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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 (CNS). 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 pro-inflammatory 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 the CNS administration. Sustained increase of cytokines and chemokines in the CNS 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.

### Introduction

Chronic pain is a major health concern that affects one in three Americans and costs the US economy \$635 billion dollars each year<sup>1,2</sup>. Acute pain is often elicited by acute inflammation and has biological significance to protect the wounded tissue. Chronic pain is maladaptive and has no beneficial biological significance and is characterized by spontaneous pain (e.g., burning) as well as evoked pain in response to noxious (hyperalgesia) or non-noxious (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 (DRG) and trigeminal ganglia<sup>3–5</sup>, and central sensitization of pain-processing neurons in the spinal cord and brain<sup>6–9</sup>.

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The perception of pain is typically associated with inflammation, a complex biological 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 A $\delta$ -fibers that terminate in skin, muscle, joint, and visceral organs, with their cell bodies located in DRG and trigeminal ganglia. Nociceptors are activated or sensitized by inflammatory mediators such as bradykinin, prostaglandins, nerve growth factor, as well as pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$  or TNF), interleukin 1 $\beta$  (IL-1 $\beta$ ) and pro-inflammatory chemokines (e.g., CCL2, CXCL5)<sup>3,10,11</sup> that directly bind and stimulate G-protein coupled receptors (GPCRs), ionotropic receptors, and tyrosine kinase receptors. It is noteworthy that all these receptors are expressed on the terminals and/or cell bodies of nociceptors<sup>3,5</sup>. Cytokine profiles observed in human skin are also associated with inflammation and pain and thus may serve as biomarkers for chronic pain<sup>12,13</sup>. 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 (TRPs, such as TRPA1, TRPV1, and TRPV4), sodium channels (e.g., subtypes Nav1.7/1.8/1.9)<sup>3,4,14</sup>, and mechanosensitive piezo ion channels<sup>15,16</sup>. MicroRNA (miRNA) 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 (TLR7) and TRPA1<sup>17</sup>. Furthermore, the activation of protein kinases such as MAP kinases, protein kinase A (PKA), and protein kinase C (PKC) in primary sensory neurons critically contributes to the induction and maintenance of peripheral sensitization. For instance, p38 MAP kinase activation in DRG neurons initiates peripheral sensitization by increasing TRPV1 activity in response to TNF<sup>18</sup> and also by increasing Nav1.8 activity in response to IL-1 $\beta$ <sup>32</sup> and, furthermore, this activation maintains peripheral sensitization and chronic pain by increasing TRPV1 expression<sup>19,20</sup>. In parallel, peripheral TNF and IL-1 $\beta$  have been strongly implicated in the pathogenesis of inflammatory and neuropathic pain<sup>21–25</sup>.

Of note nociceptors and immune cells have bidirectional interactions<sup>26</sup>. 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<sup>27,28</sup>. Like immune cells, nociceptors express cytokines, chemokines, and TLRs that are essential for immune modulation<sup>29–32</sup>. 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 DRG. For example, nociceptor-produced chemokine CCL2 regulates local macrophage activation in DRG after chemotherapy via TLR signaling, resulting in neuropathic pain<sup>32,33</sup>. Activation of nociceptors, especially C-fibers also produces neurogenic inflammation via releasing neuropeptides such as substance P and calcitonin gene related peptide (CGRP) or prostanoids<sup>27</sup> (Table-1). Neurogenic inflammation occurs immediately following intradermal administration of capsaicin and mustard oil via respective activation of TRPA1 and TRPV1. Neurogenic inflammation results in rapid plasma extravasation and edema,

even prior to 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 (CRPS) following bone fracture<sup>34</sup>. 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 CGRP<sup>35</sup>.

Peripheral inflammation with resulting persistent nociceptive input also leads to the increased release of neurotransmitters (glutamate, substance P, CGRP, BDNF) 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<sup>36,37</sup>. Activation of postsynaptic glutamate NMDA receptors and plasma cell membrane surface insertion of AMPA receptors are essential steps for the induction and maintenance of central sensitization<sup>6,38</sup>.

## 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 years, jumping from 12 in 2008 to 150 in 2016. There is also a similar increase in the number of “microglia and pain” publications in last 10 years (Fig. 1). Neuroinflammation is a localized form of inflammation that occurs in the peripheral nervous system (PNS, 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). While the CNS is normally protected by the blood-brain-barrier (BBB), increased permeability of the BBB 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 neurological and neuropsychiatric diseases such as Alzheimer’s disease, Parkinson disease, multiple sclerosis, depression, bipolar disorder, and autism, as well as postoperative complications like neurocognitive disorders (e.g., delirium)<sup>39–41</sup>.

It is noteworthy that neuroinflammation is associated with various painful insults and pathologies (Fig. 2)<sup>39</sup>, including but not limited to trauma such as traumatic brain injury (TBI), stroke, spinal cord injury (SCI), major surgeries including both cardiac and non-cardiac 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 PNS and CNS<sup>26,42</sup>. Furthermore, painful neuroinflammation (e.g., glial activation) can be induced by chemotherapy drugs such as paclitaxel, anti-HIV treatment, and chronic opioid

treatment<sup>43–46</sup>. Immune therapies are increasingly appreciated for treating cancers by boosting the activities of immune cells such as T cells and other immune cells<sup>47,48</sup>. These therapies also change pain sensitivity by modulating inflammation and neuroinflammation. Deficiency of the negative immune regulator B7-H1, also called program death ligand 1 (PD-L1), enhances inflammation and neuropathic pain after chronic constriction injury of mouse sciatic nerve<sup>49</sup>. Notably, PD-1, the receptor for PD-L1, is not only present in immune cells but also expressed in nociceptor neurons of mouse and human DRG. Furthermore, PD-L1 can potently suppress nociceptor activities via regulating sodium and potassium ion channels in mouse and human nociceptors<sup>50</sup>. 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 may also be triggered by the dorsal root reflex in the spinal cord following 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<sup>51,52</sup>. Furthermore, neuroinflammation in the spinal cord and brain can also be neurogenic following neuronal activation in the CNS<sup>53</sup>.

While it is widely believed that chronic pain persists after the observable signs and symptoms of inflammation have resolved<sup>54</sup>, 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 neurocircuit, mediators of neuroinflammation are highly effective in modulating pain sensitivity. Especially, inflammation and neuroinflammation are differentially correlated with chronic pain. For example, HIV-infected patients with neuropathic pain show permanent neuroinflammation in the spinal cord<sup>55</sup>. Patients with fibromyalgia, a chronic widespread musculoskeletal pain condition<sup>56</sup>, also exhibit small fiber neuropathy together with chronic neuroninflammation<sup>57</sup>. 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 Clark and co-workers has shown a distinct role of inflammation and central neuroinflammation in acute versus chronic phase of pain behavior in a rat model of complex regional pain syndrome (CRPS)<sup>58</sup>. While peripheral IL-1 $\beta$  levels only increased at 4 weeks, spinal levels of IL-1 $\beta$  increased at both 4 weeks (acute phase) and 16 weeks (chronic phase). Importantly, systemic administration of anakinra, a peripherally restricted IL-1 receptor antagonist, only inhibited nociceptive behaviors at 4 weeks but not 16 weeks, but intrathecal injection of anakinra reduced nociceptive behaviors at both 4 and 16 weeks<sup>58</sup>. This study supports a critical role of central neuroinflammation in maintaining chronic pain in a rodent model of CRPS.

