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REVIEW

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Sleep disturbances and severe stress as glial activators: key targets for treating central sensitization in chronic pain patients?

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ABSTRACT

Introduction: The mechanism of sensitization of the central nervous system partly explains the chronic pain experience in many patients, but the etiological mechanisms of this central nervous system dysfunction are poorly understood. Recently, an increasing number of studies suggest that aberrant glial activation takes part in the establishment and/or maintenance of central sensitization.

Areas covered: This review focused on preclinical work and mostly on the neurobiochemistry studied in animals, with limited human studies available. Glial overactivation results in a low-grade neuroinflammatory state, characterized by high levels of BDNF, IL-1β, TNF-α, which in turn increases the excitability of the central nervous system neurons through mechanisms like long-term potentiation and increased synaptic efficiency. Aberrant glial activity in chronic pain might have been triggered by severe stress exposure, and/or sleeping disturbances, each of which are established initiating factors for chronic pain development.

Expert opinion: Potential treatment avenues include several pharmacological options for diminishing glial activity, as well as conservative interventions like sleep management, stress management and exercise therapy. Pharmacological options include propentofylline, minocycline, β -adrenergic receptor antagonists, and cannabidiol. Before translating these findings from basic science to clinical settings, more human studies exploring the outlined mechanisms in chronic pain patients are needed.

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Pain; fibromyalgia; low back pain; sleep; stress; neuroinflammation

1. Introduction

Modern pain neuroscience has advanced our understanding about pain, including the role of central sensitization (CS, or central hyperexcitability) in the presence and amplification of (persistent) pain experiences. CS is defined as 'an amplification of neural signaling within the central nervous system that elicits pain hypersensitivity' [1] and 'increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input' [2]. In many patients with chronic pain, a clear origin for nociceptive input is lacking or is not severe enough to explain the severe pain and other symptoms experienced by the patient. In such patients, CS is often present and can explain the clinical picture. It is now well established that the mechanism of sensitization of the central nervous system partly explains the chronic pain experience in many patients, including those with neuropathic pain [3], whiplash [4], chronic low back pain [5], osteoarthritis [6], headache [7], pain following cancer treatment [8], fibromyalgia [9], chronic shoulder pain [10], chronic fatigue syndrome [11], rheumatoid arthritis [12], temporomandibular disorders [13], patellar tendinopathy [14], orofacial pain [15], and lateral epicondylalgia [16,17].

CS encompasses various related dysfunctions within the central nervous system, all contributing to altered, often increased responsiveness to a variety of stimuli-like mechanical pressure, chemical substances, light, sound, cold, heat, stress, and electricity [18]. Such central nervous system dysfunctions include sensitization of spinal cord neurons [19], altered sensory processing in the brain [20] with increased brain activity in areas known to be involved in acute pain sensations (primary and secondary somatosensory cortices, thalamus, insula, anterior cingulate cortex, and prefrontal cortex) [21], involvement of several brainstem nuclei (nucleus cuneiformis, periaqueductal gray, parabrachial nucleus, etc.), altered brain neurochemistry [22], poor functioning of descending antinociceptive mechanisms [23,24], and increased activity of brain-orchestrated nociceptive facilitatory pathways [20]. These facilitatory pathways probably relate to the increased brain activity as described above, and they might be (further) activated by cognitive–emotional factors such as

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Article highlights

- An increasing amount of animal research data supports the idea of aberrant glial activity as a potential underlying, even etiological, mechanism of central sensitization.
- A recent human study using positron emission tomography-magnetic resonance imaging (PET/MRI) identified a pattern consistent with brain glial activation in patients with chronic low back pain, and other human cerebrospinal fluid studies support the idea of neural inflammation in chronic pain patients.
- Stress and poor sleep can trigger glial overactivation and subsequent low-grade neuroinflammatory state, characterized by high levels of BDNF, IL-1β, TNF-α, which in turn increases the excitability of the central nervous system neurons through mechanisms like long-term potentiation and increased synaptic efficiency.
- Several potential pharmacological treatment avenues for diminishing glial activity in chronic pain patients are available, including minocycline antibiotics, β-adrenergic receptor antagonists and propentofylline. Human causation and effectiveness studies exploring these pharmacological options are emerging.
- Potential conservative treatment options for normalizing glial activity in chronic pain patients include sleep management (e.g., cognitive behavioral therapy for insomnia), stress management and exercise therapy, but studies examining whether such interventions can actually normalize glial activity in chronic pain patients are needed.

