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Supraspinal neuroimmune crosstalk in chronic pain states

Paul J Austin and Nathan T Fiore

The neural circuits underling sensory, affective, and cognitive dimensions of pain are well-defined, and there is strong evidence that these circuits are compromised by the activation of glial cells and the release of immune mediators in chronic pain states. Immune mediators can modulate glutamatergic and GABAergic signaling, synaptic plasticity, neurogenesis, and neurotrophic factors. Recent neural imaging studies highlight astrocyte and microglial activation within the sensory-discriminative circuits in chronic pain patients. There is also emerging pre-clinical evidence that individuals with neuropathic pain that are 'susceptible' to co-morbid affective disturbances have neuroinflammation in the interconnected prefrontal cortex-ventral hippocampal circuitry. The therapeutic potential of anti-inflammatory agents to mitigate these detrimental supraspinal neuroimmune interactions should be considered in chronic pain patients, particularly those with elevated peripheral immune biomarkers and co-morbid affective disturbances.

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Introduction

The experience of pain is multi-dimensional and can be broadly classified into sensory-discriminative, affective-motivational, and cognitive dimensions. The results from human and experimental animal investigations have suggested that these different pain dimensions are encoded by distinctly different supraspinal structures. For example, the sensory-discriminative dimension, which includes location, intensity, duration, and modality of pain is coded in the primary somatosensory cortex (S1), whereas the affective-motivational and cognitive dimensions are likely coded in the anterior cingulate and prefrontal cortices, respectively [1]. In addition to higher brain regions coding aspects of the pain experience, ascending projections containing nociceptive input also project to areas of the brainstem, including to the midbrain periaqueductal grey matter (PAG) [2]. The PAG coordinates some important unconscious responses to pain, including defensive behaviors that are coupled with alterations in cardiovascular, respiratory, and even analgesic responses.

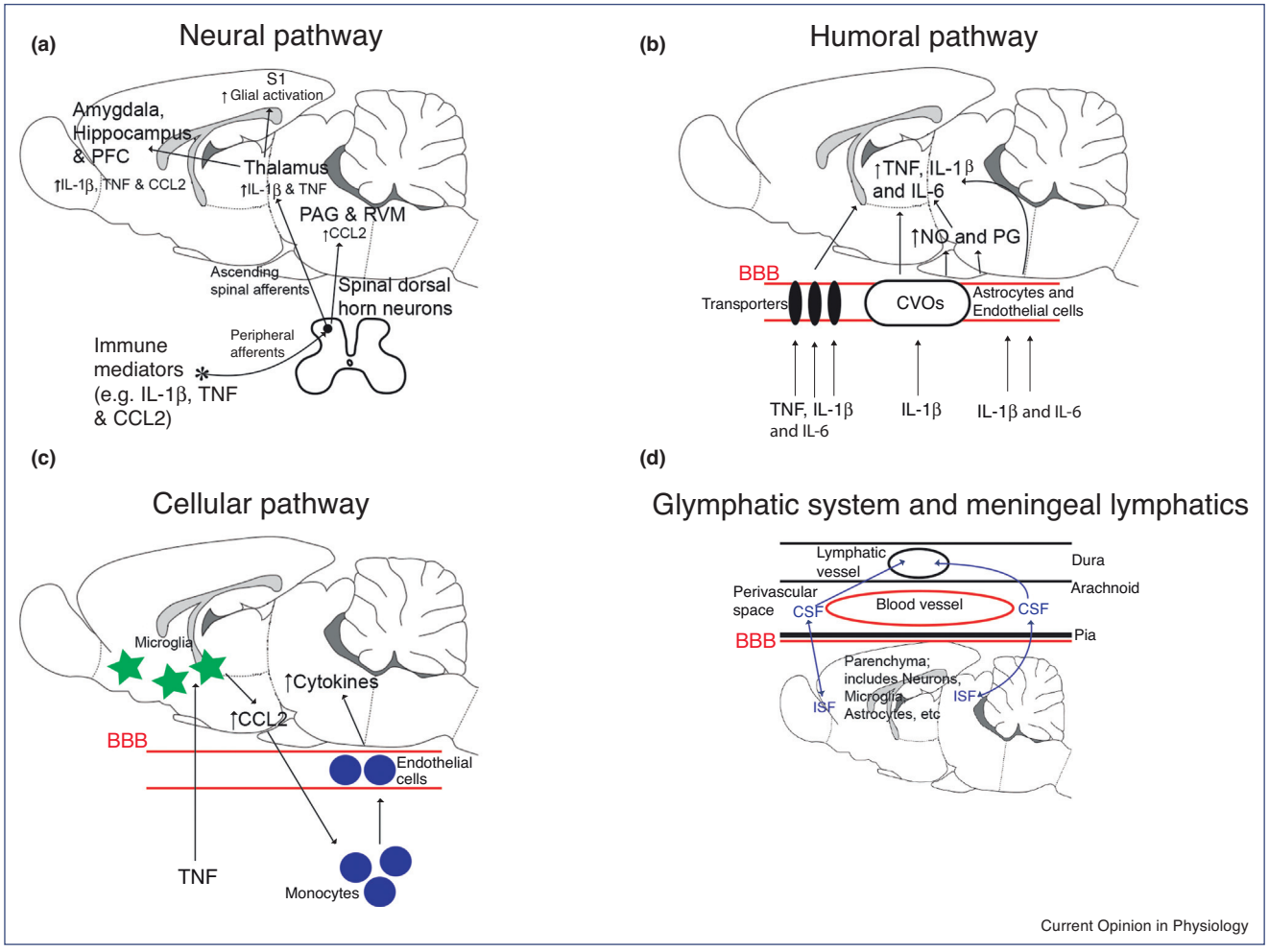
It is the synchronous activation of these multiple brainstem and higher brain structures that is proposed to result in the experience of pain, most commonly as a consequence of a noxious stimulus. While acute noxious activation warns an individual of actual or potential tissue damage and serves to remove them from the threat of damage, chronic pain, that is pain lasting for longer than three months, has no benefit. It is now understood that chronic pain is not simply the prolonged activation of peripheral nociceptors, but associated with neural plasticity or 'central sensitisation' in acute pain networks, and as a consequence, the perception of pain persists long after the acute injury has healed.