## **Neuroinflammation in chronic overlapping pain conditions and widespread pain**

Emerging evidence suggests that neuroinflammation contributes to the pathophysiology of co-prevalent or co-existing chronic pain conditions which are referred to as chronic

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overlapping pain conditions (COPCs) which include but are not limited to fibromyalgia, headache, temporomandibular disorder, back pain, irritable bowel syndrome, primary headaches, pelvic pain, and vestibulodynia. COPCs are characterized by symptoms consistent with the dysregulation of sensory, inflammatory, and psychological domains<sup>13,56,59,60</sup>. There is increased evidence that patients with COPCs exhibit increased basal and stress-induced levels of catecholamines (epinephrine and norepinephrine) in circulation<sup>61–64</sup> and reduced activity of catechol-O-methyltransferase (COMT)<sup>65,66</sup>, a ubiquitously expressed enzyme that metabolizes catecholamines<sup>67</sup>. COPC patients have functional variants in the *COMT* gene that result in reduced COMT activity<sup>68–72</sup>. The ‘low COMT activity’ variants are associated with increased fibromyalgia<sup>73–77</sup> and temporomandibular disorder<sup>78</sup> onset and increased pain in response to experimental stimuli<sup>78,79</sup> and stressful events<sup>71,80,81</sup>. Consistent with clinical syndromes, in rodents sustained delivery of the COMT inhibitor OR486 results in pain at multiple body sites that persists for weeks and altered pain- and anxiety-related volitional behaviors<sup>68,82–84</sup>. Persistent COMT-dependent pain is initiated by peripheral  $\beta_2$ - and  $\beta_3$ -adrenergic receptors ( $\beta_2$ - and  $\beta_3$ ARs) through release of nitric oxide, TNF $\alpha$ , IL-1 $\beta$ , IL-6, and CCL2 in plasma and maintained by increased TNF in central tissues<sup>68,84–86</sup>. Higher levels of nitric oxide derivatives (e.g. nitrite and nitrate) and pro-inflammatory cytokines have been found in patients with chronic musculoskeletal pain conditions<sup>13,87–91</sup>. Of note, patients with site-specific pain conditions (e.g., localized temporomandibular disorder or localized vestibulodynia) exhibit a balance in pro- and anti-inflammatory cytokines, whereas those with COPCs fail to exhibit a compensatory increase in anti-inflammatory cytokines. Compared to patients with localized pain, those with COPCs also exhibit dysregulation in microRNAs (e.g., miR-let-7f) that augment immune response and pro-inflammatory cytokine production<sup>60</sup>. 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 COPCs may be attributed to prior injury or stressful events that produce long-lasting changes in nociceptor function, a phenomenon known as hyperalgesic priming. Pre-clinical studies have demonstrated that acute painful inflammation or nerve injury as well as non-painful stress ‘primes’ nociceptors so that they respond to a subsequent insult (e.g., PGE<sub>2</sub> or epinephrine) for a prolonged duration due to activation of distinct kinase and gene transcription pathways<sup>4,92–95</sup>. In line with these findings, individuals with the ‘low activity’ COMT genotype report enhanced and prolonged pain after motor vehicle collisions or psychological strain<sup>71,80,96</sup>, which are events that stimulate the sympathetic release of epinephrine known to sensitize nociceptors<sup>97,98</sup> and promote inflammation<sup>99–107</sup>. 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 (LPS) 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<sup>108</sup>.

## 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 DRG and trigeminal ganglia, and central glia including microglia, astrocytes, and oligodendrocytes in the spinal cord and brain<sup>109,110</sup>. 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, and “glial activation” is emerging as a powerful mechanism underlying pathogenesis of chronic pain<sup>111–117</sup>, and chronic pain may also manifest as a “gliopathy”<sup>110</sup>.

After painful injuries, there are different activation states of microglia and astrocytes. The morphological activation (glial reaction or gliosis) is the most studied activation state, although this type of glial activation may not directly cause pain<sup>110</sup>. Glial reaction is characterized by increased expression of microglial markers such as CD11b, IBA1, and CX3CR1 and astroglial markers GFAP (glial fibrillary acid protein), as well as morphological 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)<sup>116,118</sup>. Subcutaneous formalin injection into a hind paw of rat or mouse is probably the most used animal model of inflammatory pain, which lasts for less than one hour. Of interest, in 1999 Maixner and coworkers showed that subcutaneous formalin also caused a robust microglial reaction (labeled with CD11b) in the spinal cord days after the injection. This microglial reaction from formalin-induced nerve injury is associated with the development of mechanical allodynia<sup>119</sup>. 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<sup>120</sup>. In 2003, Eisenach and coworkers showed that incision and nerve injury causes upregulation of cyclooxygenase-1 (COX-1) in spinal glial cells, which is important for the development of postoperative pain and neuropathic pain<sup>121,122</sup>.

### Signaling mechanisms in glial regulation of allodynia and hyperalgesia

Several neuromodulators such as ATP, chemokines (CX3CL1, CCL2, CXCL13), and neuropeptides (SP and CGRP), as well as colony-stimulating factor 1 (CSF1) are involved in glial activation after painful insults<sup>110,123,124</sup> (Table-2). The up-regulations 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<sup>125,126</sup>. Peripheral nerve injury increases the expression of ATP P2Y receptors (P2X4, P2X7, and P2Y12) in spinal microglia, and each up-regulation was implicated in neuropathic pain sensitization (mechanical allodynia)<sup>127,128</sup>. 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<sup>129</sup>. Glucocorticoids induce ATP release from spinal astrocytes, leading to microglial activation and diurnal exacerbation of allodynia<sup>130</sup>. Vesicular nucleotide transporter regulates ATP release from spinal cord neurons after nerve injury<sup>131</sup>. Microglial pannexin-1 channel was also shown to facilitate ATP release in neuropathic pain<sup>132</sup>. Nerve injury results in cleavage and activation of chemokine CX3CL1/fractalkine by protease cathepsin S, leading to microglia activation through stimulation of CX3CR1 receptor<sup>115</sup>. CX3CR1, one of best-known markers of microglia, is strongly up-regulated after nerve injury, as revealed in *Cx3cr1*-GFP mice (Fig. 3A,B). This microglial receptor is also critical for the development of neuropathic pain symptoms, since mechanical allodynia after nerve injury is reduced after spinal administration of the CX3CR1 neutralizing antibody and abrogated in *Cx3cr1* knockout mice<sup>115,133,134</sup>. Following chronic constriction injury (CCI) of the sciatic nerve, CX3CR1 up-regulation peaks within 10 days. In the late phase of mechanical allodynia (>3 weeks), microglial CX3CR1 expression markedly declines (Fig. 3A,B). Microglia may play a role in maintaining persistent hyperalgesia and allodynia after bone cancer<sup>135</sup>. Orthopedic surgery such as bone fracture also results in nerve injury and microglial activation in the spinal cord. It was proposed that spinal microglial activation also contribute to postoperative cognitive dysfunction such as delirium<sup>136</sup>. Substance P signaling from C-fiber afferent terminals in the spinal cord results in microglia activation and central sensitization after bone fracture<sup>137</sup>.

After painful insults, gliopathy is also characterized by dysfunction of astrocytes, such as downregulations of the glutamate transporters (GLT-1 and GLAST) in spinal cord astrocytes, resulting in glutamate accumulation in synaptic clefts that causes neuronal hyperactivity<sup>110,138</sup>. Connexin-43 (Cx43) is a critical astrocytic signaling molecule that controls the release of astroglial mediators including glutamate and ATP<sup>139</sup>. Of note, Cx43 upregulation in spinal cord astrocytes is sustained after spinal cord injury and nerve injury (Fig. 3F) and contributory to the development and maintenance of mechanical allodynia<sup>140,141</sup>. Additionally, chronic pain-associated gliopathy could manifest as a functional switch of Cx43 from gap junction communication to hemichannel regulation, so that astrocytes become “leaky” during this switch, resulting in increased secretion of cytokines (IL-1 $\beta$ ) and chemokines (CCL2, CXCL1)<sup>141,142</sup>. Chemokines regulate bidirectional interactions of neurons and glial cells<sup>143</sup>. 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., CXCL13) derived from neurons<sup>124</sup>. Astrocytes also release thrombospondin 4 (TSP4) to modulate synapse formation, synaptic plasticity, and behavioral hypersensitivity<sup>144</sup>(Table-2).