This box summarizes key points contained in the article.

pain catastrophizing, stress, hypervigilance, lack of acceptance, depressive thoughts, and maladaptive illness perceptions (e.g. perceived injustice, low self-efficacy).

CS is also accompanied by an exaggerated central nervous system response, to nociceptive [25] or even non-nociceptive [26] input, resulting in pain that is disproportional to the stimulus [27]. CS is often associated not only with severe pain but also with various other symptoms like sleep disturbances and stress intolerance [28,29]. In spite of our increased understanding of the mechanisms explaining (hypersensitivity) symptoms in patients with chronic pain, there is much to learn about the development of chronic pain, including the etiological mechanisms underlying CS. Why do some patients develop CS pain while others do not?

Glia are nonneuronal cells that reside within the nervous system. A subset of glial cells, in particular microglia and astrocytes, resembles physiological functions of immune cells and therefore can be referred to as immune-like [30] or immune competent [31] cells. For too long, neuroscientists have focused on neurons and neglected the glia, probably relating to the fact that basic neuroscience was historically developed from methods monitoring electrical signaling in (between) neurons [32]. Yet, glia far outnumber neurons, as well as having much higher cellular diversity and functions than neurons [33].

An increasing number of preclinical studies suggest that aberrant glial activation might explain the establishment and/or maintenance of CS, and persistent pain [30,34–37], and that poor sleep [38] and severe stress [31,39,40] each can result in such aberrant glial activation. Here, we update the reader with our current understanding of the potential role of aberrant glial activity in explaining (the onset of) CS in patients with chronic pain, together with severe or long-term stress and sleep disturbances as triggers for aberrant glial activation. Finally, potential pharmacological and non-pharmacological treatment avenues for these neuro-immune interactions are discussed. This review focused on preclinical work and mostly on the neurobiochemistry studied in animals, with limited human studies available.

2. Glial activation, neuroinflammation, and the etiology of CS pain

The three major glial cell types are microglia, astrocytes, and oligodendrocytes. While oligodendrocytes have recently been implicated in the development of central pain [41], here we will focus mostly on the first two classes of glia, as their role in pain is more established. Microglia are sometimes referred to as the resident macrophages in the brain: in the presence of injury or infection, they become activated and work together to repair the damage and restore brain homeostasis [31,42]. To allow microglia to perform this important physiological function, it is crucial that they are not static cells but rather hold a high degree of motility within the central nervous system. While in the past, authors would characterize microglia as being either in a 'resting state' or an 'activated state,' today most accept that microglial cells, when not activated, are in a 'surveillance' rather than 'resting' state. Microglia are in fact continuously protracting and retracting their processes to scan the molecular and cellular microenvironment, including synapses, in their proximity [31].

In addition, microglia play a crucial role in synapse formation, synapse elimination (e.g. microglia can phagocytize hippocampal synapses), and refinement of neuronal circuits with complement proteins C1q and C3 as critical mediators [31]. Synapse formation/elimination is a crucial mechanism for many functions, including learning (i.e. long-term potentiation and long-term depression) and protection against injury. Rapid eye movement sleep is important in selectively eliminating and maintaining newly formed synapses, which in turn contributes to learning and memory consolidation [43]. Also, microglia motility is regulated by different modes of neurotransmission: increased by glutamatergic neurotransmission and decreased by GABAergic neurotransmission [44]. Hence, microglia and neurons have bidirectional interactions and are constantly 'fine-tuning' each other.

Upon activation, microglia transform morphologically from a ramified (resting) to an ameboid (phagocytic) phenotype [42]. Not every activated microglia present a pro-inflammatory pattern. Microglia inflammatory actions depend on the activation subtype: the pro-inflammatory subtype (i.e. M1) secretes pro-inflammatory cytokines (e.g. tumor necrosis factor α [TNF-α], interleukin 1β [IL-1β], IL-6), neurotrophic factors (e.g. brain-derived neurothrophic factor or BDNF), and free radicals that are toxic to the surrounding cells [42], while the anti-inflammatory subtype (i.e. M2) secretes anti-inflammatory cytokines (e.g. IL-10) for resolving inflammation and trophic factors for promoting tissue healing [45]. Such generation of inflammatory responses is mediated through the activation of p38 mitogen-activating kinase (MAPK) in spinal microglia, at least in male (but not in female) animals [46], which plays a pivotal role in wide dynamic range neuronal hyperexcitability [47].

