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Neuroinflammation and Central Sensitization in Chronic and Widespread Pain

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ABSTRACT

Chronic pain is maintained in part by central sensitization, a phenomenon of synaptic plasticity, and increased neuronal responsiveness in central pain pathways after painful insults. Accumulating evidence suggests that central sensitization is also driven by neuroinflammation in the peripheral and central nervous system. A characteristic feature of neuroinflammation is the activation of glial cells, such as microglia and astrocytes, in the spinal cord and brain, leading to the release of proinflammatory cytokines and chemokines. Recent studies suggest that central cytokines and chemokines are powerful neuromodulators and play a sufficient role in inducing hyperalgesia and allodynia after central nervous system administration. Sustained increase of cytokines and chemokines in the central nervous system also promotes chronic widespread pain that affects multiple body sites. Thus, neuroinflammation drives widespread chronic pain *via* central sensitization. We also discuss sex-dependent glial/immune signaling in chronic pain and new therapeutic approaches that control neuroinflammation for the resolution of chronic pain. **(Anesthesiology 2018; 129:343-66)**

CHRONIC pain is a major health concern that affects one in three Americans and costs the U.S. economy $635 billion dollars each year.[1,2] Acute pain is often elicited by acute inflammation and has biologic significance to protect the wounded tissue. Chronic pain is maladaptive, has no beneficial biologic significance, and is characterized by spontaneous pain (*e.g.*, burning) as well as evoked pain in response to noxious (hyperalgesia) or nonnoxious (allodynia) stimuli. It is generally believed that neuronal plasticity in pain-coding pathways and circuits results in chronic pain. Neuronal plasticity consists of peripheral sensitization in primary sensory neurons of dorsal root ganglia and trigeminal ganglia[3–5] and central sensitization of pain-processing neurons in the spinal cord and brain.[6–9]

The perception of pain is typically associated with inflammation, a complex biologic response of the somatosensory, immune, neuronal, autonomic, and vascular/circulatory system to tissue damage, pathogens, or irritants. Acute inflammation, which generally results in perception of pain, serves an important protective or survival role by removing harmful stimuli, initiating the healing process, and restoring tissue integrity (table 1). Primary afferents that respond to tissue injury (*i.e.*, nociceptors) include unmyelinated C-fibers and myelinated Aδ-fibers that terminate in skin, muscle, joints, and visceral organs, with their cell bodies located in dorsal root ganglia and trigeminal ganglia. Nociceptors are activated or sensitized by inflammatory mediators such as bradykinin, prostaglandins, nerve growth factor, and proinflammatory cytokines such as tumor necrosis factor-α, interleukin 1β, and proinflammatory chemokines (*e.g.*, [CC motif] ligand 2, CXC motif chemokine 5)[3,10,11] that directly bind and stimulate G-protein–coupled receptors, ionotropic receptors, and tyrosine kinase receptors. It is noteworthy that all of these receptors are expressed on the terminals and/or cell bodies of nociceptors.[3,5] Cytokine profiles observed in human skin are also associated with inflammation and pain and thus may serve as biomarkers for chronic pain.[12,13] Activation of a mosaic of peripheral receptors results in hypersensitivity and hyperexcitability of nociceptor neurons (peripheral sensitization) through modulation of various ion channels, such as transient receptor potential ion channels (*e.g.*, transient receptor potential ion channels A1, V1, and V4), sodium channels (*e.g.*, subtypes $Na_v1.7/1.8/1.9$),[3,4,14] and mechanosensitive Piezo ion channels.[15,16] MicroRNA may serve as a novel inflammatory and pain-evoking mediator. For example, miR-let-7b induces spontaneous pain *via* activation of Toll-like receptor 7 and transient receptor potential ion channel A1.[17] Furthermore, the activation of

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Table 1. Comparison of Inflammation, Neurogenic Inflammation, and Neuroinflammation with Special Focus on Location, Features, and Role in Pain

	Inflammation	Neurogenic Inflammation	Neuroinflammation
Location	Peripheral tissues Skin, muscle Internal organs except brain	Peripheral tissues Especially skin tissue	PNS: nerves, dorsal root, and trigeminal ganglia CNS: spinal cord, brain
Features	Disruption of vasculature and edema Infiltration of immune cells Production of inflammatory mediators	Activation of C-fibers Release of neuropeptides Edema	Disruption of BBB, infiltration of immune cells Activation of peripheral and central glial cells Production of inflammatory cytokines
Role in pain	Induction and resolution of acute pain Transition from acute to chronic pain	Induction of pain Migraine, CRPS	Transition from acute to chronic pain Maintenance of chronic pain

BBB = brain–blood barrier; CNS = central nervous system; CRPS = complex regional pain syndrome; PNS = peripheral nervous system.

protein kinases such as mitogen-activated protein kinases, protein kinase A, and protein kinase C in primary sensory neurons critically contributes to the induction and maintenance of peripheral sensitization. For instance, activation of p38 mitogen-activated protein kinase in dorsal root ganglia neurons initiates peripheral sensitization by increasing transient receptor potential ion channel V1 activity in response to tumor necrosis factor[18] and also by increasing $Na_V1.8$ activity in response to interleukin-1β, and furthermore, this activation maintains peripheral sensitization and chronic pain by increasing transient receptor potential ion channel V1 expression.[20,21] In parallel, peripheral tumor necrosis factor and interleukin-1β have been strongly implicated in the pathogenesis of inflammatory and neuropathic pain.[22–26]

Of note, nociceptors and immune cells have bidirectional interactions.[27] Nociceptors not only listen to immune cells by responding to inflammatory mediators but also talk to immune cells and modulate the immune response to inflammation.[28,29] Like immune cells, nociceptors express cytokines, chemokines, and Toll-like receptors that are essential for immune modulation.[19,30–32] Release of cytokines and chemokines from nociceptors can rapidly regulate resident immune cells and attract circulating cells to the area of local inflammation that engage primary afferents and cell bodies in the nerve and dorsal root ganglia. For example, nociceptor-produced chemokine (CC motif) ligand 2 regulates local macrophage activation in dorsal root ganglia after chemotherapy *via* Toll-like receptor signaling, resulting in neuropathic pain.[19,33] Activation of nociceptors, especially C-fibers, also produces neurogenic inflammation *via* releasing neuropeptides such as substance P and calcitonin gene–related peptide or prostanoids[28] (table 1). Neurogenic inflammation occurs immediately after intradermal administration of capsaicin and mustard oil *via* respective activation of transient receptor potential ion channels A1 and V1. Neurogenic inflammation results in rapid plasma extravasation and edema, even before the infiltration of immune cells. Neurogenic inflammation plays an important role in inflammatory diseases such as asthma and psoriasis but also contributes to pain conditions such as migraine and complex regional pain syndrome after bone fracture.[34] Nociceptors may differentially regulate inflammation in a context-dependent manner. For example, ablation of nociceptors decreases neurogenic inflammation but also enhances inflammation after bacterial infection by releasing calcitonin gene–related peptide.[35]

Peripheral inflammation with resulting persistent nociceptive input also leads to the increased release of neurotransmitters (glutamate, substance P, calcitonin gene–related peptide, and brain-derived growth factor) from the primary afferent central terminals in the spinal cord and trigeminal nucleus. Through signal transduction, these neurotransmitters produce a state of neuronal hyperactivity and hyperexcitability in the spinal cord and brain known as central sensitization.[36,37] Activation of postsynaptic glutamate *N*-methyl-D-aspartate (NMDA) receptors and plasma cell membrane surface insertion of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are essential steps for the induction and maintenance of central sensitization.[6,38]

Neuroinflammation Is Associated with Various Insults That Evoke Painful Sensations

A PubMed Search on September 30, 2017, using the keywords "neuroinflammation and pain" reveals a substantial increase in the number of publications in the last 10 yr, jumping from 12 in 2008 to 150 in 2016. There is also a similar increase in the number of "microglia and pain" publications over the same period of time (fig. 1). Neuroinflammation is a localized form of inflammation that occurs in the peripheral nervous system (including peripheral nerves and ganglia) and central nervous system (CNS, including the spinal cord and brain). Characteristic features of neuroinflammation are (1) vasculature changes that result in increased vascular permeability, (2) infiltration of leukocytes, (3) activation of glial cells, and (4) production of inflammatory mediators including cytokines and chemokines (table 1). Although the CNS is normally protected by the blood–brain barrier, increased permeability of the blood–brain barrier is an important feature of neuroinflammation, leading to increased leukocyte invasion to the CNS. It is becoming increasingly appreciated that neuroinflammation is a major cause of several neurologic and neuropsychiatric diseases such as Alzheimer

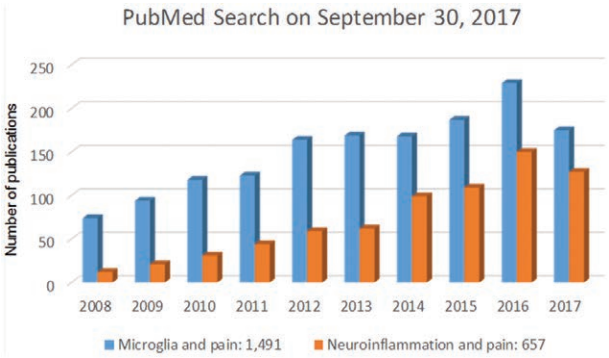

Fig. 1. A PubMed Search on September 30, 2017, shows the number of publications on "neuroinflammation and pain" and "microglia and pain" in the last 10 yr. The *numbers* at the bottom show the total number of publications in PubMed.

disease, Parkinson disease, multiple sclerosis, depression, bipolar disorder, and autism, as well as postoperative complications like neurocognitive disorders (*e.g.*, delirium).[39–41]