There is a growing appreciation that activity in neural networks can be modulated by immune mediators, such as cytokines, chemokines and prostaglandins. These immune mediators act on, and are released by, CNS-infiltrating immune cells, endothelial cells, glial cells, and neurons. There are well-described immune-to-brain transmission routes that detail how cytokines and immune cells can enter the CNS [3], while the recent discoveries of the glymphatic system and meningeal lymphatic vessels have contributed to the re-conceptualization of the brain as a non-immune privileged organ [4] (Figure 1). The ability of brain resident glia and neurons to produce immune mediators has highlighted that a 'neural' immune-to-brain transmission route may be the most critical pathway [5]. In particular, in chronic pain states that result from tissue or nerve damage, there is an abundance of peripheral immune mediators capable of activating afferent nerves and propagating immune-to-brain transmission. In this review we will discuss the critical role of immune mediators as neuromodulators (Figure 2) and consider how neuroimmune interactions in neural networks regulate sensory-discriminative, affective-motivational and cognitive dimensions in chronic pain states.

Peripheral immune activation

Ongoing activation of the immune system has been reported in a variety of chronic pain conditions, with increased Interleukin-1 (IL-1), IL-2, IL-8 and tumor

Figure 1

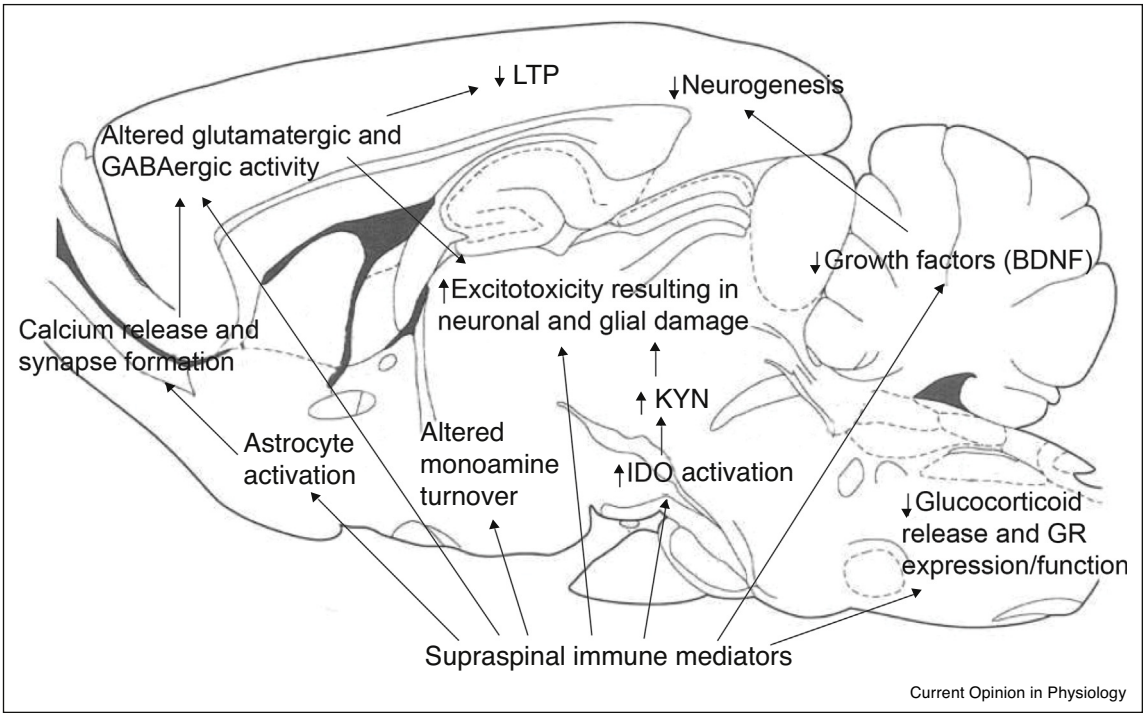


Transmission of peripheral immune mediators to the non-immune privileged brain. Immune-to-brain transmission routes include; **(a)** Neural transmission; the most prominent example is via the vagus nerve, where IL-1β activates vagal afferents, leading to the activation of neurons and release of IL-1β by neighboring glial cells within the brain [44]. Neural transmission is prominent following peripheral nerve injury, where the milieu of immune mediators released activate peripheral nociceptors and are relayed to the thalamus as well as rostroventromedial medulla (RVM) and periaqueductal grey (PAG) via ascending spinal afferents. Thalamic relays then propagate these signals to the sensory-discriminative, affective-motivational and cognitive forebrain regions, including the primary somatosensory cortex (S1), prefrontal cortex (PFC), amygdala and hippocampus. **(b)** Humoral transmission; pro-inflammatory cytokines enter the systemic circulation and are either actively transported across the BBB, pass through into circumventricular organs or act on astrocytes and endothelial cells within the BBB to induce their production in the brain, either directly or indirectly via the release of nitric oxide (NO) and prostaglandins (PGs) [45]. **(c)** Molecular transmission; systemic cytokines, such as TNF, trigger the release of CCL2 from microglia within the brain that facilitates monocyte adhesion and rolling along the endothelial cells, thus allowing monocyte infiltration across the BBB which results in the propagation of neuroinflammation [46,47]. **(d)** The glymphatic system enables passive circulation of interstitial fluid (ISF) between the brain parenchyma and the subarachnoid CSF along perivascular spaces [48], and into the meningeal lymphatic vessels that carry leukocytes and drain into both deep and superficial cervical lymph nodes [49].

necrosis factor (TNF) serum levels and gene expression in peripheral blood mononuclear cells in individuals with painful polyneuropathies and lumbar disc herniations [6–8]. Furthermore, serum TNF levels correlated with depression scores in painful neuropathy [7]. In addition to these previous investigations, we recently reported expanded populations of central memory T lymphocytes (CD4+ and CD8+) with increased NFκB signalling in patients with long-term Complex Regional Pain Syndrome, with T lymphocyte numbers correlating with stress and anxiety scores [9].

There is extensive pre-clinical evidence that circulating monocytes and T lymphocytes can enter the spinal cord after nerve injury, and in conjunction with activated spinal microglia and astrocytes, and their immune mediators, contribute to pain hypersensitivity [see detailed reviews 10,11]. Further, monocytes have been shown to