M1–M2 polarization of microglia also has different effects on synaptogenesis: M2-microglia stimulate synaptogenesis, while M1-microglia in inflammatory state result in synapse elimination (synaptic stripping, a mechanism linked to learning processes) [31]. The concept of M1 versus M2 polarization of microglia is currently being debated, since it may represent a continuous process rather than different subtypes [48]. Also, the fact that glial cells have different dynamics and a different order of activation after stimulus might also explain differences across patients, possibly even explaining in part why some people develop chronic pain after a certain event (e.g. physical trauma), while others do not.

In the acute or subacute phases of injury and pain, glial activation likely plays an adaptive role, as it favors tissue healing and recovery. When glial activation does not resolve, and becomes chronic, it can become pathogenic and lead to collateral damage of nearby neurons and other glia [42]. Resulting low-grade chronic neuroinflammation is an underlying feature of many neurological disorders like major depressive disorder [42], Alzheimer's disease [42], Parkinson's disease [42], schizophrenia [42], traumatic brain injury [49], diabetic retinopathy [50], and brain tumors [51]. Similar chronic neuroinflammation might also be involved in the pathogenesis of chronic pain [36,52–55].

Aberrant glial activity has the potential to initiate CS through several mechanisms. Activated microglia have been identified as a major source for the synthesis and release of BDNF which is responsible for increasing neuronal excitability by causing disinhibition in dorsal horn neurons in the spinal cord [56,57]. This microglia-to-neuron communication includes not only attenuation of the pain inhibitory action of gamma-aminobutyric acid (GABA) but also of glycine receptor-mediated inhibition [57]. Increased glial synthesis of BDNF in the nociceptive pathways in patients with CS pain can be considered a pathophysiological response [58].

Elevated IL-1β gene expression in microglia has been linked to long-term potentiation induction and maintenance (and consequently to learning, fear, and memory processes) [31]. Likewise, gliosis is accompanied by increased TNF-α availability, which in turn induces long-term potentiation [59] and consequent enhanced synaptic efficacy [31] and pain sensitization [59]. Long-term potentiation and enhanced synaptic efficacy are (partly overlapping) key mechanisms underlying increased excitability of the central nervous system pain [60–62] and the formation of (maladaptive) pain memories [63,64] in patients with chronic pain and CS, possibly coordinated by gliosis. Likewise, pro-inflammatory mediators, produced by activated microglia during neuroinflammation, directly activate nearby neurons, which express receptors for these pro-inflammatory cytokines, which in turn become hyperexcitable [31].

While structurally and functionally very different from microglia, astrocytes have been convincingly demonstrated to play a key role in the pathogenesis of persistent pain in animal models [65–67]. Astrocytes are capable of detecting the presence of an insult, such as an inflammatory challenge [68] or nerve injury [69], to which they respond by exhibiting hypertrophy, and increasing expression of a variety of molecules that contribute to hyperalgesia and allodynia, such as the enzyme nitric oxide synthase [70], the pro-inflammatory cytokine IL-1 [68], and the chemokines CXCL1 [71].

In addition to the above-explained mechanisms, other pathways could link aberrant glial activation and CS pain. This includes the expression and function of the adenosine A1 receptor (A1AR) on microglia [72]. Selective stimulation of A1AR inhibits the morphological activation of microglia, possibly by suppressing the Ca(2+) influx induced by ATP treatment, and microglial cells, pretreated with the A1AR agonist, exhibit lower capability to facilitate spinal nociceptive neurons [72]. In addition, proteinase-activated receptors are a family of G-protein-coupled receptors available in neurons, microglia, and astrocytes. They are activated by proteases, initiating a series of intracellular signal pathways that result in not only sensitization of pain pathways [73–75] but also activation of the nuclear factor NK-κβ [76], which is important for neural plasticity in the nervous system, including synaptogenesis [77]. Activated NK-κβ has the capacity to increase the expression of BDNF in central neurons [77].

Importantly, pharmacological disruption of astroglial and microglial function, or of the action of its products, can reduce, reverse, or prevent nocifencive behaviors in animal models [68–70,78]. Taken together, these studies demonstrate that astrocytes, just like microglia, play an important role in the pathogenesis of persistent pain in animals.