It is noteworthy that neuroinflammation is associated with various painful insults and pathologies (fig. 2),[39] including but not limited to trauma such as traumatic brain injury, stroke, spinal cord injury, major surgeries including both cardiac and noncardiac procedures (*e.g.*, amputation, thoracotomy, and mastectomy), and autoimmune diseases (rheumatoid arthritis and multiple sclerosis). Other painful conditions, such as osteoarthritis, peripheral cancers (*e.g.*, bone cancers), and viral infections (*e.g.*, shingles/herpes zoster by varicella-zoster virus) also induce neuroinflammation in the peripheral nervous system and CNS.[27,42] Furthermore, painful neuroinflammation (*e.g.*, glial activation) can be induced by chemotherapy drugs such as paclitaxel, anti–human immunodeficiency virus (HIV) treatment, and chronic opioid treatment.[43–46] Immune therapies are increasingly appreciated for treating cancers by boosting the activities of immune cells such as T cells and other immune cells.[47,48] These therapies also change pain sensitivity by modulating inflammation and neuroinflammation. Deficiency of the negative immune regulator B7-H1, also called programmed death ligand 1, enhances inflammation and neuropathic pain after chronic constriction injury of mouse sciatic nerve.[49] Notably, PD-1, the receptor for programmed death ligand 1, is not only present in immune cells but also expressed in nociceptor neurons of mouse and human dorsal root ganglia. Furthermore, programmed death ligand 1 can potently suppress nociceptor activities *via* regulating sodium and potassium ion channels in mouse and human nociceptors.[50] Many of these painful insults involve brain and spinal trauma and nerve injury resulting from major surgeries, drug treatments, and diabetic neuropathy in neuropathic pain conditions. Neurogenic inflammation of the skin and joints is not only induced by activation of peripheral C-fibers but

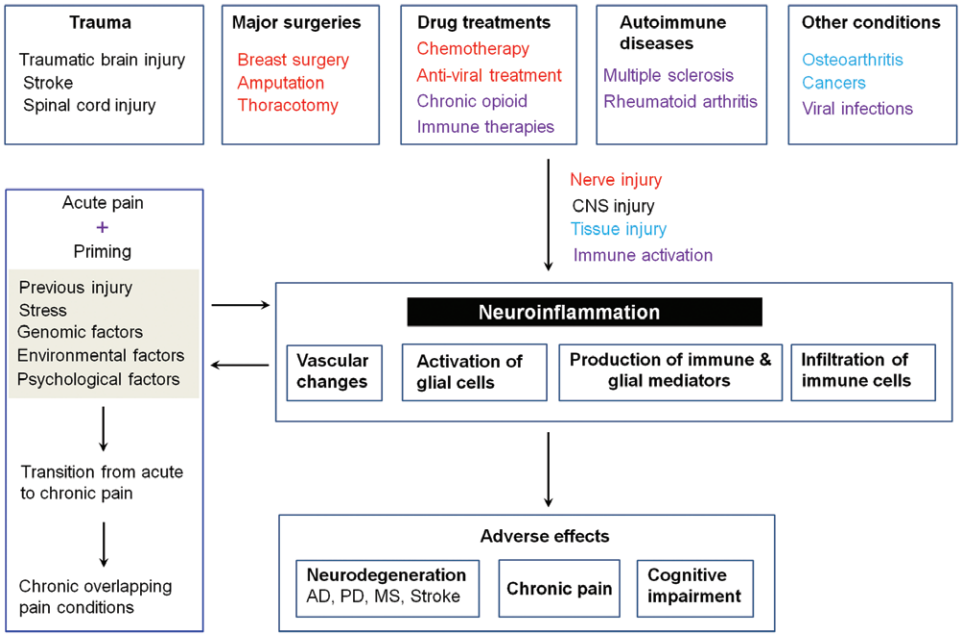

Fig. 2. Neuroinflammation is associated with various insults that evoke painful sensations. These insults include but are not limited to trauma, major surgeries, drug treatments, autoimmune disease conditions, and other painful insults and tissue damage. Some of these insults such as major surgeries (breast surgery, amputation, thoracotomy), chemotherapy, and antiviral treatment will cause nerve injury, as highlighted in *red*. Others will cause immune activation (highlighted in *purple*) and tissue injury (highlighted in *light blue*). Neuroinflammation results in several adverse effects, such as chronic pain and neurodegenerative diseases including Alzheimer disease (AD), Parkinson disease (PD), multiple sclerosis (MS), and stroke. Neuroinflammation is also associated with chronic overlapping pain conditions. After priming of the nociceptive circuit by previous injury, stress, or existing genomic, environmental, and psychologic factors, acute insult may cause transition from acute pain to chronic pain. CNS = central nervous system.

may also be triggered by the dorsal root reflex in the spinal cord after either orthograde or anterograde neuronal activation, which permits peripheral inflammatory responses to occur *via* local insults or by CNS "top-down" activation of primary afferents.[51,52] Furthermore, neuroinflammation in the spinal cord and brain can also be neurogenic after neuronal activation in the CNS.[53]

Although it is widely believed that chronic pain persists after the observable signs and symptoms of inflammation have resolved,[54] our understanding of neuroinflammation is changing this perspective. We now recognize that neuroinflammation is associated with and perhaps mediates the persistence and chronification of human pain conditions. Given the close proximity to pain neurocircuits, mediators of neuroinflammation are highly effective in modulating pain sensitivity. In particular, inflammation and neuroinflammation are differentially correlated with chronic pain. For example, HIV-infected patients with neuropathic pain show permanent neuroinflammation in the spinal cord.[55] Patients with fibromyalgia, a chronic widespread musculoskeletal pain condition,[56] also exhibit small fiber neuropathy together with chronic neuroinflammation.[57] The degree to which acute and chronic pain perception is mediated by the same cast of neuroinflammatory mediators is an interesting and open question.

It is noteworthy that a recent study by Wei *et al.*[58] has shown distinct roles for inflammation and central neuroinflammation in the acute phase *versus* the chronic phase of pain behavior in a rat model of complex regional pain syndrome. Although peripheral interleukin-1β levels only increased at 4 weeks, spinal levels of interleukin-1β increased at both 4 weeks (acute phase) and 16 weeks (chronic phase). Importantly, systemic administration of anakinra, a peripherally restricted interleukin-1 receptor antagonist, only inhibited nociceptive behaviors at 4 weeks but not at 16 weeks, though intrathecal injection of anakinra reduced nociceptive behaviors at both 4 and 16 weeks.[58] This study supports a critical role of central neuroinflammation in maintaining chronic pain in a rodent model of complex regional pain syndrome.

Neuroinflammation in Chronic Overlapping Pain Conditions and Widespread Pain

Emerging evidence suggests that neuroinflammation contributes to the pathophysiology of coprevalent or coexisting chronic pain conditions that are referred to as chronic overlapping pain conditions, which include but are not limited to fibromyalgia, headache, temporomandibular disorder, back pain, irritable bowel syndrome, primary headaches, pelvic pain, and vestibulodynia. Chronic overlapping pain conditions are characterized by symptoms consistent with the dysregulation of sensory, inflammatory, and psychologic domains.[13,56,59,60] There is increased evidence that patients with chronic overlapping pain conditions exhibit increased basal and stress-induced levels of catecholamines (epinephrine and norepinephrine) in circulation[61–64] and reduced activity of catechol-*O*-methyltransferase,[65,66] a ubiquitously expressed enzyme that metabolizes catecholamines.[67] Chronic overlapping pain condition patients have functional variants in the *COMT* gene that result in reduced catechol-*O*-methyltransferase activity.[66, 68–70] The "low catechol-*O*-methyltransferase activity" variants are associated with increased fibromyalgia[71–75] and temporomandibular disorder[76] onset and increased pain in response to experimental stimuli[76,77] and stressful events.[70,78,79] Consistent with clinical syndromes, in rodents sustained delivery of the catechol-*O*-methyltransferase inhibitor OR486 results in pain at multiple body sites that persists for weeks and altered pain- and anxiety-related volitional behaviors.[80,81–83] Persistent catechol-*O*-methyltransferase–dependent pain is initiated by peripheral β_2- and β_3-adrenergic receptors through release of nitric oxide, tumor necrosis factor-α, interleukin-1β, interleukin-6, and chemokine (CC motif) ligand 2 in plasma and maintained by increased tumor necrosis factor in central tissues.[80,83–84] Higher levels of nitric oxide derivatives (*e.g.*, nitrite and nitrate) and proinflammatory cytokines have been found in patients with chronic musculoskeletal pain conditions.[13,85–89] Of note, patients with site-specific pain conditions (*e.g.*, localized temporomandibular disorder or localized vestibulodynia) exhibit a balance in pro- and antiinflammatory cytokines, whereas those with chronic overlapping pain conditions fail to exhibit a compensatory increase in antiinflammatory cytokines.[13,60] Compared to patients with localized pain, those with chronic overlapping pain conditions also exhibit dysregulation in microRNAs (*e.g.*, miR-let-7f) that augment immune response and proinflammatory cytokine production.[60] Together, these findings suggest that localized and anatomically widespread patterns of chronic pain are associated with distinct inflammatory profiles.

Widespread patterns of chronic pain exhibited by patients with chronic overlapping pain conditions may be attributed to previous injury or stressful events that produce long-lasting changes in nociceptor function, a phenomenon known as hyperalgesic priming. Preclinical studies have demonstrated that acute painful inflammation or nerve injury, as well as nonpainful stress, "primes" nociceptors so that they respond to a subsequent insult (*e.g.*, prostaglandin E_2 or epinephrine) for a prolonged duration due to activation of distinct kinase and gene transcription pathways.[4,90–93] In line with these findings, individuals with the "low activity" catechol-*O*-methyltransferase genotype report enhanced and prolonged pain after motor vehicle collisions or psychologic strain,[70,78,94] which are events that stimulate the sympathetic release of epinephrine known to sensitize nociceptors[95,96] and promote inflammation.[97–105] Thus, repeated environmental exposures to injury, inflammation, and stress in genetically predisposed individuals results in the transition from acute pain to chronic pain, in part through neuroinflammation (fig. 2). Of note, when a challenge of lipopolysaccharide is preceded by a surgical procedure, it has been shown that this early priming of spinal microglial cells

increases the duration and intensity of pain through contributions to neuroinflammation.[106]

Glial Activation as a Primary Feature of Neuroinflammation and a Driver of Chronic Pain

Glial Activation, Gliosis, and Gliopathy after Painful Injuries

Neuroinflammation is characterized by activation of peripheral glia including Schwann cells in the nerve and satellite glial cells in the dorsal root ganglia and trigeminal ganglia, and central glia including microglia, astrocytes, and oligodendrocytes in the spinal cord and brain.[107,108] In this review we focus on central glia, especially microglia and astrocytes and the mechanisms by which glia-produced mediators modulate synaptic plasticity and central sensitization. The previous decade has seen an exponential increase in literature documenting the role of microglia and astrocytes in the pathogenesis of chronic pain; "glial activation" is emerging as a powerful mechanism underlying pathogenesis of chronic pain,[109–115] and chronic pain may also manifest as a "gliopathy."[108]

After painful injuries, there are different activation states of microglia and astrocytes. The morphologic activation (glial reaction or gliosis) is the most studied activation state, although this type of glial activation may not directly cause pain.[108] Glial reaction is characterized by increased expression of microglial markers such as cluster of differentiation molecule 11B, ionized calcium-binder adapter molecule 1, and CX3C chemokine receptor 1 and astroglial markers glial fibrillary acid protein, as well as morphologic changes such as hypertrophy or process retraction/extension of microglia and astrocytes. Microglial reaction in the spinal cord is very rapid and dramatic, whereas astrocyte reaction in the spinal cord is more persistent and occurs in more painful conditions (fig. 3, A–F).[114,116] Subcutaneous formalin injection into a hind paw of rat or mouse is probably the most studied animal model of inflammatory pain, which lasts for less than 1 h. Of interest, in 1999, Fu *et al.*[117] showed that subcutaneous formalin also caused a robust microglial reaction (labeled with cluster of differentiation molecule 11B) in the spinal cord days after injection. This microglial reaction from formalin-induced nerve injury is associated with the development of mechanical allodynia. This is one of the earliest reports to support a possible role of spinal microglia in pain regulation. Functional imaging also reveals glial activation in patients with chronic pain.[118] In 2003, Zhu *et al.*[119] and Zhu and Eisenach[120] showed that incision and nerve injury causes upregulation of cyclooxygenase-1 in spinal glial cells, which is important for the development of postoperative pain and neuropathic pain.