Figure 2



A schematic overview of six mechanisms by which supraspinal immune mediators may act as neuro- and glial-modulators in chronic pain states. (i) Direct effects on glutamatergic and GABAergic receptors and neural activity, resulting in a reduction in long-term potentiation (LTP) and synaptic connectivity, and excitotoxicity resulting in neuronal and glial damage [33••,50,51]. (ii) Astrocyte activation leading to Ca^{2+} and possible gliotransmitter release, synapse formation, and modulation of extracellular glutamate [15,20••,21]. (iii) An increase in production of the enzyme, indoleamine 2,3-dioxygenase (IDO) that facilitates the conversion of tryptophan to kynurenine (KYN). In microglia KYN can be converted into the neurotoxic metabolite QUIN, an agonist of the NMDA receptor. Increased expression of KYN and QUIN has been linked to depression in humans, as well as depressive-like behavior in chronic pain models [34,52–54]. (iv) Suppression of growth factors, particularly BDNF, leading to a reduction in neurogenesis [33••,34,55, Fiore and Austin, unpublished] (v) Changes in monoamine turnover [56–58]. (vi) Reduced glucocorticoid release as well as a decrease in GR expression and function [59].

traffic to the amygdala in a chemokine (CCL2/CCR2) dependent manner and contribute to anxiety-like behavior after nerve injury [12]. While it is becoming clear that disrupted peripheral immune cells and their trafficking into the CNS occurs in pre-clinical models of chronic neuropathic, their effect on supraspinal sensory, affective, and cognitive processing in chronic pain states remains unclear.