In fact, nearly all of the evidence that microglia might play an important role in the maintenance of chronic pain was derived from animal models. The first human study on glial activity used a sensitive *in vivo* marker of glial cell activation monitored using positron emission tomography (PET) to show that limb denervation (n = 7) induces glial activation, beyond the first-order projection area of the injured neurons, in the human thalamus but not somatosensory cortex [79]. Only the one patient with bilateral nerve injury showed bilateral microglia activation in the thalamus, while the other six patients had only contralateral activation. A more recent human study using PET-magnetic resonance imaging identified a pattern consistent with brain glial activation in patients with chronic low back pain [36], a condition known to be related to CS [5]. Other human studies using cerebrospinal fluid also support a potential role for altered central cytokines and neurotrophic factors, consistent with aberrant glial activation and related neuroinflammation, in a number of chronic pain states despite varying etiologies [35]. Hence, the available evidence supports an association between aberrant glial activity and neuroinflammation in humans with chronic pain, but direct evidence showing that aberrant glial activity and neuroinflammation lead to the development of CS in humans is currently unavailable and requires more research.

If aberrant glial activation or gliosis explains the onset of CS, the next question will be what triggers aberrant glial activation? Neuroscience has provided us with a number of potential answers, including severe stress and sleep disturbances that may primarily target the brain. In the spinal cord, glial activation is mainly triggered by peripheral tissue inflammation or nerve damage. Still, in some instances,

aberrant glial activation may not suffice for pain hypersensitivity, necessitating to look beyond glial-triggered neuroinflammation toward the consequences of such aberrant glial activation and related neuroinflammation on brain morphology and functioning.

3. Stress as a trigger of glial activation and consequent morphological brain changes

Stress can be defined as the continuous struggle of living organisms to preserve an internal dynamic state of equilibrium (homeostasis) [80]. Any factor, being either physical, psychosocial, or emotional, that challenges homeostasis, is labeled as a stressor [80]. High levels of anxiety in the time period surrounding the traumatic musculoskeletal injury predict development and maintenance of moderate-to-severe chronic pain [81]. Likewise, childhood maltreatment (including emotional abuse) is not only associated with increased risk of chronic (low back) pain, it also relates to long-lasting CS to nociceptive stimuli as seen in a subgroup of (highly debilitated) people with chronic low back pain [82].

In response to long-term stress, the body is exposed to high levels of glucocorticoids. Glucocorticoids coordinate the expression of subsets of genes involved in signal transduction, neuronal structure, vesicle dynamics, neurotransmitter catabolism, encoding of neurotrophic factors and their receptors [80]. Altogether, stress results in a highly coordinated set of changes in gene expression underlying neuronal plasticity [80], glial activation and consequent neuroinflammation with increased IL-1β and IL-6 mRNA expression [31,39,40]. Indeed, stress and the resulting noradrenaline plus glucocorticoids activate microglia (which express α1, α2, β1, and β2-adrenergic and glucocorticoid receptors), resulting in neuroinflammation (with increased availability of pro-inflammatory cytokines like IL-1, TNF-α, and IL-6)/gliosis as described above [31] (Figure 1). Stress activates glia in a way that they transform into the pro-inflammatory subtype [39,40], which could then induce CS. Long before they became activated, microglia might have been primed by a traumatic event so that a second 'hit' (e.g. another trauma or stress) induces an exaggerated glial response (the 'double-hit' hypothesis as described in other neuroinflammatory disorders [83]).

Not surprisingly, in response to stress, microglia are found to become activated in several brain regions, including the prefrontal cortex, the lateral, basolateral, central, and basomedial nuclei of the amygdala and in the CA3 and dentate gyrus of the hippocampus [31]. These brain areas undergo morphological changes in response to stress [84–86] and also play a cardinal role in chronic pain [63,87–89]. Microglia, through several mechanisms including the production of BDNF [90], may explain such stress-induced morphological changes in the brain and even CS (Figure 1). The pro-inflammatory mediators produced by activated microglia during neuroinflammation directly activate receptors on nearby neurons, inducing their hyperexcitability [31].

Surprisingly, the morphological brain changes in response to chronic stress are somewhat site-specific rather than diffuse, and primarily involve hypertrophy in the amygdala and atrophy in the prefrontal cortex and hippocampus [84,85]. This

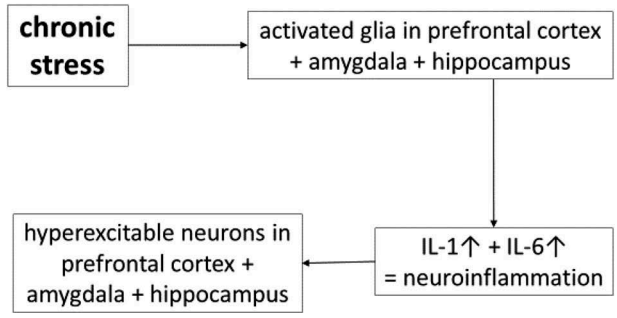

Figure 1. Schematic presentation of the mechanism of stress-induced aberrant glial activation resulting in neuro-inflammation and consequent hyperexcitable central nervous system neurons.