A critical step of glial activation in persistent pain is the activation of intracellular signaling pathways especially the mitogen-activated protein kinase (MAPK) pathways. There are three major members in the MAPK family: ERK (extracellular signal-regulated kinase 1 and 2), p38, and JNK (c-Jun N-terminal kinase) is a<sup>145</sup>. Phosphorylation of p38 (P-p38) in spinal microglia occurs in different pain conditions after surgery, nerve injury (Fig. 3C), and opioid tolerance and results in increased synthesis and release of microglial mediators (TNF, IL-1 $\beta$ , and BDNF) and pain hypersensitivity<sup>134,135,146–149</sup>. Inflammation or nerve injury also activates JNK in astrocytes<sup>150,151</sup>, leading to increased secretion of CCL2 and CXCL1 and enhanced pain states<sup>141,152</sup>. Nerve injury also causes sequential activation of ERK in

microglia (early phase) and astrocytes (late phase)<sup>145</sup>, 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<sup>2+</sup> transients and secretion of thrombospondin 1 (TSP1) from astrocytes, leading to new synapse formation and mechanical allodynia<sup>153</sup>. Toll-like receptor 4 (TLR4) plays a critical role in glial activation and neuroinflammation in the spinal cord, as well as hyperalgesia and allodynia<sup>154,155</sup>. TLR4 also contributes to neuroinflammation in the prefrontal cortex and visceral pain after chronic stress. Increased expression of TLR4 is associated enhanced glia activation in the prefrontal cortex and increased levels of proinflammatory cytokines. Administration of a TLR4 specific antagonist TAK-242 in the prefrontal cortex is sufficient to attenuate visceral hypersensitivity<sup>156</sup>. Peripheral nerve injury causes microglia activation within the mesolimbic reward circuitry, leading to a disruption of dopaminergic signaling and reward behavior<sup>157</sup>. 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<sup>153</sup>. 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<sup>158,159</sup>. Future studies are warranted to investigate how microglia and astrocytes regulate different forms of synaptic plasticity in different brain regions following 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 TMD occurs more frequently in women<sup>160,161</sup>. In 1993 Maixner and coworker reported gender differences in pain and cardiovascular responses to forearm ischemia in humans<sup>162</sup>. Paradoxically, the majority, if not all, pain-related studies were conducted in male animals<sup>163</sup>. However, it was fortunate to test males because spinal microglia play little or no role in regulating inflammatory and neuropathic pain primarily in female rodents<sup>164–167</sup> (Fig 4). Mogil and coworkers demonstrated that spinal TLR4, an important receptor for microglia activation, regulates hyperalgesia and allodynia resulting from inflammation or nerve injury exclusively in male mice<sup>164</sup>. Of note, morphological 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<sup>165,166</sup>. However, mechanical allodynia after nerve injury was exclusively attenuated in male mice after intrathecal injection of microglial inhibitor (minocycline), microglial toxin, or P2X4 blocker, or following special deletion of *Bdnf* in microglia<sup>165</sup>. Furthermore, nerve injury activates p38 in spinal microglia of male but not female mice; and in agreement, spinal administration of p38 inhibitor reduces neuropathic pain only in male mice<sup>166</sup>. Caspase-6 is a microglial activator and released from axonal terminal in the spinal cord following tissue and nerve injury, which can act on

microglia to release TNF<sup>168</sup>. Sex dimorphism was also revealed in caspase-6-mediated microglial signaling, and caspase-6 regulates neuropathic pain exclusively in males<sup>167</sup>. It appears some of male-specific microglial responses require testosterone, as 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<sup>165</sup>.

## 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<sup>169,170</sup>. In this review, we highlight the role of glial cells and neuroinflammation in promoting central sensitization and widespread chronic pain. In 1983 Woolf presented evidence for a central component of post-injury pain hypersensitivity<sup>36</sup>. The International Association for the Study of Pain (IASP) describes central sensitization as increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input<sup>171</sup>. Central sensitization may also include conditions like increased central responsiveness due to dysfunction of endogenous pain control systems, regardless if there is functional change of peripheral neurons. In 2003, Ji and Woolf defined central sensitization as the increased synaptic efficacy established in somatosensory neurons in the dorsal horn of the spinal cord following intense peripheral noxious stimuli, tissue injury, or nerve damage. This heightened synaptic transmission results in reduction in pain threshold, an amplification of pain responses and a spread of pain sensitivity to non-injured areas<sup>6</sup>. In 2009, Latremoliere and Woolf 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 is 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<sup>38</sup>.

### Mechanisms of central sensitization

As shown in Fig. 5, activation of NMDA receptor (NMDAR) is an essential step in initiating and maintaining the central sensitization and pain hypersensitivity after tissue and nerve injury<sup>172,173</sup>. Glutamate is a primary excitatory neurotransmitter in the pain pathway. Under normal circumstances NMDAR channel is blocked by Mg<sup>2+</sup> ions, but this blockade is removed by membrane de-polarization following activation of nociceptive primary afferents. Activation of NMDAR boosts synaptic efficacy and causes Ca<sup>2+</sup> influx, which can activate intracellular signaling pathways that initiate and maintain central sensitization<sup>6,38</sup>. In particular, tissue and nerve injury increases the expression of the NMDAR-NR2B (GluN2B) subunit, which regulates spinal synaptic plasticity in persistent pain conditions together with

NR1 subunit<sup>174</sup>. Interestingly, NR2B/GluN2B receptor activity and surface expression in spinal cord dorsal horn (SCDH) neurons is negatively regulated by  $\beta$ -arrestin 2<sup>175</sup>, a scaffold protein that was traditionally known as an inhibitor of GPCRs. Deficiency of  $\beta$ -arrestin 2 results in enhanced acute opioid analgesia, produced by morphine and DAMGO, a selective  $\mu$  opioid receptor agonist<sup>175,176</sup>. Paradoxically, DAMGO-induced hyperalgesia is also potentiated after  $\beta$ -arrestin 2 deficiency, as a result of enhanced surface and synapse expression of GluN2B that results in hyperactivity of the receptor. Loss of  $\beta$ -arrestin 2 also leads to a prolongation of inflammatory and neuropathic pain, as these pain conditions critically depend on GluN2B<sup>175</sup>. It appears that for the resolution of persistent pain, it is more important for  $\beta$ -arrestin 2 to regulate NMDA receptors via ERK signaling pathway (Fig. 5). Furthermore, surface trafficking of AMPA receptor (AMPA), especially calcium-permeable subunit (GluR1/GluR-A), play a critical role in spinal cord synaptic plasticity and pain hypersensitivity after tissue injury<sup>177,178</sup>. The scaffold protein Homer1a operates in a negative feedback loop to regulate the calcium signaling and excitability of the spinal cord pain pathway in an activity-dependent manner<sup>179</sup>. Given a critical role of AMPAR in direct control of excitatory synaptic transmission in the pain circuits, NMDAR-independent central sensitization may also exist.

Activation of intracellular pathways by protein kinases, such as protein kinase A (PKA), protein kinase C (PKC),  $\text{Ca}^{2+}$ /calmodulin-dependent kinase-II (CaMKII), Src (a tyrosine kinase encoded by sarcoma oncogene), and extracellular signal-regulated kinases (ERK, including ERK1 and ERK2) are important for the generation of central sensitization<sup>6,37,180</sup>. Notably, activation of ERK via phosphorylation of ERK (pERK) in spinal cord dorsal horn (SCDH) neurons is nociceptive-specific and serves as a marker of central sensitization<sup>181-183</sup>. pERK is a common pathway following the activation of various ionotropic and metabotropic receptors and protein kinases (PKA, PKC, and Src) and also a downstream event of  $\text{Ca}^{2+}$  signaling<sup>184-186</sup>. pERK induces central sensitization via rapid post-translational regulation, such as suppression of potassium channel Kv4.2 activity, leading to hyperactivity of SCDH<sup>187,188</sup>. pERK also contributes to rapid upregulation of NMDAR (GluN2B) function in SCDH neurons in response to inflammatory mediators<sup>152,189</sup>. Furthermore, translocation of pERK to the nuclei of SCDH neurons activates the transcription factor cAMP response element binding protein (CREB), leading to increased expression of pronociceptive genes encoding for c-Fos, NK-1, and prodynorphin<sup>184,190</sup>.