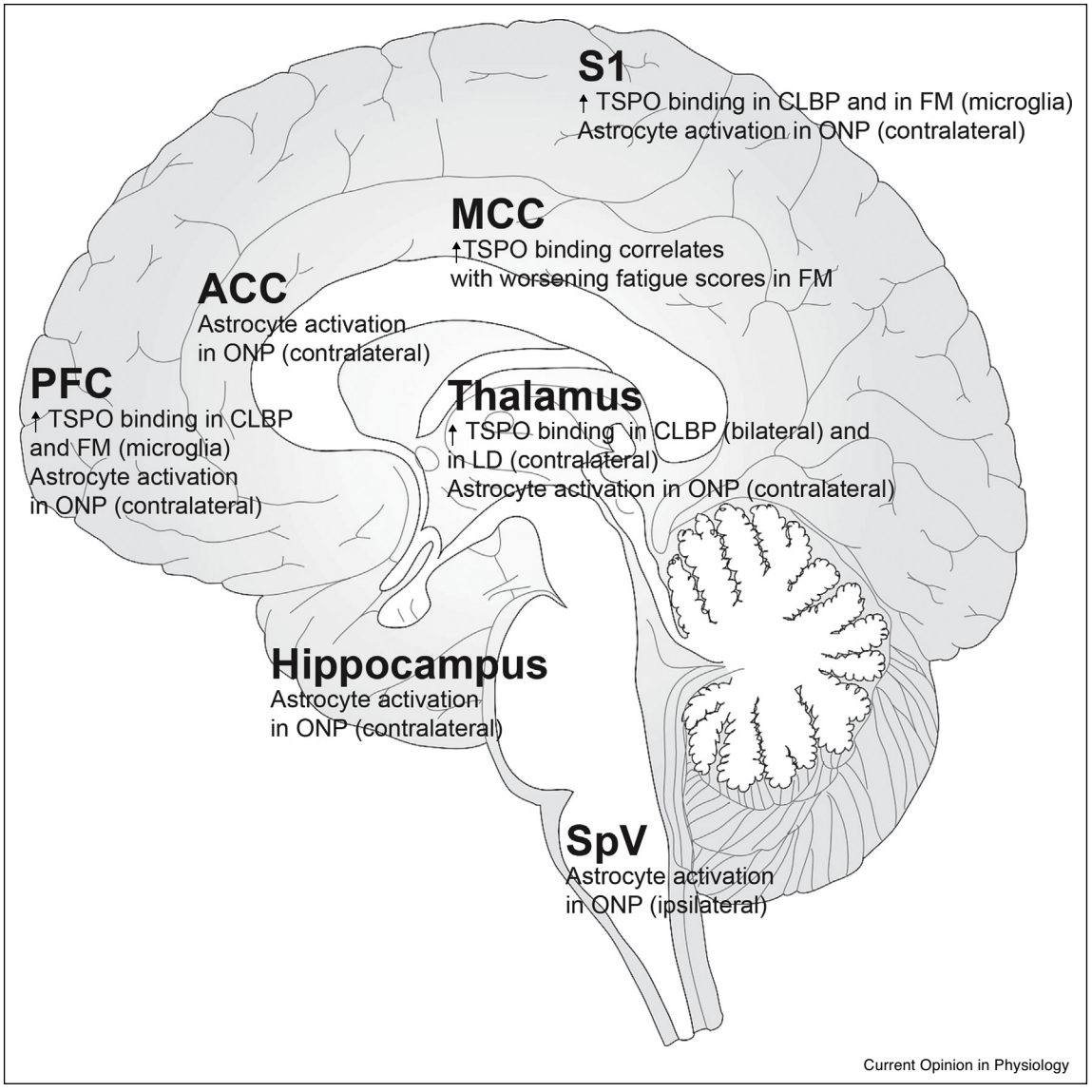
Neurally mediated immune effects

Sensory-discriminative pathways

In addition to ongoing immune activation in the periphery, there is growing evidence of central neuroimmune changes that can modulate activity in various pain-related brain regions. For example, recent pre-clinical investigations in models of chronic neuropathic pain have revealed: (i) activation of microglia in the contralateral (to nerve injury) ventroposterior thalamus and S1 [13,14•]; (ii) astrocyte activation in S1 that is associated with spontaneous Ca^{2+} currents, synapse formation, elevated extracellular glutamate levels and the onset of allodynia [15]; and (iii) increased TNF and IL-1β expression in the thalamus [16]. These data strongly suggest that astrocytic and microglial activation in sensory-discriminative pain pathways facilitates nociceptive signalling which may lead to spontaneous pain, allodynia and hyperalgesia.

While microglial and astrocyte activation cannot be directly explored in living humans, recent advances in human brain imaging studies have led to a number of studies investigating markers of supraspinal astrocyte and microglial activation (Figure 3). Human positron emission tomography (PET) brain imaging using a ligand directed against the translocator protein (TSPO), earlier called peripheral benzodiazepine receptor, a marker of activated glia, demonstrated thalamic glial activation after limb amputation, that often results in the development of chronic phantom limb pain [17]. More recently, Loggia *et al.* used the same technique to show thalamic and cortical glial activation in individuals with chronic lower back pain (CLBP) and fibromyalgia (FM) [18,19]. Unexpectedly, TSPO levels negatively correlated with clinical pain and circulating

Figure 3



