav1.3, Nav1.6 Nav1.7 and Nav1.8, and reverses the upregulation of the sodium channels produced by TNF-α.[141,142] The results indicate that TNF-α and IL-10 oppositely regulate Navs in sensory neurons. As IL-10 is upregulated in injured DRG neurons,[41] the downregulation of Navs in injured DRG neurons may be resulting from IL-10 upregulation. The data demonstrate that the abnormal expression of Navs in sensory neurons is caused by imbalance of pro- and anti-inflammatory cytokines following peripheral nerve injury. The changes in expression of TNF-α, IL-1β, IL-10, Navs and other ion channels in injured and intact DRG neurons following peripheral nerve injury are summarized in Figure 2.

Nuclear factor-kappa B (NF-ĸB), a potent transcription factor, plays key roles in a wide variety of physiological and pathological processes by controlling inflammation response.[143–145] NF-ĸB is also critically involved in chronic pain.[146] Intrathecal injection of NF-ĸB inhibitor (pyrrolidine dithiocarbamate, PDTC) prevents the mechanical allodynia induced by peri-sciatic application of TNF-α[70] and by L5-VRT. PDTC also prevents the upregulation of Nav1.3 in DRG neurons

induced by L5-VRT and by TNF-α in cultured DRG neurons.[147] Intrathecal injection of PDTC attenuates mechanical allodynia and thermal hyperalgesia, and Nav1.7 upregulation in DRG neurons in rats with diabetic neuropathy.[148] The data suggest that TNF-α/NF-ƘB signaling contributes to persistent pain by transcriptional upregulation of sodium channels.

In addition, TNF-α is also capable of acutely enhancing excitability of sensory neurons. With a preparation of L4 and L5 DRGs attached with the sciatic nerve, Zhang et al have shown that application of TNF-α (1 ng/mL) for 15 min not only evokes discharges in silent fibers, but also enhances ongoing activity in spontaneously active fibers and sensitivity to electrical stimulation of the peripheral nerves.[149] The mechanism underlying the acute effect of TNF-α is unclear. A recent work[150] shows that phospho-p65 (*p*-p65), an active form of NF-ƘB subunit, reversibly interacts with Nav1.7 channels in the membrane of DRG neurons in rats with neuropathic pain induced by either antineoplastic agent vincristine or L5-SNL. The interaction enhances Nav1.7 currents via slowing inactivation of Nav1.7 channels and facilitating their recovery from inactivation. In cultured DRG neurons TNF-α increases the membrane *p*-p65 and enhances Nav1.7 currents within 5 min but does not affect nuclear *p*-p65 within 40 min, indicating that *p*-p65 is translocated to cell membrane at first, then to nucleus after NF-ƘB activation (Figure 3). This non-transcriptional effect on Nav1.7 may contribute to the acute effect of TNF-α on excitability of sensory neurons. Recently, we found that forkhead protein FOXO1, another transcription factor, also interacted with Nav1.7 in DRG neuron membrane and increased Nav1.7 currents, leading to mechanical allodynia.[151] Although the effect of TNF-α on FOXO1 in DRG neurons has not been investigated, it has been shown that TNF-α increases FOXO1 activity in GnRH neuronal cell line GT1-7.[152] The inflammatory cytokine-mediated non-transcriptional regulation of sodium channels by activation of traditional transcription factors may contribute to acute and persistent pain.

Voltage-Gated Calcium Channels

According to electrophysiological properties, Cavs are divided into low voltage activated (LVA) channels and high voltage activated (HVA) channels, which are activated at membrane potential near −60 mV and −30 mV, respectively. Based on the differences in pore-forming α_1 subunits, the Cav channels are classified into Cav 1, Cav 2 and Cav 3. HVA channels, but not LVA channels, are associated with $\alpha_2\delta$ and β subunits. LVA channels are also called T-type channels
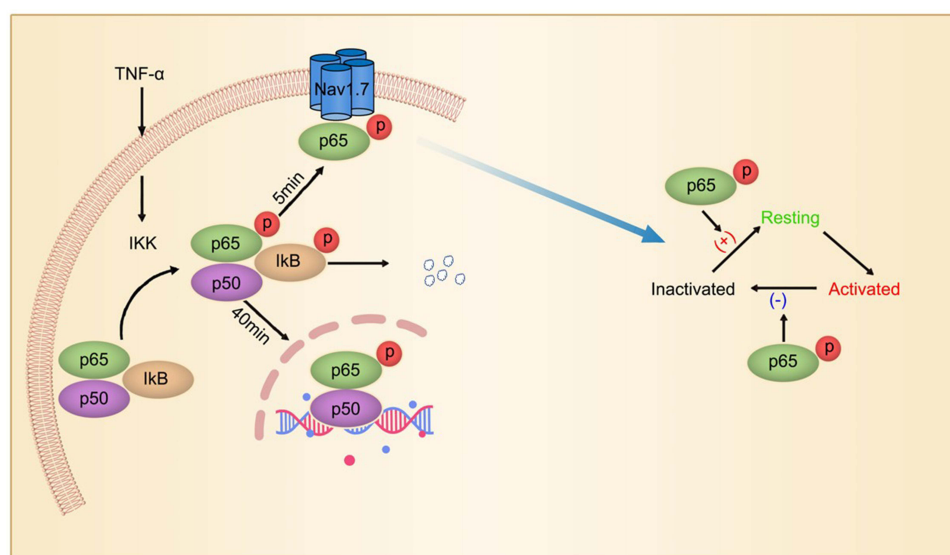
Figure 3 Non-transcriptional regulation of Nav1.7 by TNF-α/NF-ƘB signaling. NF-kB p50/p65/inhibitor of NF-kB (IkB) complex is located in the cytoplasm. On activation by TNF-α, both p65 and IkB are phosphorylated, and then p-IkB is degenerated after ubiquitination. p-p65 is translocated to membrane and increases the excitability of DRG neurons by interaction with Nav1.7 within 5 min, and then into the nucleus, where it regulates gene transcription. Adapted from iScience. Volume: 19. Xie MX, Zhang XL, Xu J, et al. Nuclear Factor-kappa B gates Nav1.7 channels in DRG neurons via protein-protein interaction. iScience.Page numbers: 623–633, Copyright (2019), with permission from Elsevier.[150]

including Cav 3.1-Cav 3.3. The HVA channels are further classified into L-type channels (Cav 1.1–Cav 1.4), P/Q-type (Cav 2.1), N-type (Cav 2.2), and R-type (Cav 2.3) (see[153] for a review).

Emerging evidence indicates that Cav 3.2 and Cav 2.2, which are also expressed in all parts of nociceptive DRG neurons, are critically involved in nociceptive and neuropathic pain (see[42] for a review). Cav 3.2 is upregulated in injured DRG neurons in many chronic pain models, such as chronic compression of DRG, SNL, CCI, partial sciatic nerve ligation and paclitaxel,[154] and also in intact L4 DRG neurons in L5-SNL model.[155] N-type channels, consisting of $\alpha1_B$ (Cav 2.2), $\alpha_2\delta$ and β subunits, are exclusively expressed in neurons and neuroendocrine cells.[156] Similar to sodium channels, Cav 2.2 is upregulated in intact L4 DRG neurons and downregulated in injured L5 DRG neurons in rat L5-SNL model.[41] Activation of Cav 2.2 expressed in presynaptic terminals is essential for neurotransmitter release and, therefore, blockage of Cav 2.2 reduces the synaptic transmission, including nociceptive signals. Upregulation of Cav 2.2[41] and Cav 3.2[155] in cell bodies of DRG neurons contributes to neuropathic pain by increasing neuronal excitability.

In clinic, anticonvulsants (pregabalin and gabapentin), which interrupt membrane trafficking of Cavs, especially N-type channels, by binding to $\alpha2\delta1$ subunit,[157] are the first-line drug for treatment of neuropathic pain. Intrathecal injection of prialt (ziconotide), a potent and selective N-type calcium channel peptide blocker, was approved in United States and European Union to treat severe chronic pain patients who are intolerant or refractory to other treatment, such as systemic analgesics, adjunctive therapies, or intrathecal morphine.[158] As the N-type channel is widely distributed in nervous system, its blockers have many severe side effects including cognitive impairment, hallucinations, and changes in mood and consciousness.[159]

In L5-SNL model, the upregulation of Cav 2.2 in intact L4 DRG neurons is accompanied by IL-1β upregulation, while its downregulation in injured L5 DRG neurons by IL-10 upregulation.[41] The data suggest that the differential cytokine expression may lead to the opposite changes in Cav 2.2. The notion is supported by the data showing that intrathecal injection of IL-1β upregulates Cav 2.2 in both L4 and L5 DRG neurons in naive rats, while intrathecal injection of IL-10 reverses Cav 2.2 upregulation in intact L4 DRG neurons produced by L5-SNL. Furthermore, in cultured DRG neurons, IL-1β and IL-10 up- and downregulates Cav 2.2, dose-dependently. Therefore, the upregulation of Cav 2.2 in intact DRG neurons is resulting from increased IL-1β, and its downregulation in injured DRG neurons from increased IL-10. It has been shown that IL-6 is involved in the upregulation of Cav 3.2 in injured L5 DRG neurons in L5-SNL model.[160]

Potassium Channels

Potassium channel families are consisted of the voltage gated (Kv) channels, Ca^{2+} activated K^+ channels, inwardly rectifying K^+ channels and tandem pore domain channels. All of them are expressed in DRG neurons, and majority of them are downregulated in DRG neurons in neuropathic pain condition. K^+ channel downregulation increases the excitability, axonal conduction and neurotransmitter release from primary afferent terminals in the spinal dorsal horn. Importantly, selective impairment of just one subtype of K^+ channel in DRG neurons can produce signs of pain in vivo (see[43] for a review).