### Does central sensitization require peripheral input?

Historically, it has been believed that the central sensitization to noxious stimuli requires a 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<sup>38</sup>. 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 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 NMDAR<sup>191</sup>. Dis-inhibition of GABAergic and glycinergic synaptic transmission in the spinal cord pain circuitry is critical to the generation of chronic pain<sup>86–89</sup>. Gate control theory describes a tonic inhibition in the spinal cord pain circuit via inhibitory neurons<sup>192</sup>. 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<sup>86</sup>.

It is noteworthy that some central etiologies/injuries such as spinal cord injury, traumatic brain injury, multiple sclerosis may cause neuroinflammation, central sensitization and chronic pain without a peripheral insult<sup>193</sup> (Fig. 2). 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<sup>194</sup>, 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 DRGs. Thus, there could be bi-directional 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<sup>152,195–197</sup>. In 2001 Samad et al. showed that spinal IL-1 $\beta$  contributes to central sensitization and inflammatory pain hypersensitivity via transcriptional regulation that causes up-regulations of cyclooxygenase-2 (COX-2) and prostaglandin E2 (PGE2)<sup>195</sup>. Notably, unilateral inflammation in the hindpaw of rats caused widespread and bilateral increase of COX-2 in the spinal cord and brain, due to possible increase of IL-1 $\beta$  level in the CSF<sup>195</sup>. This may partially explain widespread pain in some chronic pain conditions (Fig. 4). However, contribution of the spinal COX/PGE2 pathway to central sensitization is not supported by a clinical trial in post-operative pain<sup>198</sup>. Despite a critical contribution of PGE2 to peripheral sensitization, the involvement of this important inflammatory mediator in regulating central sensitization is not well studied but see<sup>199</sup>. In 2008, Kawasaki et al. demonstrated a direct induction of central sensitization by the pro-inflammatory cytokines TNF, IL-1 $\beta$ , and IL-6. These cytokines elicit very rapid increases (within 1 minute) in excitatory synaptic transmission on spinal cord neurons<sup>196</sup>. In support of this direct modulation, TNF, IL-1 $\beta$ , and IL-6 rapidly modulate the function of neurotransmitter receptors such as AMPAR, NMDAR, GlyR, and GABAR, which results in enhanced excitatory synaptic transmission and suppressed inhibitory synaptic transmission in the spinal pain circuit<sup>196</sup>. In agreement with this finding, intrathecal injection of TNF, IL-1 $\beta$ , or IL-6 elicits rapid pain hypersensitivity in naive animals<sup>196</sup>. Because these cytokines are elevated and circulating in

the CSF in chronic pain conditions<sup>200,201</sup>, they are possible mediators of widespread pain, as a result of widespread central sensitization (Fig. 4).

TNF can be produced by microglia, astrocytes, and even DRG primary sensory neurons<sup>202,203</sup>. However, in the spinal cord TNF is primarily produced by microglia, as indicated by single-cell analysis in microglia, astrocytes, and neurons<sup>168</sup>. Electrophysiological analysis reveals that TNF increases glutamate release in TRPV1<sup>+</sup> C-fiber terminals, leading to enhanced excitatory synaptic transmission in lamina IIo excitatory SCDH interneurons<sup>204</sup>. These lamina IIo interneurons synapse to lamina I projection neurons to form a pain circuit and potentiate pathological pain<sup>85, 86</sup>. TNF also increases NMDA currents in IIo excitatory interneurons via ERK activation<sup>189</sup>. Additionally, TNF inhibits spontaneous action potentials in GABAergic neurons in the dorsal horn<sup>205</sup>. Both type I and type II receptor of TNF (TNFR1 and TNFR2) are involved in behavioral manifestations of central sensitization following intrathecal TNF treatment or during formalin-induced 2<sup>nd</sup> phase pain<sup>206</sup>. Notably, caspase-6 triggers TNF release from microglia to elicit central sensitization via TNF receptor signaling<sup>168</sup>.

IL-1 $\beta$  is expressed by both microglia and astrocytes in the spinal cord<sup>112,149,207</sup>. IL-18, a highly-related family member of IL-1 $\beta$ , is induced in microglia by nerve injury and bone cancer<sup>135,208</sup>. Caspase-1 cleaves and activates IL-1 $\beta$  and IL-18 and acts as a key component of inflammasome, which contributes to the pathogenesis of chronic pain<sup>209</sup>. In addition, matrix metalloproteases MMP-9 and MMP-2 have been implicated in IL-1 $\beta$  cleavage and activation and regulation of glial activation in early vs. late-phase of neuropathic pain<sup>210</sup>. IL-1 $\beta$  induces central sensitization via both presynaptic modulation (increasing glutamate release)<sup>196</sup> and post-synaptic regulation (phosphorylation of NMDAR<sup>211,212</sup> and enhancement of NMDA current<sup>196</sup>). Endogenous IL-1 $\beta$  also potentiates presynaptic NMDAR function in neuropathic pain<sup>213</sup>. Furthermore, IL-1 $\beta$  suppresses inhibitory synaptic transmission (IPSCs) and GABA and glycine-induced currents in spinal lamina IIo neurons<sup>196</sup>. IL-18 also causes hyperactivity of spinal wide dynamic range (WDR) neurons following mechanical stimuli in vivo<sup>135</sup>. Thus, cytokines regulate central sensitization through multiple mechanisms that involve presynaptic and post-synaptic modulation as well as excitatory and inhibitory synaptic transmission modulation.

Astrocyte-produced chemokines CCL2 and CXCL1 also play an important role in central sensitization and chronic pain<sup>141,152,214</sup>. CCR2, the major receptor for CCL2, is expressed in neurons including DRG and SCDH neurons<sup>152,215,216</sup>. Bath application of CCL2 to spinal cord slices induces rapid ERK activation and causes ERK-dependent potentiation of NMDA currents in dorsal horn neurons via the activation of CCR2 receptors<sup>152,216</sup>. Intrathecal injection of CXCL1 induced ERK and CREB activation in spinal neurons via the stimulation of CXCR2 receptors<sup>214</sup>, which is epigenetically regulated after injury<sup>217</sup>. Notably, late-phase neuropathic pain and synaptic plasticity (EPSC increase) in the spinal cord pain circuit, 3 weeks after nerve injury, is transiently reversed by the CXCR2 antagonist SB225002. These results suggest an active role of CXCL1/CXCR2 in the maintenance of central sensitization<sup>141</sup>.

Glial cells also produce growth factors such as brain-derived growth factor (BDNF) and basic fibroblast growth factor (bFGF, also called FGF-2) to enhance central sensitization and chronic pain<sup>218,219</sup>. BDNF release from central terminals of primary afferents elicits central sensitization via activation of ERK and potentiation of NMDAR<sup>184,220–222</sup>. BDNF signaling in spinal microglia also facilitates central sensitization, neuropathic pain, and morphine tolerance<sup>218,223</sup>. Exposure of spinal lamina I projection neurons to BDNF results in a depolarizing shift in the anion reversal potential, causing disinhibition of GABAergic system, a key regulatory mechanism in chronic pain<sup>218,223</sup>. BDNF also modulates excitatory synaptic transmission in SCDH lamina I neurons via activation of protein kinase Fyn<sup>180</sup>.