IL-1: interleukin type 1; IL-6: interleukin type 6.

may be partially related to the distribution of glucocorticoid receptors, which are widely expressed in the brain but have high densities in the medial prefrontal cortex [91], and speciality in the hippocampus and amygdala [80,91]. Initial experiments regarding the morphological changes in key brain areas in response to chronic stress were conducted on animals, later on backed up in human (mainly observational) studies [84,85]. These morphological changes can be induced by repeated exposure to mild stressors or brief but intense stress [91]. It is important to emphasize that these stress-induced morphological changes may be reversible [91] and hence do not reflect genuine brain damage but rather a form of plasticity. These morphological changes might protect brain areas from permanent excitotoxic damage and therefore be adaptive [90].

4. Sleep as a trigger of glial activation and neuroinflammation

In the absence of other intrinsic sleep disorders and shift work, insomnia is defined as >30 min sleep latency and/or minutes awake after sleep onset for >3 days/week for >6 months [92,93]. Stress, sleep, and pain are closely interconnected. Insomnia is highly prevalent among patients with chronic pain, with 53–90% of chronic pain patients suffering from a clinically significant degree of insomnia [94–97]. This might in part be attributed to the role of stress. First, daily life stress (e.g. worry about going to work the next morning) can perturb sleep [98]. Likewise, major stressful life events and/or traumatic events such as natural disasters, combat, or a traffic accident result in sleep architecture changes that reflect poor sleep [98]. Increased awakening and decreased sleep efficiency are the most sensitive sleep architecture variables in response to stress [98]. Second, poor sleep (e.g. lying awake at night or being unable to fall asleep) can be a stressor. Third, the consequences of poor sleep (i.e. feeling itchy and fatigued) result in a marked diminished ability to cope with everyday's stressors. For all these reasons, it seems logic that sleep management is often included as part of stress management programs.

When addressing sleeping problems in the context of pain neuroscience, it is important to highlight that a single night of total sleep deprivation in healthy people is able to induce

generalized hyperalgesia and to increase state anxiety [99,100]. These findings suggest that sleep problems might not only perpetuate the central nervous system hyperexcitability in patients with chronic musculoskeletal pain but also serve as an initiating factor. Our current understanding of sleep neuroimmunology provides potential links between sleep difficulties and (the onset of) pain.

Melatonin is a neurohormone crucial for (deep) sleep and analgesia. Preclinical studies revealed that selective MT2 melatonin receptor partial agonists hold analgesic properties through modulation of ON/OFF cells of brainstem descending antinociceptive systems in neuropathic pain models [101,102].

Other neural mechanisms may contribute to the close interaction between chronic pain and poor sleep. While proper sleep facilitates immune health, sleep deprivation results in low-grade inflammatory responses [103–105]. This low-grade inflammatory response as a consequence of sleep deprivation includes increased levels of IL-6, prostaglandin E2 [104,105], and nitric oxide [38] possibly mediated by cerebral microglia [38]. Even low levels of inflammatory cytokines are known to affect brain function [106] which correlates with observations of increased sensitivity to painful stimuli following sleep restriction [100,104,107]. Sleep apnea, sometimes diagnosed in patients with chronic pain, is characterized by intermitted hypoxia, which in turn activates brain microglia toward an activated, pro-inflammatory state [108]. Taken together, sleep deprivation imparts a glia-mediated low-grade inflammatory response leading toward increased sensitivity to pain as typically seen in chronic pain sufferers.

Understanding that aberrant glial activation or gliosis possibly explains the onset of CS, as summarized in Figure 2, raises the question how to translate these findings to therapeutic targets? Likewise, understanding that aberrant glial activation can be due to chronic stress exposure and/or sleep disturbances raises the question how to account for it in clinical practice? The study of glia's role in humans with chronic pain, and the mechanism of CS in particular, is still in its infancy, making it too early to translate these findings to clinical practice. However, thinking of potential therapeutic targets provides new innovative avenues for experimental testing of these ideas in humans with CS and chronic pain within a research setting.