Signaling Mechanisms in Glial Regulation of Allodynia and Hyperalgesia

Several neuromodulators such as adenosine triphosphate (ATP), chemokines (CL1, chemokine (CC motif) ligand 2, and chemokine (CXC motif) ligand 13), and neuropeptides (substance P and calcitonin gene–related peptide), and colony-stimulating factor 1 are involved in glial activation after painful insults[108,121,122] (table 2). The upregulation of glia-specific receptors and channels are functionally correlated with pain hypersensitivity. ATP modulates glial activation *via* stimulation of ionotropic P2X receptors and metabotropic P2Y receptors.[123,124] Peripheral nerve injury increases the expression of ATP P2Y receptors (P2X4, P2X7, and P2Y12) in spinal microglia, and each upregulation was implicated in neuropathic pain sensitization (mechanical allodynia).[125,126] Accumulating evidence suggests that after tissue and nerve injury, ATP is generated from different cell types including astrocytes, neurons, and microglia. Astrocyte-expressing hemichannels connexin 43 are permeable to ATP.[127] Glucocorticoids induce ATP release from spinal astrocytes, leading to microglial activation and diurnal exacerbation of allodynia.[128] Vesicular nucleotide transporter regulates ATP release from spinal cord neurons after nerve injury.[129] Microglial pannexin 1 channel was also shown to facilitate ATP release in neuropathic pain.[130] Nerve injury results in cleavage and activation of chemokine CX3C ligand 1/fractalkine by protease cathepsin S, leading to microglia activation through stimulation of CX3C chemokine receptor 1 receptor.[113] CX3C chemokine receptor 1, one of the best-known markers of microglia, is strongly upregulated after nerve injury, as revealed in *Cx3cr1*-GFP mice (fig. 3, A and B). This microglial receptor is also critical for the development of neuropathic pain symptoms, because mechanical allodynia after nerve injury is reduced after spinal administration of the CX3C chemokine receptor 1 neutralizing antibody and abrogated in *Cx3cr1* knockout mice.[113,131,132] After chronic constriction injury of the sciatic nerve, CX3C chemokine receptor 1 upregulation peaks within 10 days. In the late phase of mechanical allodynia (greater than 3 weeks), microglial CX3C chemokine receptor 1 expression markedly declines (fig. 3, A and B). Microglia may play a role in maintaining persistent hyperalgesia and allodynia after bone cancer.[133] Orthopedic surgery and bone fracture also may result in nerve injury and microglial activation in the spinal cord. It was proposed that spinal microglial activation also contributes to postoperative cognitive dysfunction such as delirium.[134] Substance P signaling from C-fiber afferent terminals in the spinal cord results in microglia activation and central sensitization after bone fracture.[135]

After painful insults, gliopathy is also characterized by dysfunction of astrocytes, such as downregulation of glutamate transporters (glutamate transporter 1 and glutamate aspartate transporter) in spinal cord astrocytes, resulting in glutamate accumulation in synaptic clefts causing neuronal hyperactivity.[108,136] Connexin 43 is a critical astrocytic signaling molecule that controls the release of astroglial mediators including glutamate and ATP.[137] Of note, connexin 43 upregulation in spinal cord astrocytes is sustained after spinal cord injury and nerve injury (fig. 3F) and contributes to the development and maintenance of mechanical allodynia.[138,139] Additionally,

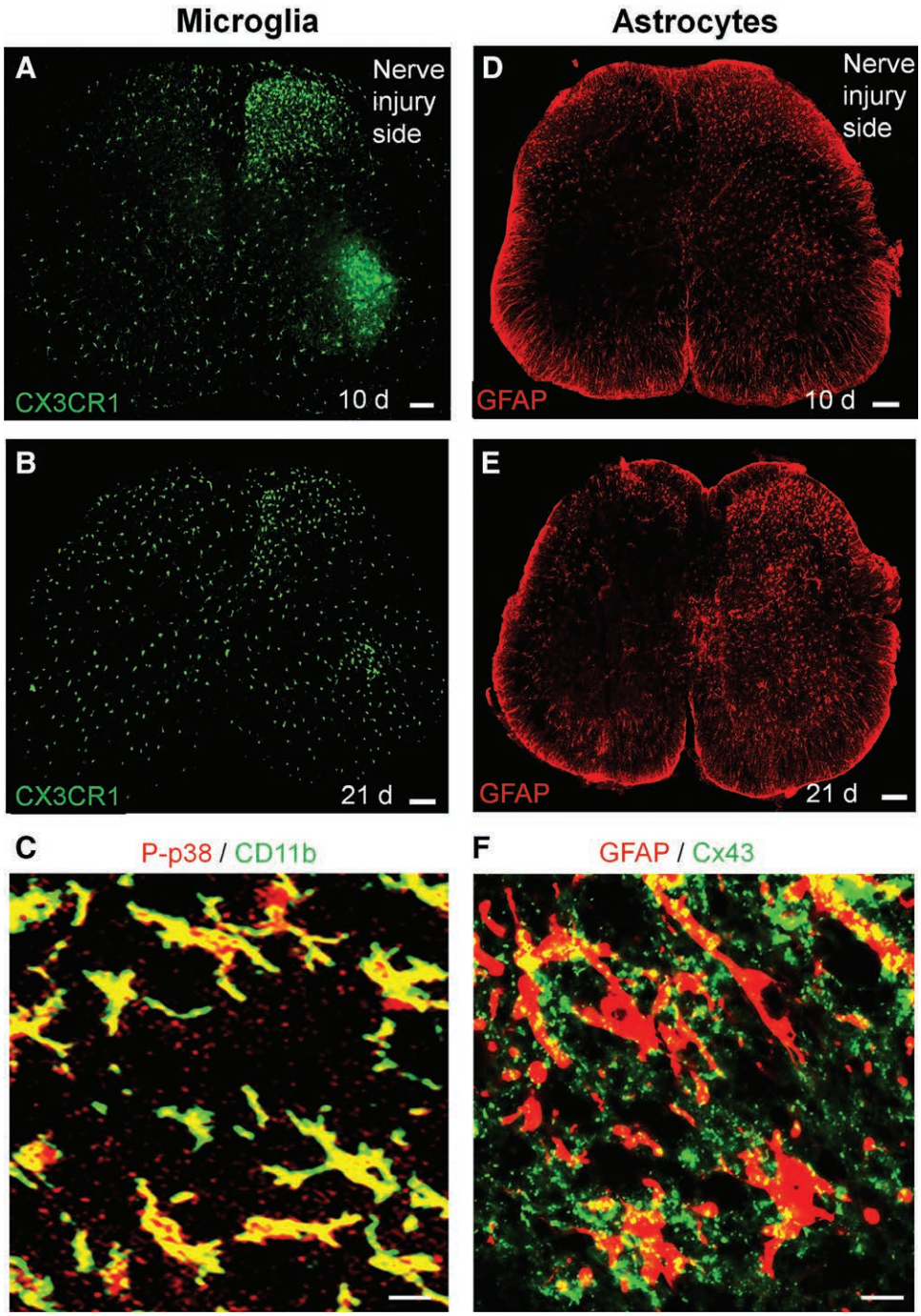

Fig. 3. Distinct and time-dependent activation of microglia and astrocytes in the spinal cord after nerve injury. Microglia activation revealed by increased CX3C chemokine receptor 1 (CX3CR1) expression in the spinal cord 10 days (*A*) and 21 days (*B*) after nerve injury in *Cx3cr1*-GFP mice. *Scale bar* = 100 μm. (*C*) Phosphorylation of p38 mitogen-activated protein kinase (P-p38) in cluster of differentiation molecule 11B (CD11b)$^{+}$ microglia in the spinal cord dorsal horn 7 days after nerve injury. *Scale bar* = 20 μm. Astroctye activation revealed by increased glial fibrillary acidic protein (GFAP) expression in the spinal cord 10 days (*D*) and 21 days (*E*) after nerve injury in mice. *Scale bar* = 100 μm. (*F*) Expression of connexin 43 (Cx43) in GFAP-positive astrocytes in the spinal cord dorsal horn 21 days after nerve injury. *Scale bar* = 20 μm. d = days.

chronic pain-associated gliopathy could manifest as a functional switch of connexin 43 from gap junction communication to hemichannel regulation, so that astrocytes become "leaky" during this switch, resulting in increased secretion of cytokines (interleukin-1β) and chemokines ([CC motif] ligand 2, [CXC motif] ligand 1).[139,140] Chemokines regulate bidirectional interactions of neurons and glial cells.[141] Astrocytes not only produce chemokines that can "talk to" neurons by modulating neuronal activity but also "listen to" neurons by responding to chemokines (*e.g.*, [CXC motif] ligand 13)

Table 2. Signal Transduction in Spinal Cord Microglia and Astrocytes after Tissue and Nerve Injury

Microglia	Astrocytes
Activators	Activators
ATP	ATP
CX3CL1	TNF
CSF1	CXCL13
LPS/HMGB1	LPS/HMGB1
CASP6	MMP-2
Receptors	Receptors
P2X4, P2X7, P2Y12	P2X/P2Y
CX3CR1	TNFR1
CSF1R	CXCR5
TLR4	TLR4
C5aR	Cx43
Intracellular signaling	Intracellular signaling
P-p38, P-ERK, P-Src	P-JNK, P-ERK
Mediators	Mediators
TNF	CCL2, CXCL1
IL-1β, IL-18	TSP1, TSP4
BDNF	bFGF, IL-1β

Painful tissue and nerve injuries induce release of glial activators, which in turn bind their respective receptors on microglia and astrocytes. Upon activation, the glial receptors cause intracellular signal transduction and activation of protein kinases (phosphorylation of mitogen-activated protein kinase and Src kinase), leading to increased synthesis and release of glial mediators that can produce central sensitization and hyperalgesia and allodynia.