### Long-term potentiation, central sensitization, and widespread pain

Spinal cord LTP (sLTP) is an important form of synaptic plasticity and a unique form of central sensitization in chronic pain<sup>224,225</sup>. sLTP of C-fiber-evoked field potentials is typically induced by high-frequency tetanic stimulation of the sciatic nerve<sup>226</sup>. sLTP is also induced by nerve injury and opioid withdrawal<sup>227–229</sup>. There are striking similarities between sLTP and central sensitization, and both show the critical requirements of NMDAR and involvement of key signaling transduction pathways including the PKC, ERK, and Src, as well as dependence of protein synthesis and gene transcription<sup>38,181,230</sup>. However, maintenance of late phase LTP (>4 h) may require additional mechanisms<sup>226</sup>. It remains to be determined if there is persistent spinal LTP months after painful insults, due to technical difficulty to follow LTP over time.

Several lines of evidence suggest an essential role of neuroinflammation in inducing and sustaining sLTP. First, spinal TNF is both sufficient and required for the induction of sLTP via both TNFR1 and TNFR2<sup>204,231</sup>. Caspase-6 also contributes to the induction and maintenance of sLTP via TNF-signaling<sup>168</sup>. Second, IL-1 $\beta$  triggers sLTP not only in excitatory synapses<sup>232</sup> but also in glycinergic synapses on GABAergic neurons in spinal cord slices<sup>233</sup>, serving as another example of cytokine-induced disinhibition. Third, activation of spinal microglial CX3CR1 via CX3CL1/fractalkine is sufficient to elicit sLTP<sup>234</sup>. Finally, the chemokine receptor CCR2 is required for the maintenance of sLTP<sup>216</sup>.

Recently, Sandkuhler and coworkers demonstrated a gliogenic sLTP that can spread widely in nociceptive pathways<sup>235</sup>. A fundamental feature of LTP induction in the brain is the requirement for coincident pre- and postsynaptic activity, which is important to restrict LTP expression to activated synapses only (homosynaptic LTP) as well as to define the input specificity of LTP. Gliogenic sLTP can travel long distances via CSF, because this LTP can be induced by glial activation and diffusible messengers, such as d-serine and TNF<sup>235</sup>. Strikingly, transfer of spinal CSF from a donor animal displaying LTP is able to induce LTP in a naïve receiver animal<sup>235</sup>. Therefore, this diffusible sLTP affects susceptible synapses at remote sites. Collectively, gliogenic LTP, 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 LTP have also been investigated in the brain, such as anterior cingulate cortex<sup>236</sup>, amygdala<sup>237</sup>, and hippocampus<sup>238</sup> 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 prior 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<sup>239,240</sup>, the same compound failed to provide beneficial reductions in neuropathic pain when administered to patients suffering from post-herpetic neuralgia<sup>241</sup>. Despite its efficacy in reducing postoperative pain in animal model, intrathecal injection of cyclooxygenase inhibitor such as ketorolac did not improve acute or chronic pain after hip arthroplasty<sup>121,198</sup>. While the clinical trial does not support a central role of COX/PGE<sub>2</sub> 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 diltapimod and losmapimod<sup>242,243</sup>, but these drugs have inconsistent levels of efficacy. An exploratory trial of diltapimod significantly inhibited nerve injury-induced neuropathic pain<sup>242</sup>, however losmapimod did not demonstrate any significant analgesic effect in comparison to placebo controls<sup>243</sup>. Intriguingly, acute postsurgical dental pain was significantly reduced when treated with another p38 inhibitor, specifically the novel p38 $\alpha$  MAPK inhibitor SCIO-469.<sup>244</sup>

The use of cytokine inhibitors and glial modulators in clinical trials has shown some encouraging results. Intractable discogenic lower back pain patients showed up to 8 weeks of pain relief when given a single intradiscal treatment of entanercept, a TNF inhibitor<sup>245</sup>. Additionally, lumbar disc herniation patients who received entanercept via transforaminal epidural injections had up to 26 weeks of pain relief following two injections in a randomized, double-blind, and placebo-controlled trial<sup>246</sup>. Another cytokine inhibitor, the IL-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<sup>247</sup>. By contrast, subcutaneous inhibition of IL-1 $\beta$  with anakinra has no beneficial effect on chronic fatigue syndrome CFS<sup>248</sup>. Microglial activation can be blocked *in vitro* by low doses of naltrexone<sup>249</sup>, and a pilot trial also showed that treatment with the drug can help to reduce symptoms related to fibromyalgia.<sup>250</sup>

Certain limitations and concerns regarding the design of the mentioned trials need to be addressed. The first concern regards lack of neuroinflammation analysis by biomarkers, as inhibition of *in vivo* glial responses in the propentofylline study notably had no biomarker validation<sup>241</sup>. 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<sup>243</sup>. In the majority

of preclinical studies, inhibitors of glial cells, MAP kinases, cytokines, and chemokines are given via intrathecal administration, which ensures direct exposure of central sites to the drug being studied<sup>110</sup>. 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<sup>165,166</sup>. 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 MAP kinase, and TNF- $\alpha$  show stronger efficacy, with more partial effects in the chronic phase according to preclinical studies<sup>110,251</sup>. 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<sup>244</sup>. It should be noted however, that the intrathecal administration of the IL-1 receptor antagonist anakinra at 16 weeks post-bone fracture in rodents can reduce chronic postoperative pain<sup>58</sup>. Finally, the mechanisms of the tested drugs need to be further elucidated. Propentofylline, although a glial modulator, also acts as a phosphodiesterase inhibitor as well as an adenosine uptake inhibitor. Thus, the administration of this drug may alter cAMP and adenosine levels in both glial and non-glial cells<sup>252</sup>.

Animal models are of exceeding importance in the understanding of the mechanisms of pain as well as testing for 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<sup>253,254</sup>. With notion 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 while on the other hand clinical patients with chronic pain suffer from their symptoms often from months to years. Thus, developing animal models that accurately reflect the various genetic, sex-dependent, psychological, 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<sup>59,255–258</sup>.

### **Alternative treatments that can modulate neuroinflammation**

Although direct targeting of neuroinflammation via inhibitors of cytokines, chemokines, and MAP kinases could be effective, these drugs may also produce side effects such as infection after long-term treatment and impair the resolution of inflammation<sup>39</sup>. In this review, we highlight the alternative approaches that can control excessive neuroinflammation, including specialized pro-resolution mediators, cell therapies, and neuromodulation (Fig. 6).

Specialized pro-resolution mediators (SPMs), including lipoxins, resolvins, protectins, and maresins are biosynthesized from polyunsaturated fatty acids, including omega-3 fatty acids

docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), two major components of fish oil. SPMs have multiple beneficial actions for treating inflammation-associated disease conditions<sup>259,260</sup>. Lipoxin A4, resolvin D1, resolvin E1, resolvin D2, neuroprotectin D1, and maresin 1, at very low doses (10–500, ng range), reduced inflammatory pain, postoperative pain, and neuropathic pain in animal models<sup>189,204,260–265</sup>. In the hippocampus, treatment with resolvin D1 prevented LTP impairment by reducing pro-inflammatory cytokines after surgery<sup>266</sup>. The benefits of these SPMs include high potency, wide safety profile, and multiple mechanisms of action including but not limited to control of inflammation in peripheral tissues, control of neuroinflammation in the PNS and CNS, resolution of synaptic plasticity, modulation of TRPA1 and TRPV1 activities, and protection against nerve injury<sup>39,228,260,266</sup>. Spinal administration of neuroprotectin D1, even 2 hours after LTP induction, is also effective in reversing LTP in the intact spinal cord<sup>204,263</sup>. SPMs are especially effective in preventing neuroinflammation, postoperative pain, and neuropathic pain after nerve injury and surgery, although post-treatment of SPMs also exhibit transient analgesic effects. Notably, thoracotomies produce a high incidence of chronic postoperative pain by nerve compression<sup>267</sup>. Strichartz and coworkers found that intrathecal and perioperative treatment with resolvin D1 and resolvin D2 effectively prevents postoperative pain in a rat model of chronic post-thoracotomy<sup>265</sup>.