Kv7.2 and Kv7.3 are the principal molecular components of the slow voltage-gated M-channel, which is named after its inhibition by muscarine acetylcholine receptor agonist. Reduction of M-channel function leads to neuronal hyperexcitability.[161] The M-channel opener retigabine (ezogabine or Potiga) was approved by FDA in 2011 as an anticonvulsant used for an adjunctive treatment of partial epilepsies, and was discontinued in 2017 due to its side effects of blue-colored appearance of the skin and eyes after prolonged intake. Flupirtine, a structural derivative of retigabine, was used as a non-opioid analgesic and was also withdrawn in 2018 for liver toxicity (see[162] for a review). As K^+ channels are widely expressed in all excitable cells in the body, they are not good targets for treatment of any disease.

Unlike other channels, K^+ channels are downregulated by TNF-alpha in DRG neurons.[163] Prolonged (5–6 days) exposure of DRG neurons to IL-1β also reduces the function of K^+ channels.[164,165]

Transient Receptor Potential Channels

The transient receptor potential (TRP) channels expressed in nociceptors sense various stimuli. In mammals, TRP superfamily has been divided into six subfamilies based on sequence homology: canonical (TRPC1 to TRPC7), vanilloid (TRPV1 to TRPV6), melastatin (TRPM1 to TRPM8), ankyrin (TRPA1), mucolipin (TRPML1 to TRPML3) and

polycystin (TRPP1 to TRPP3) (see[166] for a review). Among them, TRPV1, which responds to capsaicin and noxious heat (>43 °C),[167] and TRPA1, which responds to cold (>18 °C) and endogenous and exogenous chemical stimuli are believed to be promising targets for analgesics.[168] Animal studies show that upregulation of TRPV1 or TRPA1 in DRG neurons contributes to persistent pain in various neuropathic pain models, such as L5-SNL,[169] CCI,[170] and paclitaxel-induced peripheral neuropathy.[171,172]

Unfortunately, small-molecule inhibitors directly targeting TRPV1 and TRPA1 were unsuccessful due to their thermoregulatory function and other adverse side effects in various clinical trials.[45] However, local application of capsaicin (a TRPV1 agonist), which induces desensitization of nociceptors on repetitive application, is in use for the treatment of arthritis, muscle pain, neuropathic pain and migraine (see[173] for a review).

TNF-α is reported to upregulate TRPV1[174–177] and TRPA1[178–181] in sensory neurons in various pathological conditions. IL-β upregulates TRPV1 in cultured DRG neurons[182] and injection of IL-β into rat hind-paw increases TRPV1^{+} DRG neurons.[183] IL-β is also associated with upregulation of TRPV1 in rats with experimental autoimmune prostatitis[184] and with the upregulation of TRPA1 in vincristine-induced peripheral neuropathy in rats.[185]

Acid-Sensing Ion Channels

Acid-sensing ion channel (ASIC) was cloned and identified in DRG neurons in 1997.[186] ASICs are permeable to cations and are activated by extracellular acidosis. Now, six ASIC subunits (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3 and ASIC4) have been identified. Apart from ASIC4, all of them are expressed in peripheral nerves (see[46] for a review). A recent work[187] using an in situ hybridization technique (RNAscope) showed that, in DRG neurons of naive mice, all five subunits are expressed in NF-200^{+} and CGRP^{+} neurons with different levels; while in IB4^{+} neurons, ASIC2a, ASIC2b and ASIC3 but not ASIC1a and ASIC1b were detected. At least half of sensory neurons express multiple types of ASIC subunits. In the nerve injury mice, overall expression levels of the different ASIC subunits are not altered, as assessed by real-time qPCR. Further analysis revealed that the expression of ASICs was changed in CGRP^{+} neurons but not in IB4^{+} neurons. Namely, the percentages of ASIC1b- and ASIC3-expressing CGRP^{+} neurons in L4 DRG and in L5 DRG are increased, while ASIC1a-expressing CGRP^{+} neurons are reduced in L4 DRG.[187] Consistently, previous works show that inhibition of ASIC1b-containing channels underlies the opioid-independent inhibitory effect on inflammatory pain and neuropathic pain by mambalgins isolated from snake venom.[188] ASIC3 is involved in the inflammatory pain[189] and neuropathic pain.[190] To date, no clinical trial using ASIC ligands for treating chronic pain is available.

A brief (5 min) application of TNF-α rapidly enhanced ASIC-mediated currents in rat DRG neurons in a dose-dependent manner.[191,192] IL-1β upregulates ASIC3 mRNA in cultured fibroblast-like synoviocytes,[193] and in DRG neurons of animals with musculoskeletal pain induced by ischemia and reperfusion injury.[194,195]

Taken together, the growing experimental and clinical studies have greatly increased our understanding of the role of ion channels in persistent pain, but knowledge contributes little to pain relief in clinic. Many, if not all, ion channels in primary sensory neurons are changed in chronic pain, especially in neuropathic pain. The functional and transcriptional changes of all ion channels may contribute to the persistent hyperexcitability of sensory neurons. This explains why some specific ion channel subtype blockers tested in clinic trials are ineffective for treatment of chronic pain. Fortunately, emerging evidence indicates that the plastic changes in ion channels are tightly regulated by pro- and anti-inflammatory cytokines, which are imbalanced in chronic pain condition. Therefore, normalization of cytokine production may prevent and/or reverse the dysregulation of the ion channels, and treat chronic pain. The roles of ion channels of sensory neurons in persistent pain and their regulation by cytokines are summarized in Table 1.

Inflammatory Cytokines and Glial Cells Region-Dependently Modulate Synaptic Plasticity, Leading to Persistent Pain and Memory/Emotional Deficits

As mentioned above, the plastic changes in synaptic transmission are critical for learning/memory and chronic pain. Emerging evidence has demonstrated that overproduction of proinflammatory cytokines and glial activation induce LTP and enhance excitatory synapses in spinal dorsal horn but impair LTP and reduce excitatory synapses in hippocampus. This intriguing region-dependent effect may contribute to persistent pain and memory/emotional deficits, respectively.

Long-Term Potentiation in Spinal Dorsal Horn Contributes to Persistent Pain

LTP, discovered in the hippocampus in 1973,[196] has been intensively studied as a synaptic model of memory storage (see[62,63] for reviews), while LTP at C-fiber synapses in spinal dorsal horn was first reported in 1995[197] (Figure 4). The spinal LTP is considered as a synaptic model of persistent pain, based on following experimental and clinical data. (1) Afferent C-fiber conducts nociceptive signals and makes synapses with second order neurons in the superficial spinal dorsal horn.[198,199] (2) Pathogenic factors that cause persistent pain can reliably induce the spinal LTP, such as activation of afferent C-fibers by electrical high frequency stimulation (HFS),[200] low frequency stimulation[201] or natural stimuli,[202] peripheral nerve injury,[203,204] tissue inflammation,[201] opioid withdrawal,[205] estrogen deficit produced by ovariotomy or aging[27] and antineoplastic agent vincristine.[29] (3) LTP-inducible HFS produces long-lasting behavioral signs of pathological pain in rodent[24] and human subjects.[206] (4) The drugs that are effective to attenuate persistent pain are capable of depressing the spinal LTP, such as N-type calcium channel blockers omega-conotoxin GVIA[207] and gabapentin,[208] clonidine,[209] N-methyl-D-aspartic acid (NMDA) receptor antagonist[210] and diazepam.[211] The pathological significance of spinal LTP at C-fiber synapses is to amplify pain signals in the first order relay in pain pathway (see[47] for a review).