ATP = adenosine triphosphate; BDNF = brain-derived neurotrophic factor; bFGF = basic fibroblast growth factor; CASP6 = caspase 6; CCL2 = chemokine (CC motif) ligand 2; CSF1 = colony-stimulating factor 1; CXCL1 = chemokine (CXC motif) ligand 1; CXCL13 = chemokine (CXC motif) ligand 13; CXCR5 = CXC chemokine receptor type 5; CX3CL1 = chemokine CX3C ligand 1; CX3CR1 = CX3C chemokine receptor 1; Cx43 = connexin 43; C5aR = complement C5 receptor; HMGB1 = high-motility group box protein 1; IL = interleukin; LPS = lipopolysaccharide; MMP-2 = matrix metalloprotease 2; P-ERK = phosphorylated extracellular signal-regulated kinase; P-JNK = phosphorylated c-Jun N-terminal kinase; P-p38 = phosphorylated p38; P-Src = phosphorylated Src; TLR4 = Toll-like receptor 4; TNF = tumor necrosis factor (α); TNFR1 = TNF receptor type I; TSP = thrombospondin.

derived from neurons.[122] Astrocytes also release thrombospondin 4 to modulate synapse formation, synaptic plasticity, and behavioral hypersensitivity (table 2).[142]

A critical step of glial activation in persistent pain is the activation of intracellular signaling pathways, especially the mitogen-activated protein kinase pathways. There are three major members in the mitogen-activated protein kinase family: extracellular signal-regulated kinase 1 and 2, p38, and c-Jun N-terminal kinase.[143] Phosphorylation of p38 in spinal microglia occurs in different pain conditions after surgery, nerve injury (fig. 3C), and opioid tolerance, resulting in increased synthesis and release of microglial mediators (tumor necrosis factor, interleukin-1β, and brain-derived growth factor) and pain hypersensitivity.[132,133,144–147] Inflammation or nerve injury also activates c-Jun N-terminal kinase in astrocytes,[148,149] leading to increased secretion of chemokine (CC motif) ligand 2 and chemokine (CXC motif) ligand 1 and enhanced pain states.[139,150] Nerve injury also causes sequential activation of extracellular signal-regulated kinase in microglia (early phase) and astrocytes (late phase),[143] indicating distinct involvement of these two glial cell types in chronic pain induction and maintenance.

Glia Activation in the Brain after Painful Injuries

Accumulating evidence also suggests a role of glial activation, revealed in different brain regions, in regulating neuroinflammation and pain sensitivity. Sciatic nerve ligation induces astrocyte activation in the S1 sensory cortex, which is associated with upregulation of metabotropic glutamate receptor 5. Activation of this glutamate receptor subtype in astroglia induces spontaneous somatic Ca^{2+} transients and secretion of thrombospondin 1 from astrocytes, leading to new synapse formation and mechanical allodynia.[151] Toll-like receptor 4 plays a critical role in glial activation and neuroinflammation in the spinal cord, as well as hyperalgesia and allodynia.[152,153] Toll-like receptor 4 also contributes to neuroinflammation in the prefrontal cortex and visceral pain after chronic stress. Increased expression of Toll-like receptor 4 is associated enhanced glia activation in the prefrontal cortex and increased levels of proinflammatory cytokines. Administration of the Toll-like receptor 4–specific antagonist TAK-242 in the prefrontal cortex is sufficient to attenuate visceral hypersensitivity.[154] Peripheral nerve injury causes microglia activation within the mesolimbic reward circuitry, leading to a disruption of dopaminergic signaling and reward behavior.[155] Furthermore, nerve injury causes activation of microglia and astrocytes in the anterior cingulate cortex, and administration of microglial inhibitor minocycline in this brain region inhibited mechanical allodynia.[151] However, earlier studies showed that nerve injury did not cause microgliosis in the anterior cingulate cortex and that long-term synaptic plasticity (long-term potentiation) was not altered by minocycline.[156,157] Future studies are warranted to investigate how microglia and astrocytes regulate different forms of synaptic plasticity in different brain regions after painful insults in the peripheral tissues and the central nervous system.

Sex Dimorphism in Glial Regulation of Allodynia and Hyperalgesia

Chronic pain such as chronic orofacial pain associated with temporomandibular disorder occurs more frequently in women.[158,159] In 1993, Maixner and Humphrey[160] reported sex differences in pain and cardiovascular responses to forearm ischemia in humans. Paradoxically, the majority, if not all, pain-related studies were conducted in male animals.[161] However, it was fortunate that males were tested because spinal microglia play little or no role in regulating inflammatory and neuropathic pain primarily in female rodents[162–165] (fig. 4). Sorge[162] demonstrated that spinal Toll-like receptor 4, an important receptor for microglia activation, regulates hyperalgesia and allodynia resulting from inflammation or nerve injury exclusively in male mice. Of note, morphologic activation and proliferation of spinal microglia are identical in males and females after nerve injury. Nerve injury–evoked mechanical allodynia is also equivalent in both sexes during the tested times.[163,164] However, mechanical allodynia after nerve injury was exclusively attenuated in male mice

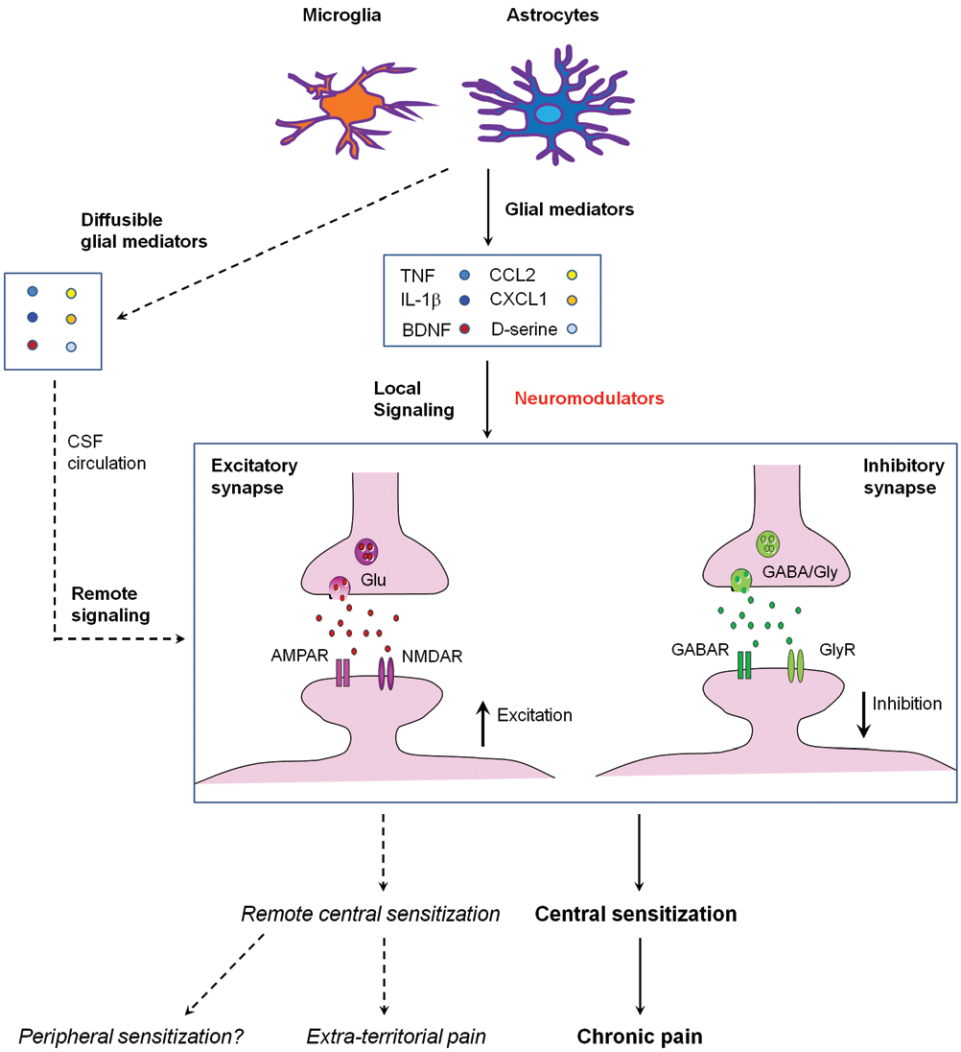

Fig. 4. Schematic illustration of local and remote central sensitization induced by glial activation and neuroinflammation in the spinal cord. Activation of spinal microglia and astrocytes by painful insults results in secretion of glial mediators such as tumor necrosis factor (TNF), interleukin (IL)-1β, chemokine (CC motif) ligand 2 (CCL2), chemokine (CXC motif) ligand 1 (CXCL1), brain-derived neurotrophic factor (BDNF), and D-serine, which can act as neuromodulators to induce local central sensitization in surrounding excitatory synapses (facilitation) and inhibitory synapses (disinhibition). During neuroinflammation, these glial mediators also affect synapses in different spinal segments to cause remote central sensitization and extraterritorial and widespread pain beyond the initial injury site. It is also possible that central sensitization may further promote peripheral sensitization via neuroinflammation. AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CSF = cerebrospinal fluid; GABA = γ-aminobutyric acid; GABAR = γ-aminobutyric acid receptor; Glu = glutamate; GlyR = glycine receptor; NMDAR = *N*-methyl-D-aspartate receptor.