Cell therapies are emerging treatments for chronic pain and neurodegenerative conditions. Implantation of bone marrow stem cells produces long-term pain relief<sup>268,269</sup>. Bone marrow stromal cells or bone marrow stem cells (BMSCs) promote tissue regeneration and tissue repair by secreting growth factors and control inflammation and neuroinflammation by secreting anti-inflammatory mediators such as transforming growth factor-beta1 (TGF- $\beta$ 1)<sup>268</sup>. A single intrathecal injection of BMSCs not only inhibits nerve injury-induced neuropathic pain for many weeks via secretion of TGF- $\beta$ 1, but also blocks nerve injury-induced neuroinflammation (glial activation and cytokine/chemokine up-regulation) in DRG and spinal cord tissues<sup>268</sup>. This paracrine modulation of neuroinflammation by BMSCs 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<sup>270</sup>. Furthermore, subcutaneous treatment with human umbilical cord blood-derived multipotent stem cells reduced peripheral neuropathic pain in rats and inhibited spinal MMP-9 and MMP-2 expression after nerve injury<sup>271</sup>. Macrophages and T cells also play a role in the resolution of pain. Pro-resolution macrophages (M2-like) may inhibit neuroinflammation and chronic pain by secreting SPMs and anti-inflammatory cytokines such as IL-10<sup>26,272</sup>. Adoptive transfer of regulatory T cells (T<sub>reg</sub>) reduced neuropathic pain, while CD8<sup>+</sup> cytotoxic T cells increased neuropathic pain after chemotherapy following intrathecal injection<sup>30</sup>. However, adoptive transfer of CD8<sup>+</sup> T cells via systemic route was also shown to resolve chemotherapy-induced neuropathic pain by increasing IL-10 receptor expression in DRG neurons<sup>273</sup>. Autologous conditioned serum (ACS) is prepared from whole blood, which is incubated with glass beads to initiate monocyte activation. ACS contains increased levels of IL-1 receptor antagonist IL-1ra as well as the anti-inflammatory cytokines IL-4 and IL-10 and demonstrates efficacy in relieving pain in patients with knee osteoarthritis<sup>274,275</sup>. Platelet-rich plasma (PRP) was

also shown to reduce clinical pain in knee osteoarthritis via possible modulation of inflammatory responses<sup>276</sup>.

Neuromodulation via electrical and magnetic stimulation, such as spinal cord stimulation (SCS), deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), transcutaneous electrical nerve stimulation (TENS), vagus nerve stimulation (VNS), auricular stimulation, and DRG stimulation, as well as acupuncture including electroacupuncture (EA), has been used to provide pain relief in patients and animals<sup>277,278</sup> via activation of specific neural pathways<sup>279,280</sup>, suppression of nociceptive neuron activities (e.g. wide-dynamic neurons and projection neurons in the spinal cord<sup>281,282</sup>), and release of pain suppressing neurotransmitters and neuromodulators<sup>283</sup>. 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<sup>284,285</sup>. While acupuncture elicits transient analgesia via releasing opioid peptides and adenosine<sup>283,286</sup>, acupuncture and sciatic nerve activation also regulates inflammation and sepsis through vagus nerve activation and dopamine release<sup>287</sup>. In 1984, Maixner and Randich demonstrated an antinociceptive role of vagal stimulation<sup>288</sup>. Notably, acupuncture also causes neuronal activation in the nucleus of solitary tract to mediate vagal responses<sup>289,290</sup>. In a rodent model of inflammatory muscle pain, acupuncture elicits anti-inflammatory effects via IL-10 release<sup>291</sup>. 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<sup>292</sup>. This has clear implications in developing novel bioelectronic technologies to treat pain and improve postoperative outcomes in vulnerable patients<sup>285</sup>.

## Concluding remarks and future directions

Neuroinflammation resulting from neuro-glial and neuro-immune interactions not only serves as a driving force for chronic pain, but is also implicated in other neurological and psychiatric diseases such as Alzheimer’s disease, Parkinson disease, multiple sclerosis, autism, major depression, and schizophrenia<sup>39</sup>. 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 neurological 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 CSF. 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 CSF 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<sup>120</sup>. In line with the use of an analgesic marker of glial activation,

translocator protein (TSPO)<sup>293</sup>, the development of additional novel radioligands to characterize different activation states of glial cells in the brain, as well as in spinal cord, DRG, and peripheral nerves is of pressing need. Specialized pro-resolution mediators (SPMs) 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 MAP kinase inhibitors. With the high incidence of postoperative pain following trauma and nerve injury resulting from surgeries<sup>267</sup> and the worsening opioid crisis in the United States<sup>294</sup>, the development of effective non-opioid treatments for the prevention and resolution of neuroinflammation and postoperative pain is of utmost importance for clinical practice and health care. The benefits of developing novel treatments may extend further towards improving cognitive function in postoperative patients, a topic that will be addressed by the authors in future reviews.

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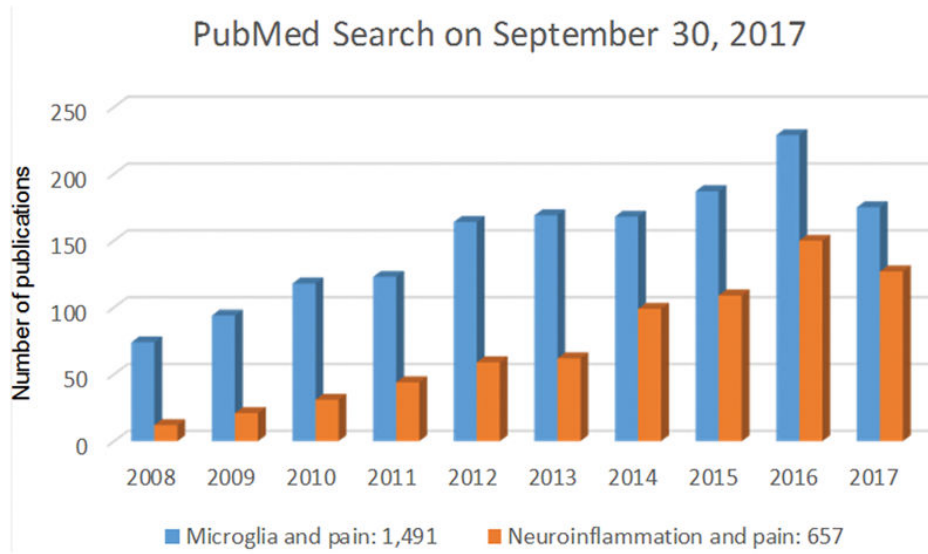
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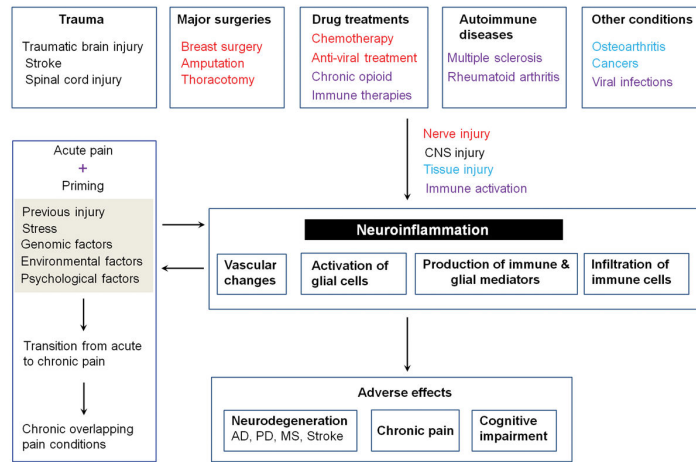
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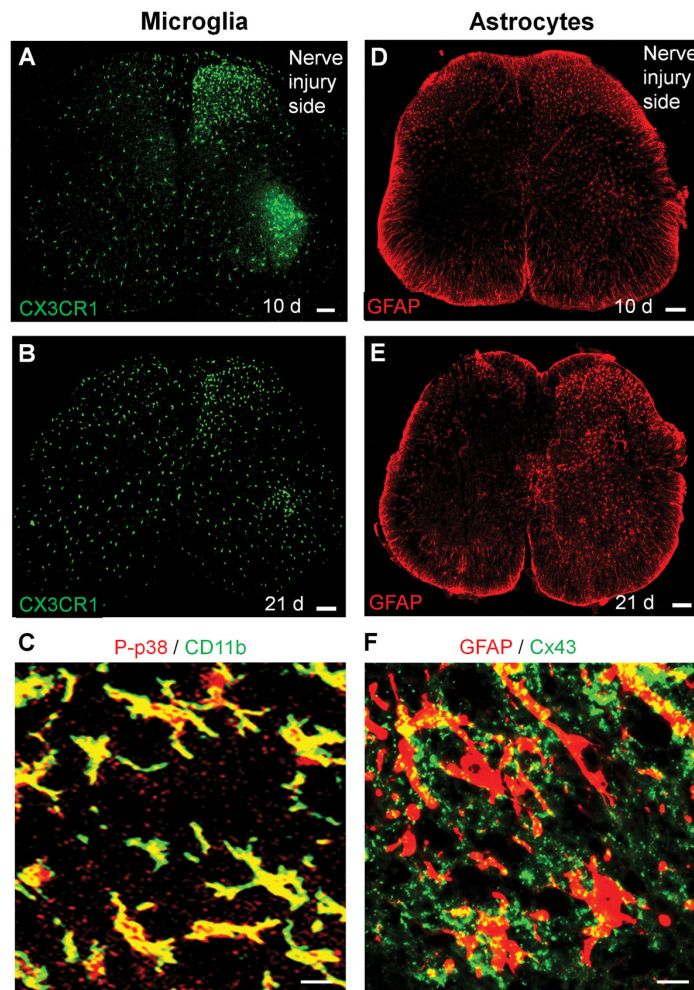
**Figure 1.**