The synaptic transmission in spinal dorsal horn is strongly controlled by descending pathway from midbrain periaqueductal gray and the rostral ventromedial medulla. The descending system exerts both inhibitory and facilitatory effects on spinal nociception.[212] Removing the descending control system by transection of spinal cord at cervical 3 level increases the amplitude of spinal C-fiber-evoked field potentials by up to 250% of control in anaesthetized adult rats.[213] Prolonged high frequency burst stimulation of the sciatic nerve at Aδ-fiber strength produces long-term depression (LTD, a persistent decrease in efficacy of synaptic transmission) of C-fiber-evoked field potentials in intact rats, but induces LTP when the descending system is removed.[213] Therefore, the descending system may not only tonically inhibit pain synaptic transmission but also determine the direction of synaptic plasticity in spinal dorsal horn. The descending system
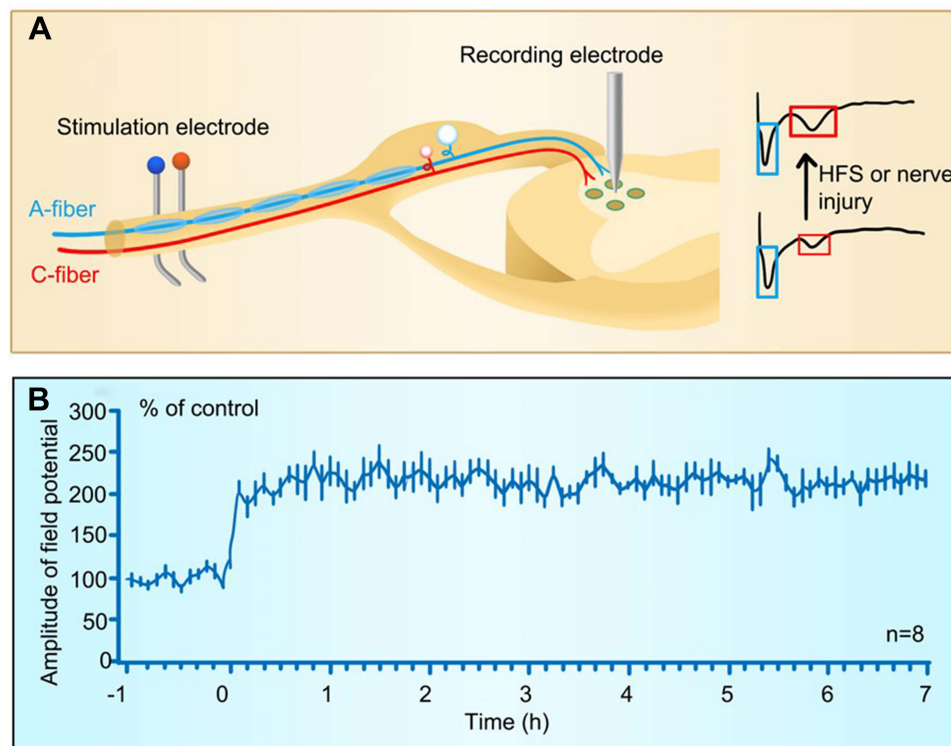
Figure 4 Long-term potentiation at C-fiber synapses in spinal dorsal horn. (**A**) Experimental setup for recording LTP of C-fiber evoked field potentials induced by electrical stimulation or injury of peripheral nerves. A-fiber and A-fiber-evoked field potentials are marked in blue and C-fiber and C-fiber-evoked field potentials in red. (**B**) The time course of the spinal LTP induced by high frequency stimulation (100 Hz, 100 pulses is given in 4 trains of 1-s duration at 10-s intervals, at the intensity sufficient to activate C-fibers) delivered to peripheral nerve. (**B**) Reprinted with permission from Liu XG, Sandkuhler J. Characterization of long-term potentiation of C-fiber-evoked potentials in spinal dorsal horn of adult rat: essential role of NK1 and NK2 receptors. J Neurophysiol. 1997;78(4):1973–1982.[200]

regulates the spinal nociception by releasing noradrenaline, serotonin (5-hydroxytryptamine) and dopamine.[214] Noradrenaline inhibits pain transmission via activation of presynaptic α_2-adrenergic receptors.[215] Spinal application of α_2-adrenergic receptor agonist clonidine not only blocks but also reverses spinal LTP at C-fiber synapses.[209] Antidepressants (duloxetine, venlafaxine), which enhance the descending inhibition via inhibition of presynaptic serotonin-noradrenaline reuptake,[216] are first-line drugs for neuropathic pain. The drugs have many side effects, including nausea, diarrhea, fatigue or somnolence, sexual dysfunction, increase in blood pressure, diaphoresis, tachycardia, tremors, and anxiety.[217] Dopamine produces anti-hyperalgesia or hypoalgesia by acting on dopamine D_2 receptors.[214] Activation of D_2 receptors by spinal application of quinpirole depresses spinal C-fiber synaptic transmission, while activation of D1/D5 receptors induces protein synthesis-dependent late-phase LTP of C-fiber-evoked field potentials in the absence of presynaptic activation.[218] As the expression of D_2 receptors is much high than D1/D5 receptors in spinal cord,[219] the net effect of dopamine on spinal nociception is inhibition. Spinal D1/D5 receptors are critically involved in translation of acute pain to chronic pain.[220,221]

Overproduction of Proinflammatory Cytokines and Glial Activation Inhibit LTP in Hippocampus but is Essential for Induction of LTP at C-Fiber Synapses in Spinal Dorsal Horn

Compelling evidence has demonstrated that overproduction of proinflammatory cytokines, such as TNF-α[28,222] and IL-1β,[223] impairs memory and LTP in hippocampus (see[224] for a review). TNF-α inhibits hippocampal LTP by activation of p38[225] and c-Jun N-terminal kinase (JNK) signaling,[226] while spinal application of either TNF-α or IL-1β at recording segments is able to induce LTP at C-fiber synapses in rats with neuropathic pain produced by L-5 VRT or SNI.[227,228] Interestingly, the spinal LTP induced by both TNF-α and IL-1β are blocked by inhibition of p38 and JNK signaling. LTP at C-fiber synapses induced by HFS is completely blocked in TNFR1 knockout mice, and the inhibitory effect of microglia inhibitors on LTP can be rescued by spinal application of TNF-α.[229] Thus, the proinflammatory cytokines inhibit LTP in hippocampus, but are essential for spinal LTP induction.

Likewise, in hippocampus activation of astrocytes[230] and microglia[231,232] impairs memory and LTP.[233] While in spinal dorsal horn the glial activation is indispensable for induction of LTP at C-fiber synapses. Our primary work showed that inhibition of microglia by spinal application of either minocycline or inhibitors of Src-family kinases (SFKs), which are exclusively activated in spinal microglia after peripheral nerve injury,[234] reversed the effect of HFS on synaptic plasticity. That is, HFS induces LTP in naive rats, but induces LTD in rats treated with microglia inhibitors.[229] The data indicate that the direction of C-fiber synaptic plasticity is decided by spinal microglia. Furthermore, spinal application of either ATP,[235] brain-derived neurotrophic factor (BDNF)[236] or opioids[205] is able to induce LTP at C-fiber synapses in the absence of conditioning activation of primary afferents. The chemical-induced spinal LTP is also dependent on activation of microglia. Sandkühler et al[237] show that combined activation of microglia and astrocytes by P2X7 receptor agonist BzATP induces LTP at C-fiber synapses in spinal lamina I neurons in the absence of presynaptic activation, which is termed gliogenic LTP. Therefore, activation of spinal glial cells is sufficient to induce the spinal LTP. Interestingly, the gliogenic LTP can be transferred between individuals, ie, application of spinal super-fusate collected from lumbar segments of animals, in which the spinal LTP has been induced by HFS, onto spinal dorsal dorsum of naive animals is capable of inducing LTP. The transferable LTP is prevented by blocking TNF-α, D-serine signaling and NMDA receptors but not by blocking glial activation in recipient animals. Accordingly, the spinal LTP is induced by the accumulated bioactive substances released by glial cells called gliotransmitters in activated site. The gliotransmitters may travel long distances via the cerebrospinal fluid and induce LTP in remote sites, and may, therefore, underlie some forms of widespread pain in intact sites. Together, glial activation is a common mechanism underlying spinal LTP induced by different approaches.[238]