Human brain imaging studies highlighting glial activation in sensory-discriminative, affective-motivational and cognitive brain regions in chronic pain states. There is strong evidence of glial activation in sensory-discriminative regions such as the ipsilateral spinal trigeminal nucleus (SpV), the contralateral 'sensory' thalamus, and the contralateral primary somatosensory cortex (S1) in chronic lower back pain (CLBP), limb denervation (LD), orofacial neuropathic pain (ONP) and Fibromyalgia (FM). Furthermore, affective-motivational and cognitive brain regions such as the ventral hippocampus, anterior (ACC) and middle (MCC) cingulate, and prefrontal (PFC) cortices show evidence of glial activation in chronic pain states. Studies were conducted by either; (i) MRI imaging examining infra-slow oscillations or T2 relaxation times, which represent astrocyte calcium waves and astrocyte activation, respectively [20••,21]; and (ii) PET imaging using a ligand against TSPO, a non-specific glial activation marker, either alone or in combination with a ligand against MAO-B, an astrocyte-specific marker [17–19]. In the FM study, the authors were able to conclude that glial activation in S1 and PFC was due to microglia, due to the presence of TSPO, but not MAO-B binding [18].

IL-6, suggesting a possible antinociceptive and anti-inflammatory compensatory role of activated glia in this population [19]. Using MRI imaging, Henderson and colleagues found infra-slow oscillations and decreases in T2 relaxation time, which may represent astrocyte calcium waves and activation, in the ipsilateral spinal trigeminal nucleus (SpV), contralateral ventroposterior thalamus and S1 in orofacial neuropathic pain patients [20••,21]. In addition, a human post-mortem study found chronic astrocyte activation and increases in TNF and IL-1β in the spinal cord dorsal horn in individuals with neuropathic pain [22]. These animal and human studies provide strong evidence of activated glial cells and release of immune mediators in the dorsal horn and in supraspinal regions of

the sensory-discriminative pain pathway, and it is possible that these changes contribute significantly to the presence of persistent pain (Figure 2).