after intrathecal injection of microglial inhibitor (minocycline), microglial toxin, or P2X4 blocker, or after special deletion of *Bdnf* in microglia.[163] Furthermore, nerve injury activates p38 in spinal microglia of male but not female mice; in agreement, spinal administration of p38 inhibitor reduces neuropathic pain only in male mice.[164] Caspase-6 is a microglial activator and released from axonal terminals in the spinal cord after tissue and nerve injury, which can act on microglia to release tumor necrosis factor.[166] Sex dimorphism was also revealed in caspase-6–mediated microglial signaling, and caspase-6 regulates neuropathic pain exclusively in males.[165] It appears some male-specific microglial responses require testosterone, because minocycline reduces allodynia in testosterone-treated females but not in castrated males. In female rodents, the role of microglia in neuropathic pain appears to be replaced by T cells.[163]

Central Sensitization Controls Augmentation and Spread of Pain Hypersensitivity

Term Development

Central sensitization is a powerful phenomenon in the pain field. As a key mechanism of chronic pain, it also guides clinical treatment for conditions associated with widespread pain.[167,168] In this review, we highlight the role of glial cells and neuroinflammation in promoting central sensitization and widespread chronic pain. In 1983, Woolf[36] presented

evidence for a central component of postinjury pain hypersensitivity. The International Association for the Study of Pain describes central sensitization as increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input.[169] Central sensitization may also include conditions like increased central responsiveness due to dysfunction of endogenous pain control systems, regardless of whether there is functional change of peripheral neurons. In 2003, Ji *et al.*[6] defined central sensitization as the increased synaptic efficacy established in somatosensory neurons in the dorsal horn of the spinal cord after intense peripheral noxious stimuli, tissue injury, or nerve damage. This heightened synaptic transmission results in a reduction in pain threshold, an amplification of pain responses, and a spread of pain sensitivity to noninjured areas. In 2009, Latremoliere and Woolf[38] described central sensitization as an enhancement in the function of neurons and circuits in nociceptive pathways caused by increases in membrane excitability and synaptic efficacy, as well as to reduced inhibition, and as a manifestation of the remarkable plasticity of the somatosensory nervous system in response to activity, inflammation, and neural injury. In this review, the authors highlighted disinhibition (reduced inhibition) and also emphasized that the net effect of central sensitization is to recruit previously subthreshold synaptic inputs to nociceptive neurons, generating an increased or augmented action potential output: a state of facilitation, potentiation, augmentation, or amplification.

Mechanisms of Central Sensitization

As shown in figure 5, activation of NMDA receptors is an essential step in initiating and maintaining the central sensitization

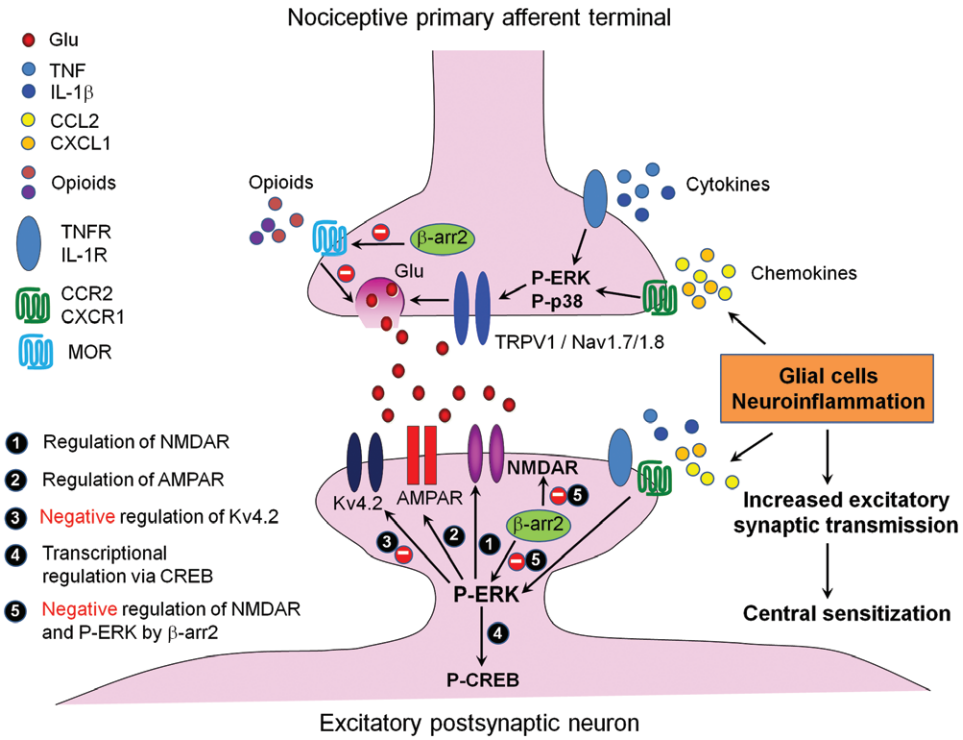

Fig. 5. Molecular mechanisms of central sensitization in first-order excitatory synapses in the spinal cord dorsal horn pain circuit and induction of central sensitization by proinflammatory cytokines and chemokines (*e.g.*, tumor necrosis factor [TNF], interleukin [IL]-1β, chemokine [CC motif] ligand 2 [CCL2], chemokine [CXC motif] ligand 1 [CXCL1]) that are produced by glial cells. At presynaptic sites (*i.e.*, central terminals of nociceptive primary afferents), activation of receptors of cytokine and chemokine receptors results in phosphorylation and activation of extracellular signal-regulated kinase (P-ERK) and p38 (P-p38), leading to glutamate (Glu) release from synaptic vesicles *via* activation of ion channels transient receptor potential ion channel V1, voltage-gated sodium ion channel 1.7 ($Na_v1.7$), and voltage-gated sodium channel subtype 1.8 ($Na_v1.8$). At postsynaptic sites, increased release of neurotransmitters (*e.g.*, glutamate) also induces phosphorylated extracellular signal-regulated kinase, which can induce central sensitization by positive modulation of *N*-methyl-D-aspartate receptor (NMDAR, step 1). Positive regulation of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR, step 2) and negative modulation of potassium channel subunit Kv4.2 (step 3) are also shown. Phosphorylated extracellular signal-regulated kinase also maintains central sensitization *via* inducing cAMP response element–binding protein phosphorylation (P-CREB, step 4). CREB is a critical transcription factor that controls the expression of pronociceptive genes. Opioids such as morphine inhibit neurotransmitter release *via* μ opioid receptors (MOR) and N-type calcium channels. The scaffold protein β-arrestin 2 (βarr2) inhibits μ opioid receptor signaling by desensitization and degradation of G-protein–coupled receptors, leading to enhanced acute opioid analgesia in β-arrestin 2 knockout mice. Paradoxically, β-arrestin 2 also inhibits *N*-methyl-D-aspartate receptor and extracellular signal-regulated kinase signaling, leading to a transition from acute pain to chronic pain.[174] CXCR2 = CXC motif chemokine receptor 2; IL-1R = interleukin-1 receptor; TNFR = tumor necrosis factor receptor.

and pain hypersensitivity after tissue and nerve injury.[170,171] Glutamate is a primary excitatory neurotransmitter in the pain pathway. Under normal circumstances NMDA receptor channels are blocked by Mg^{2+} ions, but this blockade is removed by membrane depolarization after activation of nociceptive primary afferents. Activation of NMDA receptors boosts synaptic efficacy and causes Ca^{2+} influx, which can activate intracellular signaling pathways that initiate and maintain central sensitization.[6,38] In particular, tissue and nerve injury increases the expression of the NMDA receptor-NR2B (GluN2B) subunit, which regulates spinal synaptic plasticity in persistent pain conditions together with the NR1 subunit.[172] Interestingly, NR2B/GluN2B receptor activity and surface expression in spinal cord dorsal horn neurons is negatively regulated by β-arrestin 2,[173] a scaffold protein that was traditionally known as an inhibitor of G-protein–coupled receptors. Deficiency of β-arrestin 2 results in enhanced acute opioid analgesia, produced by morphine and [D-Ala2, N-MePhe4, Gly-ol]-enkephalin, a selective μ opioid receptor agonist.[173,174] Paradoxically, [D-Ala2, N-MePhe4, Gly-ol]-enkephalin-induced hyperalgesia is also potentiated after β-arrestin 2 deficiency, as a result of enhanced surface and synapse expression of GluN2B that results in hyperactivity of the receptor. Loss of β-arrestin 2 also leads to a prolongation of inflammatory and neuropathic pain, because these pain conditions critically depend on GluN2B.[173] It appears that for the resolution of persistent pain, it is more important for β-arrestin 2 to regulate NMDA receptors *via* extracellular signal-regulated kinase signaling pathway (fig. 5). Furthermore, surface trafficking of AMPA receptors, especially calcium-permeable subunit (GluR1/GluR-A), plays a critical role in spinal cord synaptic plasticity and pain hypersensitivity after tissue injury.[175,176] The scaffold protein Homer1a operates in a negative feedback loop to regulate calcium signaling and the excitability of the spinal cord pain pathway in an activity-dependent manner.[177] Given a critical role of AMPA receptor in direct control of excitatory synaptic transmission in pain circuits, NMDA receptor–independent central sensitization may also exist.

Activation of intracellular pathways by protein kinases, such as protein kinase A, protein kinase C, Ca^{2+}/calmodulin-dependent kinase II, Src (a tyrosine kinase encoded by sarcoma oncogene), and extracellular signal-regulated kinases (including extracellular signal-regulated kinases 1 and 2), is important for the generation of central sensitization.[6,37,178] Notably, activation of extracellular signal-regulated kinase *via* phosphorylation of extracellular signal-regulated kinase in spinal cord dorsal horn neurons is nociceptive-specific and serves as a marker of central sensitization.[179–181] Phosphorylation of extracellular signal-regulated kinase is a common pathway after the activation of various ionotropic and metabotropic receptors and protein kinases (protein kinase A, protein kinase C, and Src) as well as a downstream event of Ca^{2+} signaling.[182–184] Phosphorylation of extracellular signal-regulated kinase induces central sensitization *via* rapid posttranslational regulation, such as suppression of potassium channel Kv4.2 activity, leading to hyperactivity of the spinal cord dorsal horn.[185,186] Phosphorylation of extracellular signal-regulated kinase also contributes to rapid upregulation of NMDA receptor (GluN2B) function in spinal cord dorsal horn neurons in response to inflammatory mediators.[150,187] Furthermore, translocation of phosphorylated extracellular signal-regulated kinase to the nuclei of spinal cord dorsal horn neurons activates the transcription factor cAMP response element–binding protein, leading to increased expression of pronociceptive genes encoding for c-Fos, NK-1, and prodynorphin.[182,188]

Does Central Sensitization Require Peripheral Input?