Hippocampal LTP and spinal LTP at C-fiber synapses also share many common mechanisms. Receptors in cell membrane, including NMDA receptor,[197,210] dopamine D1 receptor[218] and TrkB receptor,[204,239] and intracellular signaling molecules, such as calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC), PKA,[240] extracellular signal-regulated kinase (ERK)/cAMP response element binding protein (CREB) pathway,[241] are critical for the induction and maintenance of LTP in both hippocampus and spinal dorsal horn (see[47] for a review). The similarities and differences between hippocampal LTP and spinal LTP are summarized in Figure 5. The findings are of
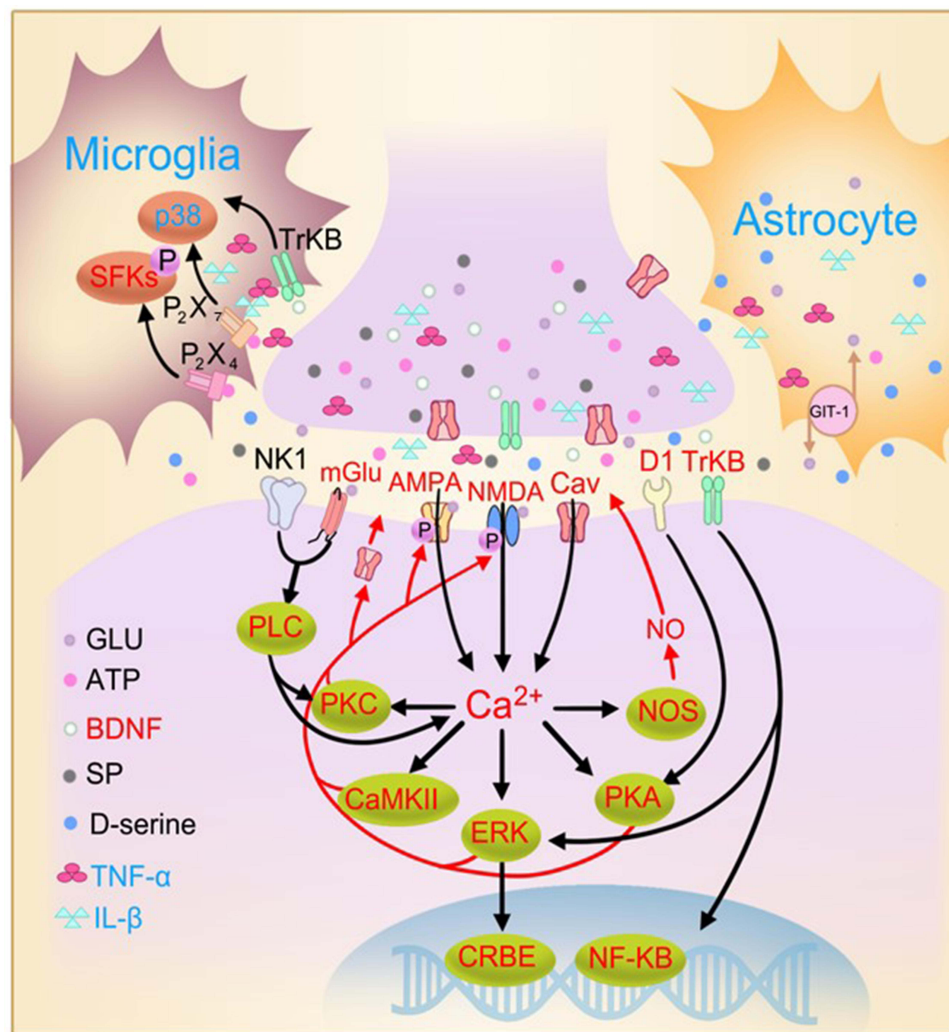
Figure 5 The similarities and differences between hippocampal LTP and spinal LTP. Similarities (indicated by red text): in both spinal dorsal and hippocampus, the induction of LTP depends on Ca^{2+} rise in postsynaptic neurons resulting from opening of NMDA receptors and Cavs; to a less extent, opening of α-amino-3hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor channels; and from Ca^{2+} release from intracellular store (not shown). The expression of LTP, referring to the enhanced efficacy of synaptic transmission manifested by persistent increase in magnitude of excitatory postsynaptic potentials, is primarily resulting from the enhancement of AMPA receptor currents produced by the receptor phosphorylation and the receptor insertion into postsynaptic membrane. The maintenance of spinal LTP is supported by activation of signal transduction pathways by Ca^{2+} rise in postsynaptic neurons. Early-phase LTP (<3 h) needs activation of PKA, PKC, CaMKII, ERK and phospholipase C (PLC) activated by neurokinin 1 (NK1) receptors and metabotropic glutamate (mGlu) receptors, and release of nitric oxide (NO). Late-phase LTP (>3 h) depends on gene transcription mediated by CREB and NF-κB and *de novo* protein synthesis (see[47] for a review). Activation of both TrkB and dopamine D1 receptors induces late-phase LTP in both hippocampus and spinal dorsal horn. Disrupting glutamate transporter 1 (GLT-1) in astrocytes, which regulates synaptic transmission via reuptaking glutamate, blocks LTP in both hippocampus[361] and spinal dorsal horn.[362] Differences (blue text): activation of either microglia or astrocytes and overproduction of TNF-α or IL-1β inhibit hippocampal LTP but are essential for spinal LTP induction. Also, activation of ligand-gated ATP receptors (P2X4 and P2X7), p38 mitogen-activated protein kinase (MAPK) and Src-family kinases (SFKs) in microglia of spinal dorsal horn is essential for induction of spinal LTP (see[47] for a review). Meanwhile, in hippocampus, blockage of P2X7 attenuates age-related LTP deficits.[363] The roles of P2X4 and SFKs in glial cells for hippocampal LTP remain elusive. Adapted with permission from Liu XG, Zhou LJ. Long-term potentiation at spinal C-fiber synapses: a target for pathological pain. Curr Pharm Des. 2015;21(7):895–905.[47]

importance for the development of pain-relieving drugs. Targeting the molecules that are shared by hippocampal LTP and spinal LTP may impair the memory function of hippocampus. While targeting the neuroinflammation may not only treat persistent pain but also improve the memory function of hippocampus.

Peripheral Nerve Injury Impairs Working Memory and Hippocampal LTP by Upregulation of TNF-α

As discussed above, the spinal LTP induced by peripheral nerve injury may contribute to persistent pain. To investigate the mechanism underlying memory deficits in chronic pain, we tested effect of the nerve injury on LTP in hippocampus, a synaptic model of memory storage, in SNI model of neuropathic pain. The results show that

SNI induces working memory and short-term memory deficits, and impairs the LTP at CA3-CA1 synapses in a time-dependent manner.[28] Neither baseline synaptic transmission nor LTP induction by HFS are affected one hour after SNI, indicating peripheral nerve injury has no acute effect on synaptic transmission and LTP induction in hippocampus. However, the synaptic potentiation induced by HFS persists for less than one hour 18 to 20 h after SNI and for less than 30 min 6–10 d after SNI. The LTP impairment produced by SNI persists for at least 1.5 months, and is also evident in contralateral hippocampus after unilateral peripheral nerve injury. Namely, SNI leads to a delayed and long-lasting LTP impairment in bilateral hippocampus. We found that TNF-α was persistently increased in both plasma and cerebrospinal fluid, as well as in hippocampal tissue after SNI. The impairments of memory and LTP by SNI are prevented by genetic deletion of TNFR1, mimicked by intracerebroventricular or intrahippocampal injection of rat recombinant TNF-α.[28] The results indicate that the memory deficits and dysfunction of hippocampus in neuropathic pain are not directly produced by peripheral nerve injury by the injury-induced overproduction of TNF-α.

Number of Excitatory Synapses is Enhanced in Spinal Dorsal Horn but Reduced in Hippocampus by TNF-α After Peripheral Nerve Injury

To elucidate the long-lasting persistent pain and memory/emotional deficits induced by peripheral nerve injury, we investigated the structural synaptic plasticity induced by SNI. The results show that SNI produces an opposite change in morphological synaptic connectivity in hippocampus and in spinal dorsal horn.[25] The dendrite lengths and spine densities are reduced significantly in hippocampal CA1 pyramidal neurons, but increased in spinal neurokinin-1-positive projection neurons in SNI mice. As most excitatory synapses are located in spines,[242] the data indicate that the excitatory synaptic connectivity is reduced in hippocampus but enhanced in spinal dorsal horn in neuropathic pain condition. Interestingly, the opposite morphological changes are again blocked by genetic deletion of TNFR1 or conditioning deletion of microglia, and are mimicked by TNF-α in cultured hippocampal and spinal cord slices.[25] Furthermore, the length of dendrites of gamma-aminobutyric acid (GABA)-nergic inhibitory interneurons in lamina II of spinal dorsal horn is significantly reduced in CCI model of neuropathic pain.[243] The opposite structural synaptic changes in spinal dorsal horn and in hippocampus may contribute to persistent pain and memory/emotional deficits, respectively (Figure 6).