A PubMed Search on September 30, 2017 shows the number of publications on “neuroinflammation and pain” and “microglia and pain” in last 10 years. The numbers on the bottom show all time publications in PubMed.

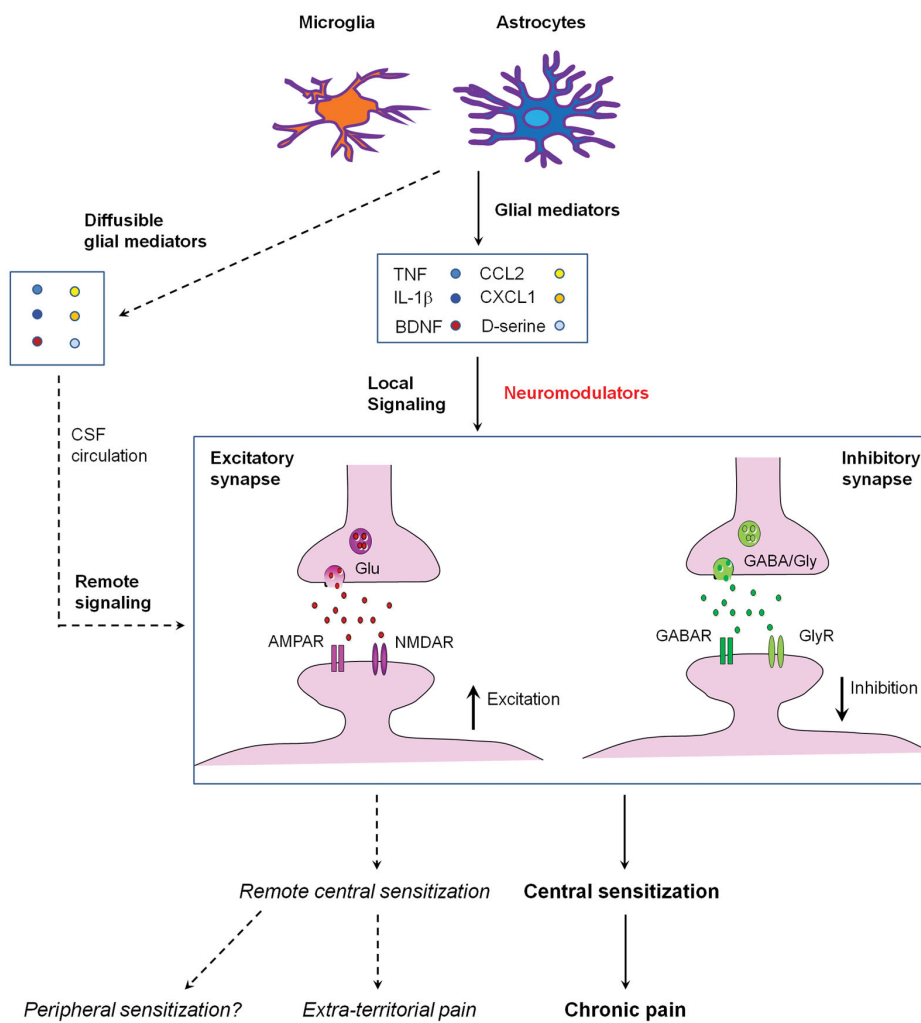


**Figure 2.**

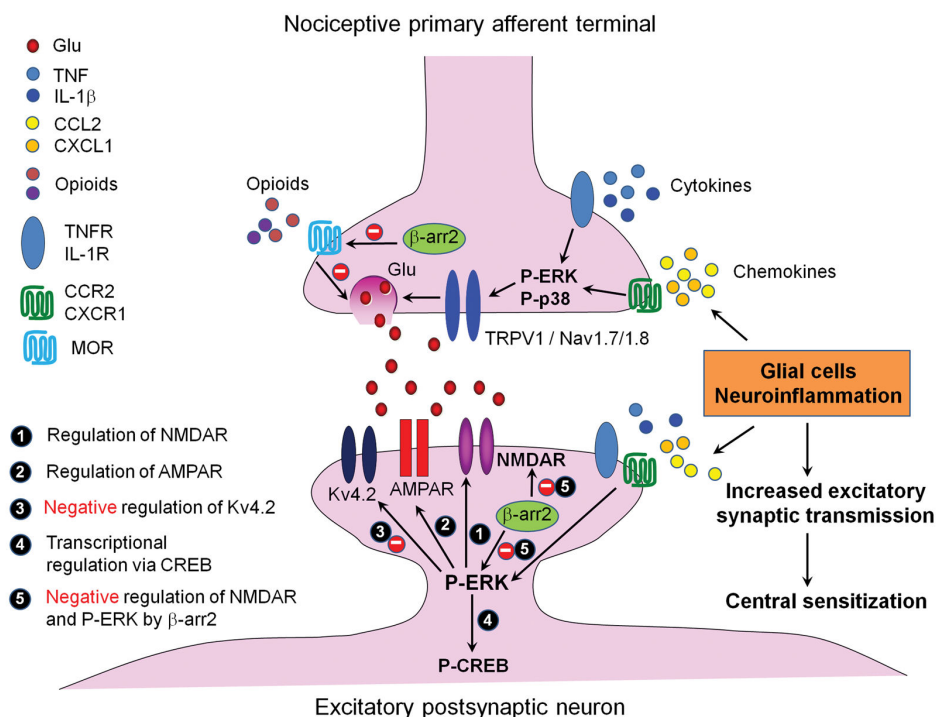
Neuroinflammation is associated with various insults that evoke painful sensations. These insults include but not limited to trauma, major surgeries, drug treatments, autoimmune disease conditions, and other painful insults and tissue damage. Some of these insults such as major surgeries (breast surgery, amputation, thoracotomy), chemotherapy, and anti-viral 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’s disease (AD), Parkinson disease (PD), multiple sclerosis (MS), and stroke. Neuroinflammation is also associated with chronic overlapping pain conditions. After priming of nociceptive circuit by previous injury, stress, or existing genomic, environmental, and psychological factors, acute insult may cause transition from acute pain to chronic pain.