5. Treatments that have the potential to normalize glial activity

Pharmacological treatments like minocycline, an antibiotic, theoretically hold the capacity to target aberrant glial activity in patients with chronic pain and CS. Animal work has demonstrated that (postoperative) minocycline antibiotics suppress microglia and astrocytes and reduce hippocampal TNF-α and IL-1β mRNA levels [109,110]. Likewise, minocycline inhibits spinal microglial activation and attenuate diabetic pain in rats with experimentally induced diabetes [111]. A recent small randomized clinical trial has shown that a brief treatment with minocycline was associated with a small, but statistically significant reduction in pain in patients with lumbar radiculopathy [112]. Since patients with lumbar radiculopathy demonstrate increased glial activity [36], it is possible that minocycline reduces pain by inhibiting microglia in humans, as it does in animals.

Another therapeutic avenue includes pretreatment with β-adrenergic receptor antagonists (e.g. propranolol), which have been shown to reduce microglial activation in an animal model [113]. *In vitro* work showed that ketamine inhibited some of the inflammatory responses of both astrocytes and microglial cells [114]. In addition, cannabidiol, a major non-psychotomimetic constituent of *Cannabis sativa*, inhibits microglial activation and consequent neuroinflammation [115]. Still, for all these treatments, it remains to be established whether they are able to normalize glial activity in humans, and if so, whether such glial effects are accompanied by analgesic effects (Table 1).

In addition, inhibitors of cytokine synthesis closely linked to glial activity, including propentofylline, have been explored for the use as therapeutic agents for the treatment of neuropathic pain [116]. Human microglia were less responsive to propentofylline treatment [116], casting doubt whether direct microglial inhibition is a relevant therapeutic target for patients with chronic pain. However, methodological concerns with these studies (the length of the trial, the dosage used, the specific

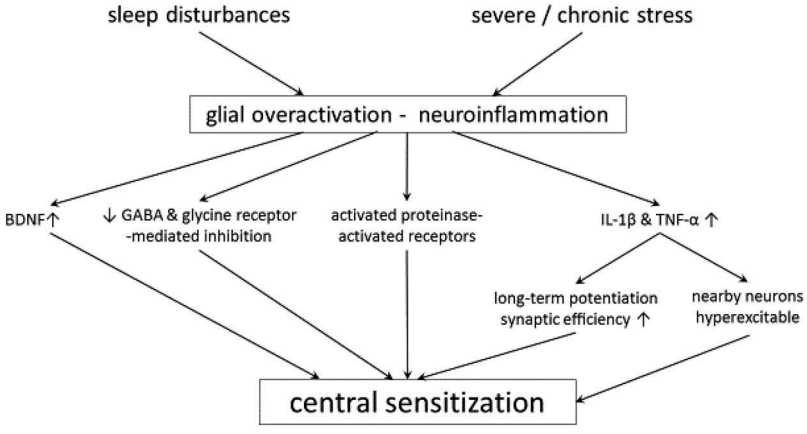

Figure 2. Mechanisms linking glial overactivation to the development of central sensitization. The displayed mechanisms are mainly based on animal studies.
BDNF: brain-derived neurotrophic factor; GABA: gamma-amino-butyric acid; IL: interleukin; TNF: tumor necrosis factor.

Table 1. Potential therapeutic avenues for normalizing glial activity in patients with chronic pain.

Name of treatment	Type of treatment	Mechanism of action
Minocycline	Antibiotic	Suppresses microglia and astrocytes [109,110]
Propranolol	β-Adrenergic receptor antagonist	Reduces microglial activation [113]
Ketamine	NMDA blocker	Inhibits inflammatory responses of astrocytes and microglia [114]
Propentofylline	Atypical methylxanthine	Inhibits cytokine synthesis [116]
Cannabidiol	A major non-psychotomimetic constituent of *Cannabis sativa*	Inhibits microglial activation [115]
Stress management	Conservative intervention	Prevents (further) glial (over)activation?
Sleep management	Conservative intervention	Prevents (further) glial (over)activation?
Exercise therapy	Conservative intervention	Reverses astrocyte and microglia hyperactivity [117]

The mechanisms of action are currently based on animal studies mainly.
Any therapeutic choice of pharmacological option for normalizing glial activity in patients with chronic pain will rely on many factors, including the type of chronic pain.
NMDA: *N*-methyl-D-aspartate.

population targeted, and potential interaction with other drugs or food intake) limit the significance of these negative study outcomes [118].