Our finding that peripheral nerve injury induces cognitive deficits is repetitively confirmed by others.[244–247] In consistence with reduction of dendrite length and spine density in hippocampus in SNI mice, Apkarian's group show that the volume of bilateral hippocampi is reduced in human patients with chronic back pain and complex regional pain syndrome, and the synaptic plasticity in hippocampus is impaired in SNI mice.[248] SNI-induced working memory deficits are associated with reduced hippocampus–PFC connectivity.[249] In consistence with the enhancement of excitatory synapses in spinal dorsal horn in neuropathic pain, we show that CGRP (a marker for peptidergic C-fiber) in spinal dorsal horn is increased in several animal models of chronic pain, including chemotherapy-induced neuropathy,[29] high frequency noxious stimulation of peripheral nerve[24] and estrogen decline produced ovariectomy and aging.[27] Importantly, the CGRP increase is paralleled with LTP at C-fiber synapses and neuropathic pain behaviors. As CGRP is colocalized with *p*-p65, TNF-α and IL-1β in DRG neurons,[27] the increased CGRP+ C-fibers may be also resulting from neuroinflammation.

Interestingly, our recent work shows that in rodents with either SNI or L5-SNL, activation of dorsal hippocampus with optogenetic and pharmacological methods relieves neuropathic pain behavior. The functional connectivity of the dorsal hippocampus with many brain regions in SNI rats and in chronic pain patients is reorganized. Therefore, the dysfunction of hippocampus may not only cause cognitive deficits but also aggravate persistent pain.[26]

Opposite Regulation of BDNF by Neuroinflammation Contributes to Region-Dependent Synaptic Connectivity in Hippocampus and Spinal Dorsal Horn

It has been shown that BDNF is essential for LTP in both hippocampus and spinal dorsal horn. Acute intrahippocampal infusion of BDNF induces LTP in hippocampus,[250] and spinal application of BDNF at recording segments induces spinal LTP at C-fiber synapses.[239] Following peripheral nerve injury, both TNF-α[28,75] and IL-1β[59] are upregulated in cerebral fluid, hippocampus and spinal dorsal horn, while BDNF is downregulated in hippocampus but upregulated in spinal dorsal horn.[251–254] The opposite changes in BDNF are again blocked by genetic deletion of TNFR1 or by inhibition or deletion of microglia.[24,25] Furthermore, TNF-α upregulates BDNF in spinal slices and downregulates BDNF in

hippocampal slices in a dose-dependent manner.[25] As BDNF is critical for synapse formation,[255] TNF-α overproduction and glial activation may cause the opposite changes in synaptic connectivity in spinal dorsal horn and in hippocampus by the differential regulation of BDNF (Figure 6).

Overproduction of proinflammatory cytokines and glial activation are also evident in other brain regions closely related to memory and emotion in chronic pain condition. The upregulation of TNF-α is reported in the anterior cingulate cortex (ACC), a forebrain structure involving pain transmission and emotion, in persistent inflammation pain induced by hind-paw injection of complete Freund's adjuvant[256] and in SNI model of neuropathic pain.[257] IL-1β[258] and TNF-α[259] are upregulated in the medial prefrontal cortex (mPFC) in neuropathic pain. The upregulation of TNF-α and *p*-p65 and microglial activation in the mPFC are also demonstrated in oxaliplatin-induced memory and emotional deficits.[30] The roles of proinflammatory cytokines for synaptic plasticity in ACC and mPFC in neuropathic pain remain elusive.

Neuroinflammation May Underlie the Comorbidity of Persistent Pain and Memory/Emotional Deficits

It has long been proposed that persistent pain and depression are independent diseases but may share common pathological mechanisms.[55,56] As discussed above, the overproduction of proinflammatory cytokines and glial activation are essential for both hypersensitivity of sensory neurons and the pathological synaptic plasticity in CNS. Previous works show that both proinflammatory cytokines and glial activation are also critically involved in memory deficits (see[84,85] for reviews) and major depression (see[86,87] for reviews) in many diseases other than chronic pain. Therefore, neuroinflammation might be the common cause for persistent pain and memory/emotional deficits in chronic pain condition. Indeed, in SNI rats the pain behaviors are co-related with neither memory scores assessed with novel object recognition test nor flatting times that measure depression-like behavior in force swimming test.[59] The data suggest that the cognitive deficits may not be caused by persistent pain in neuropathic pain condition. This notion is supported by a later work.[60] However, IL-1β is upregulated in the injured sciatic nerve and in plasma within hours, and then in the spinal dorsal horn
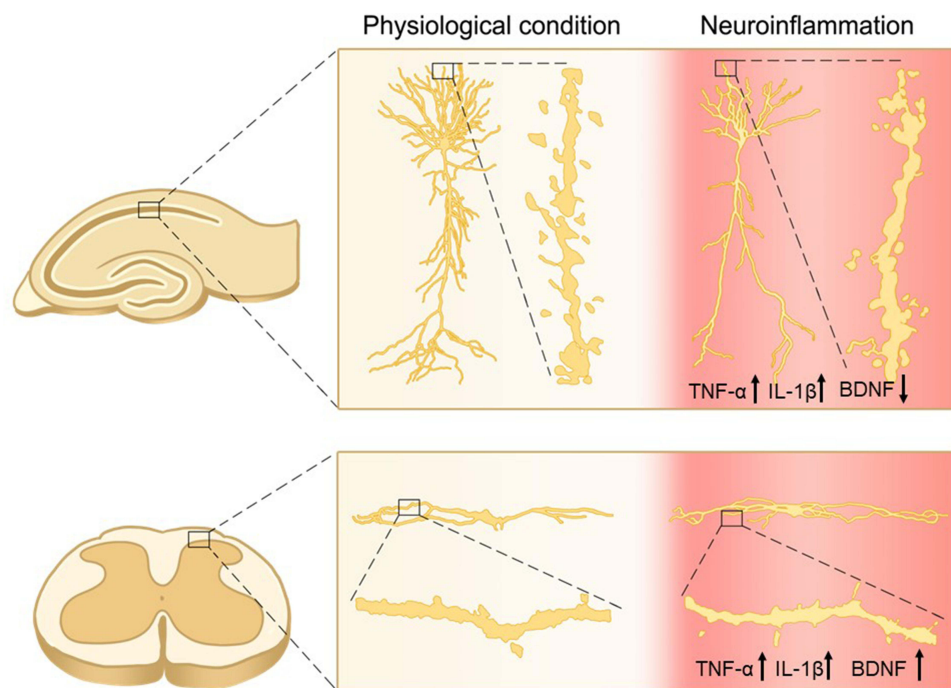
Figure 6 The opposite effect of spared nerve injury of the sciatic nerve on excitatory synaptic connectivity in hippocampus and spinal dorsal horn. The dendrite lengths and spine densities are reduced in hippocampal CA1 pyramidal neurons but increased in spinal projection neurons in SNI rats. The morphological changes are paralleled with upregulation of TNF-α and IL-1β in both hippocampus and spinal dorsal horn. Meanwhile, BDNF that is critical for synapse formation is downregulated in hippocampus and upregulated in spinal dorsal horn. These opposite changes in synaptic connectivity and BDNF expression are blocked by either deletion of TNFR1 or conditioned deletion of microglia, and are mimicked by TNF-α in cultured hippocampal and spinal cord slices. The data suggest that the differential regulation of BDNF by inflammatory cytokines may contribute to the opposite morphological changes in hippocampus and spinal dorsal horn. Adapted with permission from Liu Y, Zhou LJ, Wang J, et al.TNF-alpha differentially regulates synaptic plasticity in the hippocampus and spinal cord by microglia-dependent mechanisms after peripheral nerve injury. J Neurosci. 2017;37(4):871–881.[25]

and in the brain regions closely associated with memory and emotion, including ACC, mPFC, amygdala and hippocampus in SNI rats. The changes are accompanied by glial activation. Importantly, the SNI-induced persistent pain, memory decline and depressive behaviors, as well as the IL-1β upregulation and glial activation, are substantially prevented by local administration of IL-1β neutralizing antibody at injured nerve or deletion of IL-1R1. Furthermore, the behavioral changes and neuroinflammation induced by SNI are mimicked in naive rats by repetitive intravenous injection of recombinant rat IL-1β at a pathological concentration, determined in SNI rats.[59] Therefore, peripheral nerve injury causes persistent pain, memory deficit and mood depression by triggering neuroinflammation. Our late studies showing that activation of TNF-α/NF-ƙB and glial cells in DRG and spinal dorsal horn in vincristine-treated rats[29] and in hippocampus in oxaliplatin-treated rats[30] contribute to persistant pain and memory/emotional deficits support this notion.

Interactions of Circulating Immune Cells with Glial Cells and Neurons are Critical for Initiation and Maintenance of Neuroinflammation

The data discussed above indicate that neuroinflammation is a common cause of persistent pain and memory/emotional deficits in chronic pain condition. The next key question is how the neuroinflammation is triggered and maintained in chronic pain.