Affective-motivational and cognitive pathways

There is growing evidence of neuroimmune changes within brain regions that likely code the affective-motivational and cognitive aspects of chronic pain, and it is important to appreciate that cytokine and chemokine receptor expression is strongest in brain regions critical for affective state regulation [3]. We have previously hypothesized that supraspinal neuroimmune cross-talk in prefrontal cortex (PFC)-hippocampal circuits underlies an increase in susceptibility to develop affective-motivational disturbances associated with chronic pain [3], and evidence supporting this notion has recently strengthened. Peripheral IL-1β has been identified as a mediator of immune-to-brain signalling that leads to neuroinflammation and microglial activation, particularly in brain regions thought to mediate the affective and cognitive aspects of chronic neuropathic pain [14•,23]. Blocking peripheral IL-1β or deleting the IL-1 receptor in a pre-clinical model of neuropathic pain, prevented an increase in IL-1β expression and microglial activation in the PFC, hippocampus, amygdala and nucleus accumbens, as well as normalizing short-term memory deficits and depressive-like behaviours [23].

Several research groups have developed behavioral paradigms to identify subgroups of rats with neuropathic pain that are '*susceptible*' to co-morbid affective behavioral disturbances after nerve injury [24•,25–28,29••,30,31]. These models' have revealed increased expression of IL-1β, IL-4, IL-6, TNF and CCL2 and reduced brain-derived neurotrophic factor (BDNF) in contralateral infralimbic and prelimbic PFC subregions in only '*susceptible*' rats, whereas '*resilient*' rats with pain but without behavioral disturbances do not show alterations in immune mediators [27,29••, Fiore and Austin, unpublished] (Figure 4). Increased expression of phospho-p38 in neurons and microglia, as well as a shift to a M1 reactive microglial morphology in the infralimbic and prelimbic regions also occurs selectively in '*susceptible*' rats [30,32, Fiore and Austin, unpublished]. Normalization of these neuroinflammatory mediators with either ketamine, minocycline or a TrkB agonist reversed the affective behavioral disturbances in '*susceptible*' rats [27,29••,30].

Hippocampal neuroinflammation is linked to reduced neurogenesis, impaired LTP and decreased synaptic plasticity in pre-clinical models of neuropathic pain [see detailed review 3]. These chronic pain models display bilateral increases of TNF in the CA1 region of the hippocampus, as well as a reduction in BDNF, reduced neurogenesis, decreased excitatory synaptic connections and microglial activation, and these changes are associated with impaired memory function [33••,34]. Furthermore, these mal-adaptations and cognitive deficits were blocked by deletion of TNFR1, pharmacological or genetic ablation of microglia, and alkyl-glycerol ethers.