Also, the reasoning outlined above regarding the possible role of aberrant glial activity and neuroinflammation in relation to CS is focused primarily on the onset of CS. If CS has been initiated and the aberrant glial activity and neuroinflammation has been present for a few months or even longer, this has likely resulted in neuroplastic changes including some of the above-discussed changes in brain morphology or connectivity. When neuroplastic changes are already established, targeting aberrant glial activity might be too late or at best can prevent further acceleration of CS in chronic pain patients, rather than serving as a new potential treatment for chronic pain. This reasoning is supported by animal work, showing that after spinal nerve ligation, dorsal horn microglia are activated first, followed by the activation of astrocytes, accompanied by a decrease in microglial activity [119]. Hence, targeting the aberrant glial activity might be a new therapeutic avenue for the prevention of the development, rather than the treatment of CS and chronic pain. A preclinical study found positive outcome when glia-suppressing drugs are delivered in the early postoperative phase [110], but this idea requires experimental testing in humans. The possibility to image glial activation *in vivo* [36] may help to identify patients most likely to benefit from this therapeutic approach, and to identify optimal treatment window, duration, or dosage.

6. Conclusion

An increasing amount of animal research data support the idea of aberrant glial activity as a potential underlying, even etiological, mechanism of CS. Such glial overactivation results in a low-grade neuroinflammatory state, characterized by high levels of BDNF, IL-1β, and TNF-α, which in turn increases the excitability of the central nervous system neurons through mechanisms like long-term potentiation and increased synaptic efficiency. Other mechanisms linking aberrant glial activation to the development of CS include attenuation of the nociceptive inhibitory action of GABA and glycine receptor-mediated inhibition. In addition, aberrant glial activity in chronic pain might have been triggered by severe stress exposure, and/or sleeping disturbances, each of which are established initiating factors for chronic pain development. Potential treatment avenues include several pharmacological options for diminishing glial activity, as well as conservative interventions like sleep management, stress management, and exercise therapy. Before translating these findings from basic science to clinical settings, more human studies exploring the outlined mechanisms in chronic pain patients are needed.

The present work is focused on the role of the glia. Other neuro-immune pathways also exist. For instance, in addition to stress and sleep problems, animal work suggests that neurotrauma results in aberrant glial activation [120]. Also, neuroinflammation observed in animals in response to stress may not be attributed solely to glial activation: stress also induces monocyte trafficking to the brain (e.g. from the bone marrow to the amygdala), resulting in inflammation (i.e. IL-1)-mediated anxiety-like behavior [39,40]. This increased monocyte recruitment to the brain is a unique pathway in which the immune system communicates with the brain [39]. In addition, animal research findings revealed gender differences, including a microglia-independent pathway to mediate pain hypersensitivity [37]. According to those studies, microglia are not required for the development of mechanical pain hypersensitivity in female mice, as they achieved similar levels of pain hypersensitivity using adaptive immune cells, likely T lymphocytes [37,121,122]. Monocyte trafficking might be the preferred pathway for mediating pain hypersensitivity in females [39].

The present review focused on explaining the underlying mechanisms of CS, a mechanism now established to contribute to wide variety of pathologies. It is difficult to combine findings from a large range of different pathologies, studies at different molecular levels and from various types of experiments in one paper. However, the paper aims at creating a bridge between preclinical and clinical data, in order to contribute to translational efforts in the field (or at least stimulate translational work in this area).

7. Expert opinion: non-pharmacological treatment options to normalize glial activity

We emphasized the role of severe stress and sleeping disturbances in triggering aberrant glial activity, implying that aberrant glial activity is not the cause, but instead one part in the long chain of CS initiating events. Hence, conservative interventions like *stress* and *sleep management* seem warranted.

Within the field of conservative interventions, *exercise* is another intervention that is helpful in many chronic pain

states and could also be working in part via glial mechanisms. Exercise increases glial fibrillary acidic protein expression in hippocampal astrocytes, more specifically in the stratum radiatum, a region that contains numerous astrocytes and is important for learning and memory [123]. The increased expression of the astrocytic marker 'glial fibrillary acidic protein' implies that exercise results in a substantial increase in astroglial metabolism and protein synthesis, consistent with a healthy cellular hypertrophy in response to increased physiological demands [123]. This notion is supported by the observed change in morphology of the astrocytes in response to exercise [123]. This astrocyte activation in response to exercise can be explained by the increased production of growth factors like nerve growth factor, fibroblast growth factor, glial cell line-derived neurotrophic factor, and BDNF in response to exercise [42,124,125] – e.g. nerve growth factor and fibroblast growth factor are capable of inducing astrocytic proliferation. Astrocytic activation in response to exercise implies strengthened 'tripartite' synapses (astrocyte–presynaptic neuron–postsynaptic neuron), as astrocytes are needed (and change their morphology) around potentiated synapses to accompany neuronal plasticity as seen during long-term potentiation [123].