Transmigration of Circulating Inflammatory Monocytes into Peripheral Nerve, Spinal Parenchyma and Brain Perivascular Space Leads to Neuroinflammation

In peripheral nerves, neuroinflammation produced by injury is resulting from increased resident and infiltrating macrophages. The upregulation of chemokine C-C motif ligand 2 (CCL2) in injured DRGs causes transmigration of circulating monocytes into DRGs through CCL2 receptor expressed on monocytes (see[260] for a review).

In spinal dorsal horn, it has been shown that the colony-stimulating factor 1 (CSF1) in afferent neurons is upregulated after peripheral nerve injury (see[261] for a review) or high frequency noxious stimulation of sciatic nerve.[24] The CSF1 released from the central terminals of DRG neurons activates microglia in spinal dorsal horn by binding to its receptors, contributing to spinal neuroinflammation.[261] In addition, the blood–spinal cord barrier (BSCB) breaks down following peripheral nerve injury. The disrupted BSCB leads to the influx of inflammatory mediators and the recruitment of circulating monocytes into spinal parenchyma. The BSCB impairment can be also produced by circulating IL-β and is shut down by anti-inflammatory cytokines IL-10 and TGF-β1.[262] The CCL2/CCL2R system plays a role in the recruitment of circulating monocytes into spinal parenchyma.[263] Accordingly, glial activation induced by both CSF1 released from sensory afferents and the recruitment of circulating monocytes due to BSCB disruption may lead to neuroinflammation in spinal cord.

How can peripheral nerve injury induce remote inflammation in the brain? Previous studies have shown that the infiltration of immune cells into brain parenchyma resulting from blood–brain barrier (BBB) interruption leads to neuroinflammation and cognitive deficits in a variety of pathological conditions.[264–266] In SNI model of neuropathic pain, however, BBB is intact and no immune cell is detected in brain parenchyma, while circulating proinflammatory (classical) monocytes and C-X-C motif chemokine 12 (CXCL12) in the blood and in brain perivascular space are persistently increased.[267] In parallel, the number of perivascular macrophages (PVMs) in the brain, particularly in hippocampus, is enhanced, and both microglia and astrocytes are activated. The experiments with the transgenic CCR2 (RFP/+) and CX3CR1 (GFP/+) mice reveal that at least some of the PVMs are derived from circulating monocytes. The SNI-induced PVM increase, glial activation and memory decline are substantially prevented by either depleting circulating monocytes via intravenous injection of clodronate liposomes or by blockade of CXCL12-CXCR4 signaling, whereas intravenous injection of CXCL12 at a pathological concentration in naïve mice mimics the changes induced by SNI. Because glial activation and PVM increase are in the same spatial and temporal fashion, and glial activation is prevented by obstruction of PVM recruitment by either deleting circulating monocytes or blocking CXCL12-CXCR4 signaling, glial activation may be resulting from an increase in PVMs. Accordingly, the brain neuroinflammation induced by peripheral nerve injury may be initiated at and distributed along the blood vessels. Importantly, in chronic pain patients circulating monocytes and plasma CXCL12 are elevated, and both of them are highly correlated with memory decline

assessed by the Montreal Cognitive Assessment.[267] The data indicate that CXCL12-mediated monocyte recruitment into the perivascular space is critical for brain neuroinflammation and the resultant cognitive impairment in neuropathic pain.

Overproduction of Inflammatory Cytokines in Both Neurons and Glial Cells Contributes to Persistent Neuroinflammation

It is generally believed that bioactive substances released by activated glial cells contribute to chronic pain.[268] In DRG, activation of satellite glial cells enhances the excitability of sensory neurons by releasing ATP, glutamate and cytokines, such as TNF-α and IL-1β, and fractalkine.[269] In spinal cord TNF-α and IL-1β released by the activated microglia and astrocytes enhance synaptic transmission.[270] Glial activation is also believed to cause memory deficits in Alzheimer's disease,[271] major depression[272] and postoperative cognitive dysfunction[273] by sustained release of proinflammatory cytokines. In other words, the cytokines released by glial cells cause the neuronal disorders.

However, accumulating evidence has demonstrated that the neurons in peripheral and central nervous system are also capable of producing proinflammatory cytokines. TNF-α is shown to be upregulated in the neurons of DRG and spinal dorsal horn in both L5-VRT and SNI models,[75,274] and in the neurons of bilateral hippocampi in SNI model.[28,275] NF-ĸB that is critical for transcriptional upregulation of proinflammatory cytokines[276] is upregulated in neurons of spinal dorsal horn[277] and DRG in both inflammatory pain[278] and neuropathic pain models.[279] *Tnf-α* mRNA is upregulated in the neurons of DRG and spinal dorsal horn after coronary artery occlusion in rats.[280] TNF-α and *p*-p65 are upregulated in the neurons of DRG and spinal dorsal horn in vincristine-induced peripheral neuropathy.[29] Furthermore, *p*-p65 and TNF-α are upregulated in the neurons but not in microglia and astrocytes of hippocampus and mPFC in rats with neuropathic pain induced by oxaliplatin.[30] A recent work shows that *p*-p65, TNF-α and IL-β are upregulated predominantly in the neurons and sparsely in glial cells of spinal dorsal horn and hippocampus in ovariectomized and aged female mice, and the change is essential for both glial activation and the genesis of persistent pain, memory/emotional deficits induced by estrogen decline. The results of in situ hybridization show that *Tnf* and *Il-1β* mRNAs are expressed in the neurons of DRGs and spinal dorsal horn.[27] As the proinflammatory cytokines can potently activate glial cells,[59,281–284] the cytokines produced by neurons may initiate or maintain glial activation in pathological conditions. The neuron–glia interaction mediated by proinflammatory cytokines may contribute to neuronal disorders by producing persistent neuroinflammation.

A New Strategy for Treatment of Chronic Pain

In spite of accumulating knowledge on mechanisms of chronic pain, as discussed above, the pharmacological treatment of chronic pain is still a big challenge in clinic. According to a systematic review and meta-analysis[285] and a recent guideline,[286] antidepressants (duloxetine, venlafaxine) and anticonvulsants (pregabalin and gabapentin) are still the first-line drugs for treatment of neuropathic pain. Pain relief is only achieved in <50% of chronic pain patients with the drugs, and is particularly low for patients with neuropathic pain. In recent decades, exponential growth of experimental studies on neuropathic pain has not yet led to any major clinical applications.[287] The reasons for the insufficient translation of basic research into clinical application are complicated. Unlike infectious diseases and cancer, which are caused by invading pathogens or malignant cells, chronic pain results from the alteration of endogenous molecules, such as ion channels, cytokines and intracellular signals, which are critically involved in many physiological functions. Elimination or inhibition of pathogens and cancer cells are successful for treatment of infectious diseases and cancer, while the same strategy is inappropriate for treating chronic pain. The drugs targeting pain-related molecules, which are widely expressed in the nervous system or even the whole body, will certainly cause severe side effects, such as N-type calcium channels[159] and K$^+$ channels[162] discussed above. Meanwhile, the drugs targeting the molecules that are preferably expressed in sensory neurons are ineffective for pain relief, such as Nav1.7 blockers.[118–120] In recent years, the single-cell and/or spatial transcriptomics of DRG neurons in mice,[288] nonhuman primates[289] and humans[290,291] have identified many subtypes of nociceptors, the species differences in molecular phenotypes and new potential drug targets. Although the works have greatly increased our knowledges of nociception, we have a long way to go for determining efficacy and side effects of the new drug targets.

Compelling evidence has demonstrated that inflammation plays a key role in many, if not all, chronic diseases (see[292] for a review). As discussed above, overproduction of inflammatory cytokines causes persistent pain and memory/emotional

deficits by enhancing the excitability of sensory neurons via regulation of ion channels and region-dependent regulation of synaptic connectivity in CNS (Figure 1). Persistent inflammatory cytokine overproduction is initiated and maintained by the interactions of immune cells, glial cells and neurons. Therefore, neuroinflammation is believed to be a promising target for treatment of chronic pain.[293] However, proinflammatory cytokines and glial cells also have many important physiological functions, such as protecting the host from infection and promoting tissue repair and recovery.[294,295] In the nervous system, TNF-α/NF-κB signaling is important for memory storage.[143] Glial cells are crucial for regulating neuronal structures and functions[296] as well as for neurogenesis.[297] Thus, normal inflammation response is essential for the structures and functions of nervous system. However, persistent overproduction of inflammatory cytokines and glial activation play key roles in neuropathic pain,[298] Alzheimer's disease[299] and mood depression.[300,301] Clearly, the function of nervous system and inflammation exhibits a reverted-U relationship. Therefore, the right strategy for treating the neuronal disorders is normalization (counterbalance) of the neuroinflammation, ie, to bring it back to normal level. This cannot be achieved with the agents that simply inhibit inflammatory cytokinse and glial cells, which may lead to either insufficient or excessive inhibition of inflammation response. Consistently, clinical data show that anti-TNF agents are effective for treating osteoarthritis pain,[302] lower back pain,[303] and rheumatoid arthritis.[304] Systematic review and meta-analysis indicate the agents have many side effects, including infection[305,306] and psoriasis.[307] To date, no agent for normalization of neuroinflammation is available in clinic. However, in recent years experimental studies have brought light to this issue.