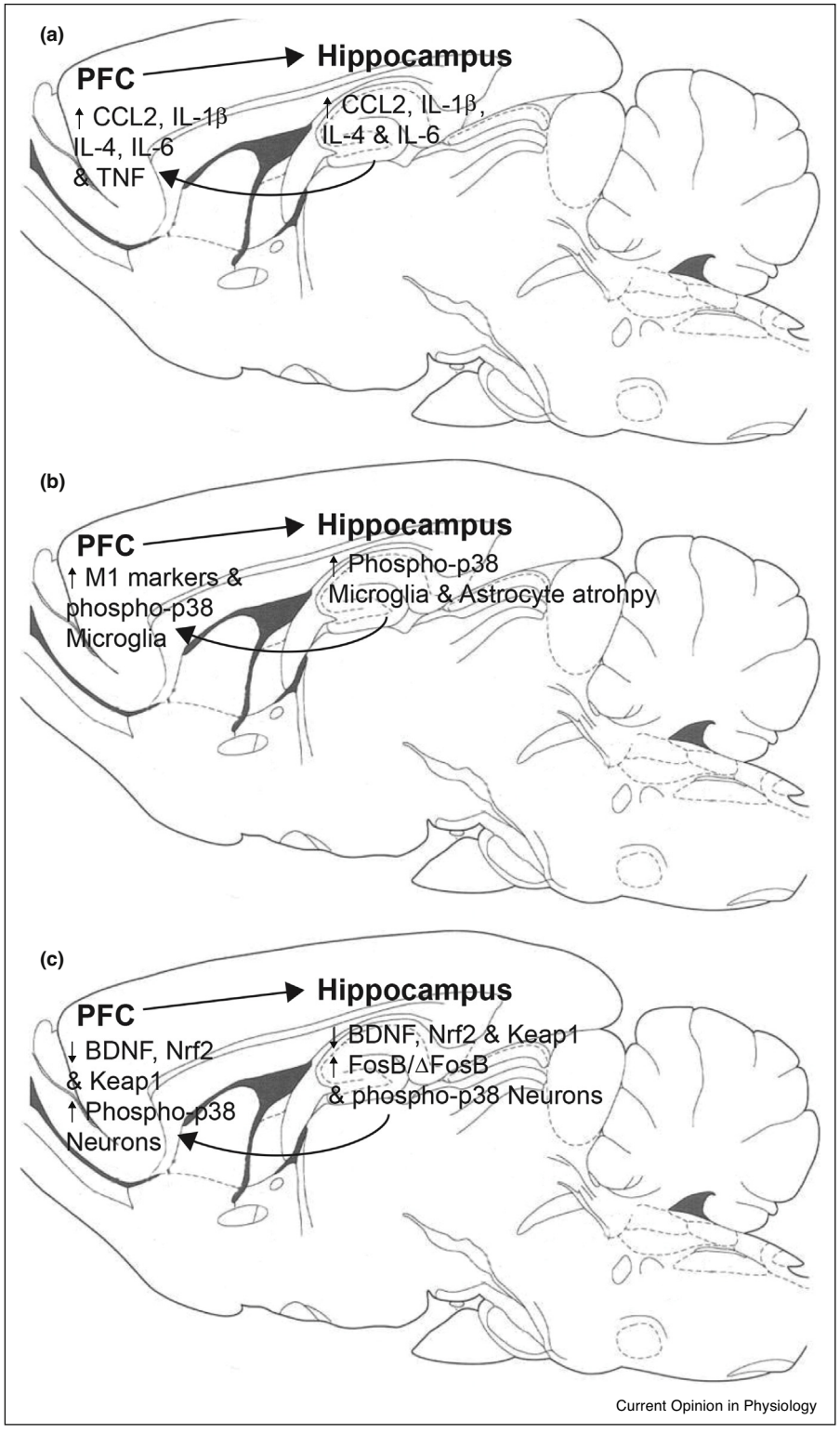
In the contralateral ventral CA1, we have identified increased expression of neuronal IL-1β, IL-6 and CCL2, as well as reactive microglia expressing phospho-p38 in '*susceptible*' rats, who also had decreased BDNF expression, astrocyte atrophy and a reduced volume in the ventral dentate gyrus [24•, Fiore and Austin, unpublished] (Figure 4). The contralateral expression pattern of immune mediators and reduced dentate gyrus volume, together with increased expression of the neural activation marker FosB/ΔFosB in the contralateral hippocampus indicate a 'neural' immune-to-brain transmission mechanism specifically in '*susceptible*' rats [24•]. Given the reciprocal connectivity between the ventral hippocampus and the infralimbic and prelimbic regions, we believe that the neuroinflammatory changes seen in the PFC and hippocampus in '*susceptible*' rats are indicative of a disrupted neural circuitry that underpins co-morbid affective disturbances after nerve injury. Further, sulforaphane, an activator of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and potent anti-inflammatory, has been shown to normalize hippocampal Keap1-Nrf2 signaling and sucrose preference in '*susceptible*' rats [28].

A recent human neural imaging study supports our hypothesis of PFC-hippocampal neuroinflammation, with evidence of astrocyte activation in the contralateral dorsolateral prefrontal cortex and ventral hippocampus in chronic neuropathic orofacial pain [20••]. Taken together, these studies suggest that in chronic pain states neuroinflammation in the neural substrates that underpin cognitive and affective processing may lead to development of co-morbid cognitive or affective-motivational disturbances in susceptible individuals.

Descending pathways

It is well known that nociceptive input can be modified at the level of the primary afferent synapse by descending pathways, and that this mechanism may be deficient in individuals with chronic pain. Work by Keay and Bandler has established that different functional columns of the PAG drive integrated behavioral responses that are specific to pain of different tissue origins [see review by 35]. Importantly, these neural columns are capable of modulating incoming noxious inputs with the ventrolateral PAG column mediating a powerful opioid-dependent analgesia. Studies show that microglia are activated and co-express the activation marker, phospho-p38, in the vlPAG [14•,36], while astrocyte activation occurs in the lateral and vlPAG columns of rats with neuropathic pain and in particular those that express behavioral disabilities during social-interactions [25]. Chronic pain results in

Figure 4



A summary of **(a)** cytokine and chemokine, **(b)** glial and **(c)** neuronal, neurotrophic and transcription factors within the PFC and hippocampus in rats with chronic neuropathic pain that are 'susceptible' to co-morbid affective behaviors [24•,27,28,29••,30].

increased expression of phospho-NFκB and CXCL1 in vlPAG astrocytes, while neurons increase their expression of the concomitant receptor, CXCR2 [37]. Moreover, interruption of the CXCL1/CXCR2 axis in the vlPAG has been shown to reverse allodynia and hyperalgesia, presumably by restoring the ability of the vlPAG to inhibit incoming noxious information.