Historically, it has been believed that the central sensitization to noxious stimuli requires sustained, intense, and repeated applications of the stimulus. More recently, it has become apparent that persistent peripheral nociceptive input may not be required to elicit central sensitization, because central sensitization can result from changes in the properties of neurons in the central nervous system that appear to be independent of peripheral input.[38] Central sensitization produces pain hypersensitivity by changing the sensory responses elicited by normal inputs, including subthreshold innocuous tactile stimulation. For example, spinal cord disinhibition by intrathecal injection of γ-aminobutyric acid (GABA) and glycine receptor antagonists is sufficient to induce central sensitization and mechanical allodynia *via* disinhibition of inhibitory signaling and subsequent activation of excitatory signaling that is mediated by the NMDA receptor.[189] Disinhibition of γ-aminobutyric acid–mediated (GABAergic) and glycinergic synaptic transmission in the spinal cord pain circuitry is critical to the generation of chronic pain.[84–87] Gate control theory describes a tonic inhibition of the spinal cord pain circuit *via* inhibitory neurons.[190] A feed-forward spinal cord glycinergic neural circuit in the laminae II–III dorsal horn gates mechanical allodynia, and nerve injury impairs glycinergic synaptic transmission and opens the gate to elicit mechanical allodynia.[84]

It is noteworthy that some central etiologies/injuries such as spinal cord injury, traumatic brain injury, and multiple sclerosis may cause neuroinflammation, central sensitization, and chronic pain without a peripheral insult (fig. 2).[191] Therefore, it is important for future studies to examine neuroinflammation in the brain, including those regions that do not receive input from primary afferents. Spinal cord injury is sufficient to produce hyperexcitability in primary sensory neurons[192] *via* possible retrograde signaling from the dorsal root reflex. Neuroinflammation in the spinal cord may also regulate gene expression of primary sensory neurons *via* diffusible inflammatory mediators that can reach to dorsal root ganglia. Thus, there could be bidirectional interactions between peripheral sensitization and central sensitization. Central sensitization is not only secondary to peripheral sensitization but may, in turn, regulate peripheral sensitization (fig. 4).

Neuroinflammation Drives Central Sensitization and Widespread Pain *via* Glia-produced Cytokines and Chemokines

An important step forward in revealing the role of central sensitization in widespread chronic pain is to demonstrate direct involvement of cytokines and chemokines (small cytokines) in the induction and maintenance of central sensitization.[150,193–195] In 2001, Samad *et al.*[193] showed that spinal interleukin-1β contributes to central sensitization and inflammatory pain hypersensitivity *via* transcriptional regulation that causes upregulations of cyclooxygenase-2 and prostaglandin E_2. Notably, unilateral inflammation in the hind paw of rats caused widespread and bilateral increase of cyclooxygenase-2 in the spinal cord and brain, due to possible increase of interleukin-1β level in the cerebrospinal fluid.[193] This may partially explain widespread pain in some chronic pain conditions (fig. 4). However, contribution of the spinal cyclooxygenase/prostaglandin E_2 pathway to central sensitization is not supported by a clinical trial in postoperative pain.[196] Despite a critical contribution of prostaglandin E_2 to peripheral sensitization, the involvement of this important inflammatory mediator in regulating central sensitization is not well studied, but see the work of Ahmadi *et al.*[197] In 2008, Kawasaki *et al.*[194] demonstrated a direct induction of central sensitization by the proinflammatory cytokines tumor necrosis factor, interleukin-1β, and interleukin-6. These cytokines elicit very rapid increases (within 1 min) in excitatory synaptic transmission on spinal cord neurons. In support of this direct modulation, tumor necrosis factor, interleukin-1β, and interleukin-6 rapidly modulate the function of neurotransmitter receptors such as AMPA receptor, NMDA receptor, glycine receptor, and GABAR, which results in enhanced excitatory synaptic transmission and suppressed inhibitory synaptic transmission in the spinal pain circuit.[194] In agreement with this finding, intrathecal injection of tumor necrosis factor, interleukin-1β, or interleukin-6 elicits rapid pain hypersensitivity in naive animals.[194] Because these cytokines are elevated and circulating cerebrospinal fluid in chronic pain conditions,[198,199] they are possible mediators of widespread pain, as a result of widespread central sensitization (fig. 4).

Tumor necrosis factor can be produced by microglia, astrocytes, and even dorsal root ganglia primary sensory neurons.[200,201] However, in the spinal cord, tumor necrosis factor is primarily produced by microglia, as indicated by single-cell analysis of microglia, astrocytes, and neurons.[166] Electrophysiologic analysis reveals that tumor necrosis factor increases glutamate release in transient receptor potential ion channel $V1^+$ C-fiber terminals, leading to enhanced excitatory synaptic transmission in lamina IIo excitatory spinal cord dorsal horn interneurons.[202] These lamina IIo interneurons synapse to lamina I projection neurons to form a pain circuit and potentiate pathologic pain.[84] Tumor necrosis factor also increases NMDA currents in IIo excitatory interneurons *via* extracellular signal-regulated kinase activation.[187] Additionally, tumor necrosis factor inhibits spontaneous action potentials in GABAergic neurons in the dorsal horn.[203] Both type I and type II receptors of tumor necrosis factor are involved in behavioral manifestations of central sensitization after intrathecal tumor necrosis factor treatment or during formalin-induced second-phase pain.[204] Notably, caspase-6 triggers tumor necrosis factor release from microglia to elicit central sensitization *via* tumor necrosis factor receptor signaling.[166]

Interleukin-1β is expressed by both microglia and astrocytes in the spinal cord.[110,147,205] Interleukin-18, a highly related family member of interleukin-1β, is induced in microglia by nerve injury and bone cancer.[133,206] Caspase-1 cleaves and activates interleukin-1β and interleukin-18 and acts as a key component of inflammasome, which contributes to the pathogenesis of chronic pain.[207] In addition, matrix metalloproteases 9 and 2 have been implicated in interleukin-1β cleavage and activation and regulation of glial activation in early *versus* late phase of neuropathic pain.[208] Interleukin-1β induces central sensitization *via* both presynaptic modulation (increasing glutamate release)[194] and postsynaptic regulation (phosphorylation of NMDA receptor[209,210] and enhancement of NMDA current[194]). Endogenous interleukin-1β also potentiates presynaptic NMDA receptor function in neuropathic pain.[211] Furthermore, interleukin-1β suppresses inhibitory synaptic transmission and GABA and glycine-induced currents in spinal lamina IIo neurons.[194] Interleukin-18 also causes hyperactivity of spinal wide dynamic range neurons after mechanical stimuli *in vivo*.[133] Thus, cytokines regulate central sensitization through multiple mechanisms that involve presynaptic and postsynaptic modulation as well as excitatory and inhibitory synaptic transmission modulation.

Astrocyte-produced chemokines (CC motif) ligand 2 and chemokine (CXC motif) ligand 1 also play an important role in central sensitization and chronic pain.[139,150,212] Chemokine (CC motif) receptor 2, the major receptor for chemokine (CC motif) ligand 2 is expressed in neurons including dorsal root ganglia and spinal cord dorsal horn neurons.[150,213,214] Bath application of chemokine (CC motif) ligand 2 to spinal cord slices induces rapid extracellular signal-regulated kinase activation and causes extracellular signal-regulated kinase-dependent potentiation of NMDA currents in dorsal horn neurons *via* the activation of chemokine (CC motif) receptor 2 receptors.[150,214] Intrathecal injection of chemokine (CXC motif) ligand 1 induced extracellular signal-regulated kinase and cAMP response element–binding protein activation in spinal neurons *via* the stimulation of CXCR2 receptors,[212] which is epigenetically regulated after injury.[215] Notably, late-phase neuropathic pain and synaptic plasticity (excitatory postsynaptic current increase) in the spinal cord pain circuit, at 3 weeks after nerve injury, is transiently reversed by the CXCR2 antagonist SB225002. These results suggest an active role of chemokine (CXC motif) ligand 1/CXC motif chemokine receptor 2 in the maintenance of central sensitization.[139]

Glial cells also produce growth factors such as brain-derived growth factor and basic fibroblast growth factor to enhance central sensitization and chronic pain.[216,217]

Brain-derived growth factor release from central terminals of primary afferents elicits central sensitization *via* activation of extracellular signal-regulated kinase and potentiation of NMDA receptor.[182,218–220] Brain-derived growth factor signaling in spinal microglia also facilitates central sensitization, neuropathic pain, and morphine tolerance.[216,221] Exposure of spinal lamina I projection neurons to brain-derived growth factor results in a depolarizing shift in the anion reversal potential, causing disinhibition of GABAergic system, a key regulatory mechanism in chronic pain.[216,221] Brain-derived growth factor also modulates excitatory synaptic transmission in spinal cord dorsal horn lamina I neurons *via* activation of protein kinase Fyn.[178]

Long-term Potentiation, Central Sensitization, and Widespread Pain

Spinal cord long-term potentiation is an important form of synaptic plasticity and a unique form of central sensitization in chronic pain.[222,223] Spinal cord long-term potentiation of C-fiber–evoked field potentials is typically induced by high-frequency tetanic stimulation of the sciatic nerve.[224] Spinal cord long-term potentiation is also induced by nerve injury and opioid withdrawal.[225–227] There are striking similarities between spinal cord long-term potentiation and central sensitization, and both show the critical requirements of NMDA receptor and involvement of key signaling transduction pathways including the protein kinase C, extracellular signal-regulated kinase, and Src, as well as dependence of protein synthesis and gene transcription.[38,179,228] However, maintenance of late-phase long-term potentiation (greater than 4 h) may require additional mechanisms.[225] It remains to be determined whether there is persistent spinal long-term potentiation months after painful insults, due to technical difficulty in following long-term potentiation over time.