Supplement Mg^{2+} by Oral Magnesium-L-Threonate Normalizes Neuroinflammation in Rodents

Clinical data have shown that Mg^{2+} deficiency is involved in many chronic diseases, such as hypertension, ischemic heart disease, stroke, metabolic syndrome, diabetes, colorectal cancer (see[308] for a review) and postmenopausal symptoms.[309,310] In nervous system, Mg^{2+} deficiency results in anxiety and depression in animals and humans (see[311] for a review). A clinical trial shows that the global cognitive ability of older adults is improved by oral application of magnesium-L-threonate (L-TAMS, also called MgT).[312] A recent review article by Morel et al[313] indicates that supplementing Mg^{2+} by intravenous administration of MgSO4 is effective for pain relief and reduction of analgesic consumption, based on 80 randomized controlled trials and 8 systematic reviews.

In experimental studies, supplementing Mg^{2+} with chronic oral administration of L-TAMS improves spatial learning in naive rats[314] and in mice with Alzheimer's disease,[315] and prevents and restores the memory deficit induced by SNI.[275] Mg^{2+} deficiency contributes to the chronic pain and memory/emotional deficits induced by antineoplastic agents, including vincristine, cyclophosphamide and oxaliplatin, and the effects are prevented by oral L-TAMS.[29,30,316] Oral L-TAMS also prevents or reverses the chronic pain and memory/emotional deficits induced by estrogen decline in ovariectomized or aging female mice.[27]

The mechanisms underlying therapeutic effects of Mg^{2+} are still in debate. It has long been shown that Mg^{2+} voltage-dependently blocks NMDA receptor in spinal dorsal horn.[317] As NMDA receptor activation is essential for neuronal hypersensitivity and LTP at C-fiber synapses in spinal dorsal horn,[197,318] blockade of central sensitization is believed to underlie the pain relief of Mg^{2+}.[319] If this is true, supplementing Mg^{2+} would impair memory function, as NMDA receptor is also indispensable for the memory function and LTP in hippocampus.[320] In contrast, it has been shown that elevating brain Mg^{2+} by oral application of L-TAMS improves memory function and enhances NMDA receptor activity by upregulation of the NR2B subunit of NMDA receptors in hippocampus of naive rats.[314] It has been repetitively shown that overexpression of NR2B in forebrain facilitates synaptic potentiation and enhances memory function in mice,[321,322] while upregulation of NR2B in spinal dorsal horn is critical for development of neuropathic pain.[323,324] Obviously, blocking NMDA receptor cannot explain the fact that Mg^{2+}supplement can relieve pain and improve memory, simultaneously.

We addressed this contradictory issue with animal model of chemotherapy-induced neuropathy, which exhibits both persistent pain[325] and memory/emotional deficits.[326,327] We found that NR2B expression was downregulated in hippocampus but upregulated in spinal dorsal horn in vincristine-treated animals, and the opposite changes were prevented by oral L-TAMS.[29] How could supplementing Mg^{2+} regulate NR2B in hippocampus and in spinal dorsal horn in the opposite direction? Experimental studies have demonstrated that Mg^{2+} deficiency in the rat induces an inflammatory syndrome characterized by leukocyte and macrophage activation, and release of inflammatory cytokines.[328] A recent

meta-analysis of randomized controlled trials indicates that Mg^{2+} supplementation significantly reduces different human inflammatory markers.[329] Therefore, neuroinflammation induced by Mg^{2+} deficiency may region-dependently regulate NR2B. Indeed, in the rats treated with vincristine, intracellular Mg^{2+} in DRG neurons is significantly reduced, and free Mg^{2+} in blood and CSF is decreased from 8 mM to 6 mM. In cultured DRG neurons, reducing Mg^{2+} from 8 mM to 6 mM in medium upregulates TNF-α and p-p65.[29] In cultured hippocampal slices, reducing Mg^{2+} to 6 mM also upregulates TNF-α and p-p65, but downregulates NR2B subunit of NMDA receptor.[30] Furthermore, single intrathecal injection of rat recombination TNF-α in naive rats upregulates TNF-α in both hippocampus and spinal dorsal horn, but down- and upregulates NR2B in hippocampus and in spinal dorsal horn, respectively. Therefore, intracellular Mg^{2+} deficiency leads to the differential expression of NR2B in hippocampus and in spinal dorsal horn by upregulation of TNF-α, contributing to persistent pain and memory/emotional neuronal deficits. It has been shown that 31% of Mg^{2+} in the body is distributed inside cells, where it functions as a co-activator for around 600 enzymes.[330] Dysfunction of the enzymes may be responsible for Mg^{2+} deficiency-induced neuroinflammation, while the underlying mechanisms remain elusive.

We used L-TAMS in our experiments because only L-TAMS, but no other magnesium compound, including $MgCl_2$, Mg citrate and Mg gluconate, is able to elevate Mg^{2+} in the CSF of rats.[314] In human patients, increasing the plasma Mg^{2+} three-fold via intravenous infusion of $MgSO_4$ does not elevate Mg^{2+} in the CSF.[331] This may explain why supplementing Mg^{2+} with $MgSO_4$ has only modest effect on pain relief,[313] as the central mechanisms of chronic pain may not be affected by intravenous $MgSO_4$. To date, effect of oral L-TAMS on chronic pain has not been tested in clinical trial. As Mg^{2+} deficiency causes neuroinflammation, supplementing Mg^{2+} may bring the neuroinflammation back to but never below normal level.

Upregulated Proteins in Neuropathic Condition Promote Recovery of Persistent Pain and Memory/Emotional Deficits by Normalization of Neuroinflammation

In disease conditions, such as neuropathic pain, many proteins are upregulated (see[332] for a review). Some of them contribute directly to neuropathic pain. Till now, all drugs are designed to target the upregulated proteins that directly cause disease, such as blockers of ion channels. As many of the upregulated proteins are widely distributed in the body and exert many physiological functions, side effects are inevitable. In recent years, studies have shown that some upregulated proteins, such as translocator protein (TSPO, 18 kDa) and liver X receptors (LXRs), promote recovery of neuropathic pain by inhibition of neuroinflammation. When persistent pain is healed, their expression returns to normal level (see below). More and more such kinds of proteins may be discovered in the future. Accordingly, a natural cure may occur when endogenous agonists for such proteins are sufficient in the body; however, in majority of cases they are probably deficient and exogenous ones are needed for recovery. A novel approach is developing agents that activate the upregulated proteins that promote recovery from diseases.

Translocator Protein (18 kDa)

Early experimental studies show that diazepam inhibits morphine tolerance[333] and nociceptive pain.[334] Clinical data have demonstrated that diazepam is effective for treatment of chronic pain associated with spinal cord injury (see[335] for a review). Our electrophysiological data[211] reveal that spinal application of diazepam not only prevents LTP at C-fiber synapses but also potently depresses the late-phase LTP (>3 h), which is protein synthesis-dependent.[336] The pharmacological effects of diazepam are mediated by the GABAA receptors in CNS and so-called peripheral benzodiazepine receptors.[337] The peripheral receptor, now called TSPO (18 kDa), localized primarily in the outer mitochondrial membrane, is critically involved in steroid synthesis by transporting the substrate cholesterol into mitochondria. In the brain, TSPO is mainly expressed in microglia and astrocytes, and is upregulated in response to injury, inflammation and disease. Therefore, TSPO is used as a biomarker for brain inflammation and reactive gliosis.[338] Meanwhile, activation of TSPO also improves neurological and psychiatric disorders by controlling neuroinflammation via enhancing neurosteroidogenesis (see[339] for a review). In spinal dorsal horn, TSPO is upregulated predominantly in astrocytes, sparsely in microglia but not in neurons after L5-SNL.[340] The TSPO expression returns to normal level when behavioral signs of neuropathic pain are naturally extinct (approximately 50 d after L5-SNL). Interestingly, a single intrathecal injection of specific TSPO agonists Ro5-4864 or FGIN-1-27 at 7 and 21 d after L5-SNL reverses the established mechanical allodynia and thermal hyperalgesia. Importantly, TSPO upregulation also returns to normal level when neuropathic

pain behavior is reversed by Ro5-4864. These findings are confirmed by another work.[341] The data suggest that the role of TSPO upregulation is to promote recovery from the neuronal disorder. Mechanically, Ro5-4864 substantially inhibits spinal astrocytes and reduces the production of TNF-α in vivo and in vitro. The effects are prevented by a neurosteroid synthesis inhibitor (AMG), suggesting that enhancing neurosteroid production contributes to the anti-neuroinflammatory effect of TSPO agonists. In addition, TSPO upregulation has been shown in many other animal models of chronic pain, such as arthritis[342] and complex regional pain syndrome.[343]

Importantly, TSPO is also upregulated in patients with major depression,[344,345] Alzheimer's disease and lumbar radiculopathy.[346] The increased TSPO is believed to improve memory by repairing cellular damage and preventing further neuronal degeneration (see[347] for a review). Recently a randomized, double-blind clinical trial showed that etifoxine (a TSPO ligand) is effective for reduction of anxiety symptoms in patients with anxiety disorder.[348] To date, no clinal study that treats chronic pain with TSPO ligand is available.