The rostroventromedial medulla (RVM) acts as final relay in the control of descending pain modulation receiving input from the PAG, and can both facilitate or inhibit nociceptive inputs [see review by 38]. Neuronally expressed CCL2 acting on microglial CCR2 in the RVM promotes hyperalgesia after nerve injury [39]. Recent pre-clinical studies have revealed systemic infusion of bone-marrow derived stromal cells (BMSCs) produces long-lasting antinociceptive responses, despite transplanted cells not surviving in the host [40••]. These effects are dependent on upregulation of NFκB signalling by BMSCs, as well as CCL4 and CCR2 chemotactic interactions between BMSC and monocytes that induces CXCL1 release in monocytes [40••,41]. CXCL1 crosses the blood brain barrier and acting via the CXCR2 receptor upregulates μ-opioid receptor expression by RVM neurons, driving descending pain inhibition. These studies demonstrate the complex role of chemokine signalling in the RVM and PAG but highlights the potential to engage the endogenous opioid system for pain relief.

Conclusions

There is substantial evidence derived from both human and animal studies that supraspinal neuroimmune interactions play a significant role in both sensory-discriminative as well as affective and cognitive dimensions of chronic pain. On the basis of the laterality of immune mediators in nerve-injured rodents and co-expression of cytokines and chemokines by neurons, as well as microglia and astrocytes, a 'neural' transmission route is likely in chronic pain states. Microglial and astrocyte activation, as well as immune mediators in the ascending ('sensory' thalamus and S1) and descending (RVM and PAG) pain pathways appear to increase neuronal excitability and contribute to symptoms of spontaneous pain, allodynia and hyperalgesia in chronic pain states. Whereas, evidence derived predominantly from rodent studies suggests neuroimmune mediators in the PFC-hippocampal circuits appear to be restricted to only individuals with neuropathic pain that are susceptible to co-morbid affective and cognitive disturbances. Moreover, immune mediators in PFC-hippocampal circuits have been shown to have diverse roles on synaptic plasticity, neuronal excitability and neurogenesis.

Although to date there is a lack of efficacy of novel anti-inflammatories to treat chronic pain in several clinical trials [3], there are promising options including systemic administration of BMSCs that engage the endogenous analgesic opioid system and minocycline, which polarizes microglia toward an anti-inflammatory M2 phenotype and has shown efficacy in treating pain and depressive-like behaviors *in vivo* and depression clinically [30,42]. It is also worthwhile to investigate agents (alone or in combination) that target-specific cytokines and chemokines, such as Infliximab (TNF blocker), Anakinra (IL-1R blocker), Tocilizumab (IL-6R blocker) that are used in the treatment of Rheumatoid arthritis, or target inflammatory signalling pathways, with inhibitors of p38 and NFκB, which have shown efficacy in experimental models. Major consideration should be given to patient selection as patient groups with elevated peripheral immune biomarkers (i.e. C-reactive protein, IL-1β and IL-6 [29••,43]) and co-morbid affective disturbances may be more responsive to anti-inflammatory agents. However, the use and timing of immunomodulators must be carefully considered given they have diverse physiological roles, including host defense and injury repair.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

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- •• of outstanding interest

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This is the first human brain imaging study to provide evidence of astrocyte activation in regions critical for sensory, affective and cognitive dimensions of pain. The study included 37 individuals with trigeminal neuropathic pain, and showed decreases in T2 relaxation times in the ipsilateral spinal trigeminal nucleus and contralateral primary somatosensory cortex, as well as dorsolateral prefrontal, cingulate and ventral hippocampal/parahippocampal cortices. The authors state that regional changes in T2 relaxation times reflect prolonged astrocyte activation, which results in altered brain rhythm and the constant perception of pain.

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