Several lines of evidence suggest an essential role of neuroinflammation in inducing and sustaining spinal cord long-term potentiation. First, spinal tumor necrosis factor is both sufficient and required for the induction of spinal cord long-term potentiation *via* both tumor necrosis factor receptors type I and type II.[202,229] Caspase-6 also contributes to the induction and maintenance of spinal cord long-term potentiation *via* tumor necrosis factor signaling.[166] Second, interleukin-1β triggers spinal cord long-term potentiation not only in excitatory synapses[230] but also in glycinergic synapses on GABAergic neurons in spinal cord slices,[231] serving as another example of cytokine-induced disinhibition. Third, activation of spinal microglial CX3C chemokine receptor 1 *via* chemokine CX3C ligand 1/fractalkine is sufficient to elicit spinal cord long-term potentiation.[232] Finally, chemokine (CC motif) receptor 2 is required for the maintenance of spinal cord long-term potentiation.[214]

Recently, Kronschläger *et al.*[233] demonstrated a gliogenic spinal cord long-term potentiation that can spread widely in nociceptive pathways. A fundamental feature of long-term potentiation induction in the brain is the requirement for coincident pre- and postsynaptic activity, which is important to restrict long-term potentiation expression to activated synapses only (homosynaptic long-term potentiation) as well as to define the input specificity of long-term potentiation. Gliogenic spinal cord long-term potentiation can travel long distances *via* cerebrospinal fluid, because this long-term potentiation can be induced by glial activation and diffusible messengers, such as D-serine and tumor necrosis factor.[233] Strikingly, transfer of spinal cerebrospinal fluid from a donor animal displaying long-term potentiation is able to induce long-term potentiation in a naïve receiver animal.[233] Therefore, this diffusible spinal cord long-term potentiation affects susceptible synapses at remote sites. Collectively, gliogenic long-term potentiation, as well as other forms of diffusible and glial-mediated central sensitization, may underlie widespread pain in chronic pain patients, especially in those with overlapping chronic pain conditions (fig. 6).

Synaptic plasticity and long-term potentiation have also been investigated in the brain, such as in the anterior cingulate cortex,[234] amygdala,[235] and hippocampus[236] after tissue and nerve injury. Further investigation is warranted to define the role of neuroinflammation and glial activation in these pain-associated synaptic changes in the brain.

Clinical Trials and Translational Gap

The current levels of enthusiasm and interest in the development of new treatments that specifically target neuroinflammation and glial activation may be tempered by the somewhat disappointing results of previous clinical trials. Although animal models, as reviewed in the previous sections, demonstrated promising results in the reduction of neuropathic pain, glial activation, and neuroinflammation when treated by the glial modulator propentofylline,[237,238] the same compound failed to provide beneficial reductions in neuropathic pain when administered to patients suffering from postherpetic neuralgia.[239] Despite its efficacy in reducing postoperative pain in animal models, intrathecal injection of cyclooxygenase inhibitor such as ketorolac did not improve acute or chronic pain after hip arthroplasty.[119,196] Although the clinical trial does not support a central role of cyclooxygenase/prostaglandin E_2 pathway in clinical pain, cytokines and chemokines can independently regulate central sensitization *via* direct actions on nociceptive neurons (fig. 5). Thus, future clinical studies are still needed to test the effects of spinal inhibition of cytokines/chemokines in clinical pain. Neuropathic pain patients demonstrate tolerance to p38 inhibitors including dilmapimod and losmapimod,[240,241] but these drugs have inconsistent levels of efficacy. An exploratory trial of dilmapimod significantly inhibited nerve injury–induced neuropathic pain[240]; however, losmapimod did not demonstrate any significant analgesic effect in comparison to placebo controls.[241] Intriguingly, acute postsurgical dental pain was significantly reduced when treated with another p38 inhibitor, specifically the novel p38α mitogen-activated protein kinase inhibitor SCIO-469.[242]

The use of cytokine inhibitors and glial modulators in clinical trials has shown some encouraging results. Intractable

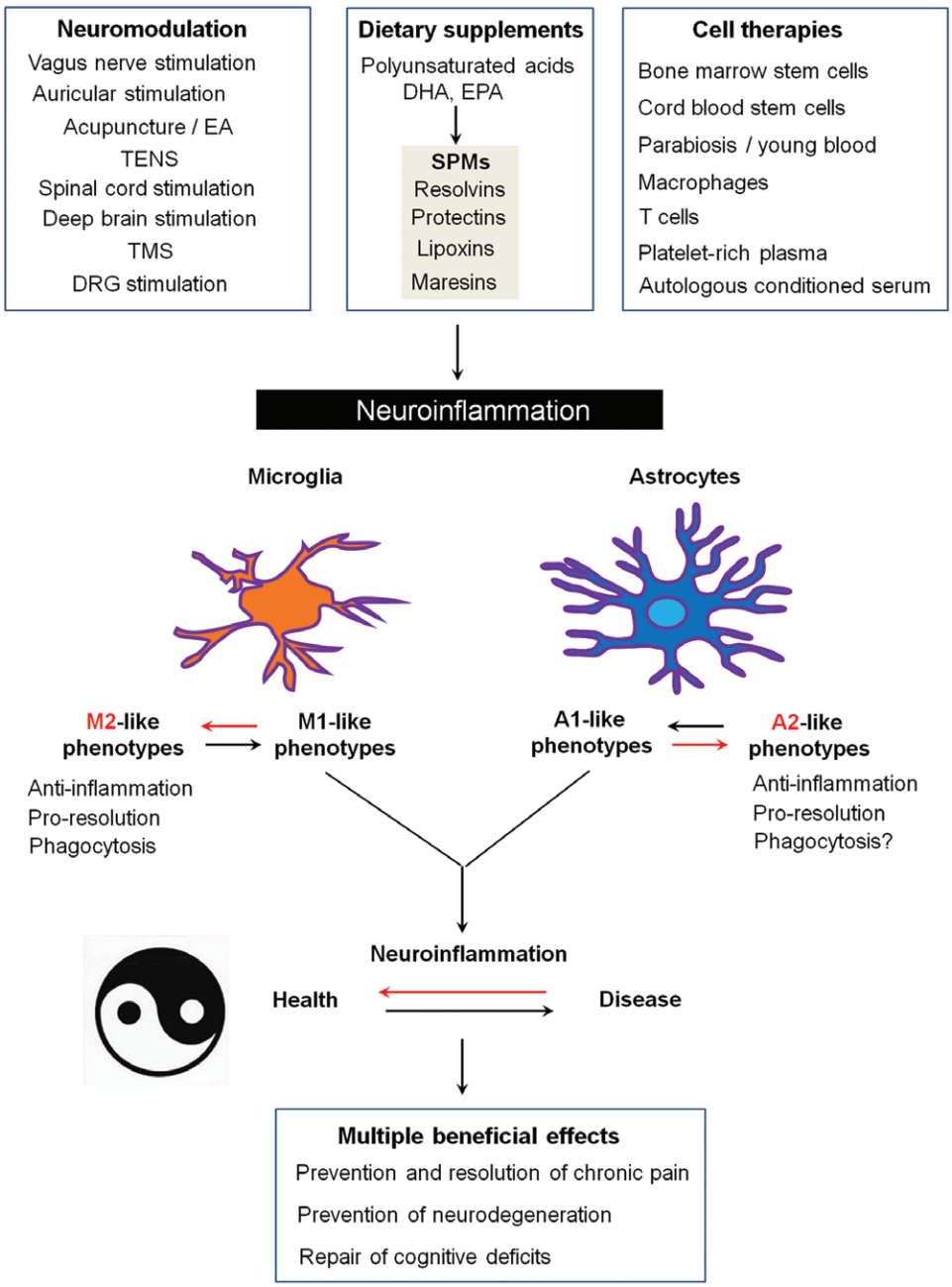

Fig. 6. Nonpharmacologic approaches that can control neuroinflammation and produce multiple beneficial effects including prevention and resolution of chronic pain, prevention of neurodegeneration, and repair of cognitive function deficits. DHA = docosahexaenoic acid; DRG = dorsal root ganglia; EA = electroacupuncture; EPA = eicosapentaenoic acid; SPM = specialized proresolution mediator; TENS = transcutaneous electrical nerve stimulation; TMS = transcranial magnetic stimulation.

discogenic lower back pain patients showed up to 8 weeks of pain relief when given a single intradiscal treatment of entanercept, a tumor necrosis factor inhibitor.[243] Additionally, lumbar disc herniation patients who received entanercept *via* transforaminal epidural injections had up to 26 weeks of pain relief after two injections in a randomized, double-blind, and placebo-controlled trial.[244] Another cytokine inhibitor, the interleukin-1 trap rilonacept, is well tolerated by patients, and in a proof-of-concept study, treatment with the drug showed pain relief for a small group of patients being treated for chronic refractory gouty arthritis.[245] By contrast, subcutaneous inhibition of interleukin-1β with anakinra has no beneficial effect on chronic fatigue syndrome.[246] Microglial activation can be blocked *in vitro* by low doses of naltrexone,[247] and a pilot trial also showed that treatment with the drug can help to reduce symptoms related to fibromyalgia.[248]

Certain limitations and concerns regarding the design of the mentioned trials need to be addressed. The first concern regards the lack of neuroinflammation analysis by biomarkers, because inhibition of *in vivo* glial responses in the propentyofylline

study notably had no biomarker validation.[239] A second concern is the lack of validation regarding whether central sites, including the spinal cord, received exposure to systemically administered drugs. For instance, it was noted by the authors of the losmapimod study that the lack of response may be a result of central sites having a lack of adequate exposure to the drug.[241] In the majority of preclinical studies, inhibitors of glial cells, mitogen-activated protein kinases, cytokines, and chemokines are given *via* intrathecal administration, which ensures direct exposure of central sites to the drug being studied.[108] The third concern is the absence of analysis of sex-dependent effects of treatments in previous studies of p38 and glial inhibitors in light of emerging evidence of sex dimorphism in microglial and p38 signaling and T-cell signaling in inflammatory and neuropathic pain.[163,164] Fourth, the timing of administration appears to be a critical component of a treatment's efficacy. During the acute phase of pain, inhibitors of microglia, p38 mitogen-activated protein kinase, and tumor necrosis factor-α show stronger efficacy, with more partial effects in the chronic phase according to preclinical studies.[108,249] In line with this point, the effective results of the p38 inhibitor SCIO-46 trial were demonstrated in dental patients during the acute phase of postsurgical pain.[242] It should be noted, however, that the intrathecal administration of the interleukin-1 receptor antagonist anakinra at 16 weeks post–bone fracture in rodents can reduce chronic postoperative pain.[58] Finally, the mechanisms of the tested drugs need to be further elucidated. Propentofylline, although a glial modulator, also acts as a phopsphodiesterase inhibitor, as well as an adenosine uptake inhibitor. Thus, the administration of this drug may alter cAMP and adenosine levels in both glial and nonglial cells.[250]