Liver X Receptors

Liver X receptors (LXR-α and LXR-β) are ligand-activated transcription factors. LXR-α is upregulated in the neurons and oligodendrocytes of spinal dorsal horn in SNI model, and a single intrathecal injection of a specific liver X receptor agonist (T0901317 or GW3965) reverses mechanical allodynia in both rats and mice.[349] GW3965 inhibits glial activation and downregulates TNF-α and IL-β but upregulates IL-10 in the spinal dorsal horn. Genetic deletion of LXR-α abolishes all the effects of GW3965, and exacerbates the neuroinflammation induced by SNI. The effects of liver X receptor agonist also depend on neurosteroid synthesis. Thus, activation of LXRs depresses neuropathic pain by normalization of neuroinflammation via neurosteroids. The similar effect of LXRs is also observed in skin/muscle incision and retraction (SMIR) model of postoperative pain. SMIR that produces a lasting mechanical allodynia in the hind-paw[350] induces LTP at C-fiber synapses.[351] This spinal LTP is accompanied by the upregulations of TNF-α, acetylated NF-κB p65, LXRα and LXRβ and the downregulation of silent information regulator 1 (SIRT1), which activates NF-κB by the direct deacetylation of NF-κB p65, in dorsal horn neurons. The spinal LTP induced by SMIR is blocked by spinal application of either TNF-α neutralization antibody, NF-κB inhibitor (PDTC) or LXRs agonist (T0901317). The effects of T0901317 on spinal LTP and acetylated NF-κB p65 and TNF-α are blocked by SIRT1 antagonist. Therefore, activation of LXRs prevents spinal LTP by inhibiting NF-κB/TNFα pathway via activating SIRT1. In LXR-α knockout mice, the mechanical allodynia induced by sciatic nerve crush lasts much longer than in wild-type mice.[352]

Recent works show that conditional deletion of LXR-β in astrocytes induces anxiety-like behaviors and increases the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) and dendritic complexity of layer V pyramidal neurons in mPFC.[353] Meanwhile, activation of LXRs by GW3965 prevents emotional and cognitive deficits induced by either chronic unpredictable mild stress or lipopolysaccharide. The activation of LXRs significantly alleviates the impairment of synaptic plasticity, prevents the upregulation of inflammatory factors and inhibits activation of NF-κB and microglial M1-polarization in both models.[354]

These data suggest that both TSPO and LXRs may be promising therapeutic targets for the treatment of persistent pain, memory decline and mood depression by anti-neuroinflammation. As their upregulations return to normal levels after cure of chronic pain, activation of such proteins may have less side effects.

In clinical practice, it is practical to monitor neuroinflammation with currently available technologies. As mentioned above, neuroinflammation is characterized by upregulation of proinflammatory cytokines and glial activation,[58] and is initiated by immune cell infiltration into nervous system. In human patients, the level of cytokines in plasma and in cerebrospinal fluid can be measured with different methods, such as ELLESA. Microglial and astrocytic activation can be detected with positron emission tomography and magnetic resonance imaging, which track TSPO ligands and mono-amine oxidase type B ligands, respectively.[338] Immune cell infiltration into nervous system is accompanied by increased circulating immune cells and chemokines. As discussed above, the increased monocytes, CCL2 and CXCL12, in blood are paralleled with monocyte infiltration into nervous system in animals with neuropathic pain. In chronic pain patients, monocytes and CXCL12 in blood are also elevated, and both of them are highly correlated with memory decline.[267] The increase in plasma CCL2 is also detected in human patients with chronic and recurrent mechanical neck pain syndrome[355]

and with nonspecific lower back pain.[356] The increased circulating monocytes, CCL2 and CXCL12, may predict infiltration of monocyte into nervous system.

Conclusions and Perspectives

It is increasingly clear that conventional strategies for treatment of chronic pain are less than successful. Targeting the changed ion channels that result in hyperexcitability of sensory neurons or the receptors and intracellular signaling molecules that lead to pathological synaptic plasticity is either ineffective or causes many serious side effects. Emerging evidence indicates that the dysfunction of ion channels in sensory neurons and the pathological plasticity in CNS, which lead to persistent pain and memory/emotional deficits, result from overproduction of inflammatory cytokines. The persistent upregulation of inflammatory cytokines is initiated and maintained by interactions of circulating immune cells, glial cells and neurons. Because all the molecules and cells that contribute to the neuroinflammation have many physiological functions, normalization (counterbalance) but not simple inhibition of the neuroinflammation is the right strategy for treating chronic pain. Experimental studies suggest that this can be achieved by supplementing Mg^{2+} with L-TAMS or activation of some upregulated proteins, such as TSPO and LXRs.

Answers to the following questions remain elusive. Mechanisms underlying opposite regulation of ion channels by pro- and anti-inflammatory cytokines in sensory neurons remain unknown. The excitability of injured DRG neurons is increased, while all Nav subtypes except for Nav1.3 are downregulated in the neurons. Although both Cav 3.2 and the auxiliary β2 subunit of Navs,[357] which plays a role in the regulation of sodium channel density and for action potential generation,[358] are reported to be upregulated, the data may be insufficient to explain the hyperexcitability of the injured DRG neurons. The mechanisms underlying the opposite regulation of BDNF, NR2B subunit of NMDA receptor and synaptic connectivity in spinal dorsal horn and in hippocampus by proinflammatory cytokines and glial activation are largely unknown. Clarifying the region-dependent effects is important for understanding and treating the neuronal disorders related to neuroinflammation. The upregulated proteins that promote recovery in disease conditions are optimal targets for treating neuroinflammation-related disorders. Further study is needed to discover more such kinds of proteins and to clarify the mechanisms of their effects with advanced techniques. It has been well established that intracellular Mg^{2+} deficiency induces inflammation, while the underlying mechanism remains unknown. As only <2% Mg^{2+} is distributed in extracellular fluid, the routinely measured serum Mg^{2+} levels do not always reflect total body magnesium status and are insensitive for detecting Mg^{2+} deficiency,[359] a simple method that can quickly measure intracellular Mg^{2+} is required. The cytokines are short lived, for instance the half-time of TNF-α in plasma is only 12 min.[360] The concentration of the cytokines should be much higher in interstitial fluid, where infiltrated immune cells, glial cells and neurons live. To investigate the function of cytokines and monitor local inflammation, a method for assessing the cytokine levels in interstitial fluid is highly desired.

Abbreviations

ACC, anterior cingulate cortex; ASIC, acid-sensing ion channels; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; BSCB, blood–spinal cord barrier; Cav, voltage-gated calcium channel; CCI, chronic constriction of sciatic nerve; CCL2, C-C motif ligand 2; CGRP, calcitonin gene-related peptide; CSF1, colony-stimulating factor 1; CNS, central nervous system; CXCL12, C-X-C motif chemokine 12; DRG, dorsal root ganglion; HFS, high frequency stimulation; HVA, high voltage activated; IB4, isolectin B4; ICD-11,11th revision of the International Classification of Diseases; JNK, c-Jun N-terminal kinase; L5/L6-SNL, lumbar 5 and /or 6 spinal nerve ligation; L5-VRT, L5 ventral root transection; IL-1β, interleukin-1beta; IL-10, interleukin-10; LTP, long-term potentiation; LTD, long-term depression; L-TAMS, magnesium-L-threonate; LVA, low voltage activated; LXR, liver X receptor; mPFC, medial prefrontal cortex; Nav, voltage-gated sodium channel; NF-200, neurofilament-200; NF-ĸB, nuclear factor-kappa B; NMDA, N-methyl-D-aspartic acid; PDTC, pyrrolidine dithiocarbamate; NP, neuropathic pain; PVM, perivascular macrophage; SIRT1, silent information regulator 1; SMIR, skin/muscle incision and retraction; SNI, spared nerve injury; TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; TNFR1,TNF receptor 1; TRP, transient receptor potential channels; TSPO, translocator protein; TTX, tetrodotoxin.

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Disclosure

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