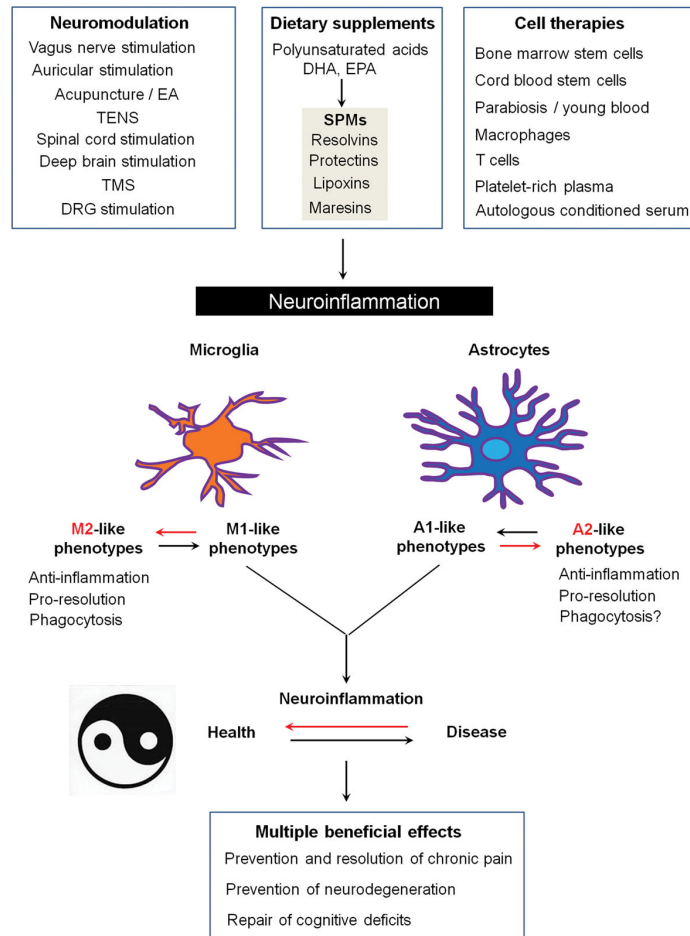
**Figure 3.** Distinct and time-dependent activation of microglia and astrocytes in the spinal cord after nerve injury. (A, B) Microglia activation revealed by increased CX3CR1 expression in the spinal cord 10 days (A) and 21 days (B) after nerve injury in *Cx3cr1*-GFP mice. Scale, 100  $\mu\text{m}$ . (C) Phosphorylation of p38 MAP kinase (P-p38) in CD11b<sup>+</sup> microglia in the spinal cord dorsal horn 7 days after nerve injury. Scale, 20  $\mu\text{m}$ . (D, E) Astrocyte activation revealed by increased GFAP expression in the spinal cord 10 days (D) and 21 days (E) after nerve injury in mice. Scale, 100  $\mu\text{m}$ . (F) Expression of Cx43 in GFAP<sup>+</sup> astrocytes in the spinal cord dorsal horn 21 days after nerve injury. Scale, 20  $\mu\text{m}$ . All the images have not been published.



**Figure 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 TNF, IL-1 $\beta$ , CCL2, CXCL1, BDNF, D-serine, which can act as neuromodulators to induce local central sensitization in surrounding excitatory synapses (facilitation) and inhibitory synapses (dis-inhibition). During neuroinflammation these glial mediators are also present in the CSF and affect synapses in different spinal segments to cause remote central sensitization and extra-territorial and widespread pain beyond the initial injury site.



**Figure 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., TNF, IL-1 $\beta$ , CCL2, 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 ERK and p38 (P-ERK, P-p38), leading to glutamate (Glu) release from synaptic vesicles, via activation of ion channels TRPV1, Nav1.7, and Nav1.8. At postsynaptic sites, increased release of neurotransmitters (e.g., Glu) also induces P-ERK, which can induce central sensitization by positive modulation of NMDA receptor (NMDAR, Step-1). AMPA receptor (AMPA, Step-2) and negative modulation of potassium channel subunit Kv4.2 (Step-3). P-ERK also maintains central sensitization via inducing CREB phosphorylation (P-CREB, Step-4). Opioids such as morphine inhibit neurotransmitter release via mu opioid receptors (MOR) and N-type calcium channels. The scaffold protein  $\beta$ -arrestin-2 ( $\beta$ arr2) inhibits MOR signaling by desensitization and degradation of GPCRs, leading to enhanced acute opioid analgesia in  $\beta$ arr2 knockout mice. Paradoxically,  $\beta$ arr2 also inhibits NMDAR and ERK signaling, leading to a transition from acute pain to chronic pain<sup>175</sup>.



**Figure 6.** Non-pharmacological 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. Abbreviations: DHA, docosahexaenoic acid; EA, electroacupuncture; EPA, eicosapentaenoic acid; VNS, vagus nerve stimulation; SPMs, specialized pro-resolution mediators; TENS, transcutaneous electrical nerve stimulation; TMS, transcranial magnetic stimulation.

**Table 1**

Comparison of inflammation, neurogenic inflammation, and neuroinflammation, with special focus on location, features, and role in pain.

	<b>Inflammation</b>	<b>Neurogenic inflammation</b>	<b>Neuroinflammation</b>
<i>Location</i>	Peripheral tissues Skin, muscle Internal organs except brain	Peripheral tissues Especially skin tissue	PNS: nerves, dorsal root and trigeminal ganglia CNS: spinal cord, brain
<i>Features</i>	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 & central glial cells Production of inflammatory cytokines
<i>Role in pain</i>	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

Abbreviations: BBB, brain blood barrier; CNS, central nervous system, CRPS, complex regional pain syndrome; PNS, peripheral nervous system

**Table 2**

Signal transduction in spinal cord microglia and astrocytes after tissue and nerve injury.

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 MAP kinase and SRC kinase), leading to increased synthesis and release of glial mediators that can produce central sensitization and hyperalgesia and allodynia.

<b>Microglia</b>	<b>Astrocytes</b>
<i>Activators</i>	<i>Activators</i>
ATP	ATP
CX3CL1	TNF
CSF1	CXCL13
LPS/HMGB1	LPS/HMGB1
CASP6	MMP-2
<i>Receptors</i>	<i>Receptors</i>
P2X4, P2X7, P2Y12	P2X/P2Y
CX3CR1	TNFR1
CSF1R	CXCR5
TLR4	TLR4
C5aR	Cx43
<i>Intracellular signaling</i>	<i>Intracellular signaling</i>
P-P38, P-ERK, P-SRC	P-JNK, P-ERK
<i>Mediators</i>	<i>Mediators</i>
TNF	CCL2, CXCL1
IL-1 $\beta$ , IL-18	TSP1, TSP4
BDNF	bFGF, IL-1 $\beta$

Abbreviations: bFGF-2, basic fibroblast growth factor 2; CASP6, caspase 6; CSF1, colony stimulating factor 1; CXCL13, chemokine (C-X-C motif) ligand 13; Cx43, connexin-43; C5aR, complement C5 receptor; HMGB1, high motility group box protein 1; LPS, lipopolysaccharide; MMP-2, matrix metalloproteinase-2; TLR4, toll-like receptor 4; TNF, tumor necrosis factor (alpha); TNFR, TNF receptor; TSP-1, thrombospondin-1; TSP-4, thrombospondin-4.