Animal models are of exceeding importance in the understanding of the mechanisms of pain, as well as for testing newly developed therapeutics. However, animal models cannot reproduce all key clinical symptoms of pain, and thus the gap in translation between preclinical studies in rodents and clinical studies in humans is a major topic in pain research discussed in numerous review articles.[251,252] With regard to methods of measurement, in animal studies pain is measured by application of von Frey filaments to cause reflex pain with subsequent quantification of paw withdrawal thresholds, whereas most clinical studies measure pain based on the visual pain scale. Additionally, the time course of pain is very different between animals and humans, with the duration of pain in preclinical studies lasting normally from days to weeks, whereas clinical patients with chronic pain often suffer from their symptoms from months to years. Thus, developing animal models that accurately reflect the various genetic, sex-dependent, psychologic, and environmental factors and sequelae of the development and maintenance of chronic pain proves to pose a continuing challenge. Although clinically effective treatments do show efficacy in animal models thus far, increasing efforts to increase the translatability of preclinical and clinical studies will prove important, for instance measurement of spontaneous pain in animal models and quantitative sensory testing in human patients.[59,253–256]

Alternative Treatments That Can Modulate Neuroinflammation

Although direct targeting of neuroinflammation *via* inhibitors of cytokines, chemokines, and mitogen-activated protein kinases could be effective, these drugs may also produce side effects such as infection after long-term treatment and impair the resolution of inflammation.[39] In this review, we highlight the alternative approaches that can control excessive neuroinflammation, including specialized proresolution mediators, cell therapies, and neuromodulation (fig. 6).

Specialized proresolution mediators, including lipoxins, resolvins, protectins, and maresins, are biosynthesized from polyunsaturated fatty acids, including the omega-3 fatty acids docosahexaenoic acid and eicosapentaenoic acid, two major components of fish oil. Specialized proresolution mediators have multiple beneficial actions for treating inflammation-associated disease conditions.[257,258] Lipoxin A4, resolvin D1, resolvin E1, resolvin D2, neuroprotectin D1, and maresin 1, at very low doses (10 to 500, ng range), reduced inflammatory pain, postoperative pain, and neuropathic pain in animal models.[187,202,258–263] In the hippocampus, treatment with resolvin D1 prevented long-term potentiation impairment by reducing proinflammatory cytokines after surgery.[264] The benefits of these specialized proresolution mediators include high potency, favorable safety profile, and multiple mechanisms of action including but not limited to control of inflammation in peripheral tissues, control of neuroinflammation in the peripheral nervous system and CNS, resolution of synaptic plasticity, modulation of transient receptor potential ion channel A1 and V1 activities, and protection against nerve injury.[39,226,258,264] Spinal administration of neuroprotectin D1, even 2 h after long-term potentiation induction, is also effective in reversing long-term potentiation in the intact spinal cord.[202,261] Specialized proresolution mediators are especially effective in preventing neuroinflammation, postoperative pain, and neuropathic pain after nerve injury and surgery, although posttreatment of specialized proresolution mediators also exhibit transient analgesic effects. Notably, thoracotomies produce a high incidence of chronic postoperative pain by nerve compression.[265] Wang and Strichartz[263] found that intrathecal and perioperative treatment with resolvin D1 and resolvin D2 effectively prevents postoperative pain in a rat model of chronic postthoracotomy.

Cell therapies are emerging treatments for chronic pain and neurodegenerative conditions. Implantation of bone marrow stem cells produces long-term pain relief.[266,267] Bone marrow stromal cells or bone marrow stem cells promote tissue regeneration and tissue repair by secreting growth factors and control inflammation and neuroinflammation by secreting antiinflammatory mediators such as transforming growth factor β1.[266] A single intrathecal injection of bone marrow stem cells not only inhibits nerve injury-induced neuropathic pain for many weeks *via* secretion of transforming growth factor β1 but also blocks nerve injury-induced

neuroinflammation (glial activation and cytokine/chemokine upregulation) in dorsal root ganglia and spinal cord tissues.[266] This paracrine modulation of neuroinflammation by bone marrow stem cells is very different from typical cell replacement strategies, such as implantation of forebrain GABAergic precursor cells into the spinal cord to generate functional GABAergic neurons for chronic pain control.[268] Furthermore, subcutaneous treatment with human umbilical cord blood–derived multipotent stem cells reduced peripheral neuropathic pain in rats and inhibited spinal matrix metalloprotease 9 and 2 expression after nerve injury.[269] Macrophages and T cells also play a role in the resolution of pain. Proresolution macrophages (M2-like) may inhibit neuroinflammation and chronic pain by secreting specialized proresolution mediators and antiinflammatory cytokines such as interleukin-10.[26,270] Adoptive transfer of regulatory T cells (T_{reg}) reduced neuropathic pain, whereas $CD8^+$ cytotoxic T cells increased neuropathic pain after chemotherapy after intrathecal injection.[30] However, adoptive transfer of $CD8^+$ T cells *via* systemic route was also shown to resolve chemotherapy-induced neuropathic pain by increasing interleukin-10 receptor expression in dorsal root ganglia neurons.[271] Autologous conditioned serum is prepared from whole blood that is incubated with glass beads to initiate monocyte activation. Autologous conditioned serum contains increased levels of interleukin-1 receptor antagonist interleukin-1ra as well as the antiinflammatory cytokines interleukin-4 and interleukin-10 and demonstrates efficacy in relieving pain in patients with knee osteoarthritis.[272,273] Platelet-rich plasma was also shown to reduce clinical pain in knee osteoarthritis *via* possible modulation of inflammatory responses.[274]

Neuromodulation *via* electrical and magnetic stimulation, such as spinal cord stimulation, deep brain stimulation, transcranial magnetic stimulation, transcutaneous electrical nerve stimulation, vagus nerve stimulation, auricular stimulation, and dorsal root ganglia stimulation, as well as acupuncture including electroacupuncture, has been used to provide pain relief in patients and animals[275,276] *via* activation of specific neural pathways,[277,278] suppression of nociceptive neuron activities (*e.g.,* wide dynamic neurons and projection neurons in the spinal cord[279,280]), and release of pain suppressing neurotransmitters and neuromodulators.[281] However, transient modulation of neuronal activity in the pain circuits during stimulation cannot explain the long-term benefits of neuromodulation. Increasing evidence suggests that neuromodulation such as vagus nerve stimulation can powerfully regulate inflammation.[282,283] Although acupuncture elicits transient analgesia *via* releasing opioid peptides and adenosine,[281,284] acupuncture and sciatic nerve activation also regulates inflammation and sepsis through vagus nerve activation and dopamine release.[285] In 1984, Maixner and Randich[286] demonstrated an antinociceptive role of vagal stimulation. Notably, acupuncture also causes neuronal activation in the nucleus of solitary tract to mediate vagal responses.[287,288] In a rodent model of inflammatory muscle pain, acupuncture elicits antiinflammatory effects *via* interleukin-10 release.[289] The identification of the "inflammatory reflex" reveals a neural circuit capable of providing information in real time to the brain about the body's inflammatory status, allowing rapid neural regulatory responses *via* the vagus nerve.[290] This has clear implications in developing novel bioelectronic technologies to treat pain and improve postoperative outcomes in vulnerable patients.[283]

Concluding Remarks and Future Directions

Neuroinflammation resulting from neuroglial and neuroimmune interactions not only serves as a driving force for chronic pain but is also implicated in other neurologic and psychiatric diseases such as Alzheimer disease, Parkinson disease, multiple sclerosis, autism, major depression, and schizophrenia.[39] Chronic pain is commonly associated with depression, anxiety, and sleep disorders, and the prevalence of chronic pain is especially high in the rapidly growing populations of aged people and females with overlapping chronic pain conditions. Thus, targeting excessive neuroinflammation will help to alleviate chronic pain and control the progression of neurologic and psychiatric diseases in aged as well as younger populations. Mechanistically, neuroinflammation drives widespread chronic pain *via* central sensitization, which can be induced and maintained by cytokines, chemokines, and other glia-produced mediators circulated in the cerebrospinal fluid. Therefore, increasing the precision with which drugs can target neuroinflammation in the CNS, namely by increasing access to the spinal cord and brain, and developing the ability to accurately measure evolving neuroinflammatory changes in the CNS particularly in the cerebrospinal fluid will be of great importance. By developing specific neuroinflammatory profiles, their creation may also reveal novel biomarkers and means to identify chronic pain states. For instance, a recent study utilizing functional imaging of patients with chronic low-back pain revealed glial activation in the brain.[118] In line with the use of an analgesic marker of glial activation, translocator protein,[291] the development of additional novel radioligands to characterize different activation states of glial cells in the brain, as well as in spinal cord, dorsal root ganglia, and peripheral nerves, is of pressing need. Specialized proresolution mediators including resolvins and protectins, as well as bone marrow stem cells and neuromodulation, can serve as alternative approaches to providing effective control of neuroinflammation with perhaps less side effects than more directly targeted neuroinflammation treatments including cytokine, chemokine, and mitogen-activated protein kinase inhibitors. With the high incidence of postoperative pain after trauma and nerve injury resulting from surgeries[265] and the worsening opioid crisis in the United States,[292] the development of effective nonopioid treatments for the prevention and resolution of neuroinflammation and postoperative pain is of the utmost importance for clinical practice and health care. The benefits of developing novel treatments

may extend further toward improving cognitive function in postoperative patients, a topic that will be addressed by the authors in future reviews.

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Competing Interests

The authors declare no competing interests.

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Streams of Unconsciousness V: Stability Reflected in the Pyriphlegethon

As represented by the Italian poet Dante, the plutonic rivers Lethe, Styx, Acheron, and Cocytus can be interpreted as supplying amnesia, hypnosis, analgesia, and akinesia, respectively—later regarded as properties of general anesthesia. However, of the five great streams of the Greco-Roman underworld, only the molten river Pyriphlegethon (Greek for "fire-flaming") boils the souls of the most violent offenders. Indeed, egregious and impulsive behaviors of tyrants, murderers, and other violent offenders melt away in the lava-like Pyriphlegethon. By flowing over and engulfing the violent, the river stabilizes the previously unstable. Perhaps this stabilizing effect of Pyriphlegethon reflects one final property of general anesthesia, cardiovascular and autonomic stability. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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