The immune-modifying, more specifically whole-body anti-inflammatory, effects of moderate *physical activity*/exercise are now well established [42]. Exercise is known to change the inflammatory state to become anti-inflammatory or neuroprotective in several neuroinflammatory diseases such as multiple sclerosis and systemic lupus erythematous [45]. Importantly, new research findings suggest that exercise can also have anti-inflammatory effects on the central nervous system [42,45]. More specifically, it seems plausible that exercise (therapy) can diminish the adverse and nonspecific activation of glial cells (gliosis) as typically seen in chronic neuroinflammation [45]. At the level of the glial cells, physical activity/exercise reduces microglial proliferation and triggers a switch toward an anti-inflammatory phenotype [42]. Such a phenotype shift is accompanied by a dramatic change in production of cytokines (i.e. from pro- to anti-inflammatory). This provides a plausible explanation for how regular and moderate exercise maintains glial activity within the healthy range, which in turn might contribute to the reduced incidence of brain disease (characterized by chronic neuroinflammation) in people who exercise regularly [42]. Still, evidence that physical activity/exercise alters glial activity in human brain areas involved in pain integration and perception is currently unavailable and represents an important focus for future research.

It remains uncertain whether we can apply this reasoning of anti-neuroinflammatory effects of exercise therapy to the treatment of CS in patients with chronic pain. One mice study supports this idea: physical training (i.e. swim exercise) in mice after nerve lesion reversed mechanical hypersensitivity, normalized nerve injury-induced nerve growth factor, and BDNF expression in the dorsal root ganglion, reversed astrocyte and microglia hyperactivity in the dorsal horn, which remained normalized after training cessation [117]. Another preclinical study showed that prolonged exercise normalizes early microglia- and astrocytes-mediated brain inflammation following myocardial infarction induction [126]. Also, the possible anti-neuroinflammatory effects of exercise therapy to the treatment of CS in patients with chronic pain are supported by studies showing decreased pain sensitivity following exercise therapy in chronic pain patients [127,128]. A final pathway through which exercise can benefit glial health [129] includes the stimulation of glial heat shock protein 72 expression [129]. Exercise induced increased neuronal and astroglial levels of heat shock protein 72 in normal spinal cord tissue, which facilitated functional recovery after experimental spinal cord injury [129]. Animal work has informed us that increased glial expression of heat shock protein 72 has anti-inflammatory effects and protects against astroglial apoptosis [129,130].

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain. 2011;152:S2–15.
2. Merskey H, NBatITFo T. Part III: pain terms, a current list with definitions and notes on usage. In: Merskey H, NBatITFo T, editor. Classification of chronic pain. 2nd ed. Seattle, USA: IASP Press; 1994. p. 209–214.
3. Rojewska E, Popiolek-Barczyk K, Jurga AM, et al. Involvement of pro- and antinociceptive factors in minocycline analgesia in rat neuropathic pain model. J Neuroimmunol. 2014;277:57–66.
4. Van Oosterwijck J, Nijs J, Meeus M, et al. Evidence for central sensitization in chronic whiplash: a systematic literature review. Eur J Pain. 2013;17:299–312.
5. Roussel NA, Nijs J, Meeus M, et al. Central sensitization and altered central pain processing in chronic low back pain: fact or myth? Clin J Pain. 2013;29:625–638.
6. Lluch Girbes E, Nijs J, Torres-Cueco R, et al. Pain treatment for patients with osteoarthritis and central sensitization. Phys Ther. 2013;93:842–851.
7. Perrotta A, Serrao M, Sandrini G, et al. Sensitisation of spinal cord pain processing in medication overuse headache involves supraspinal pain control. Cephalalgia. 2010;30:272–284.
8. Caro-Moran E, Diaz-Rodriguez L, Cantarero-Villanueva I, et al. Nerve pressure pain hypersensitivity and upper limb mechanosensitivity

in breast cancer survivors: a case-control study. Pain Med. 2014;15:1715–1723.
9. Price DD, Staud R, Robinson ME, et al. Enhanced temporal summation of second pain and its central modulation in fibromyalgia patients. Pain. 2002;99:49–59.
10. Sanchis MN, Lluch E, Nijs J, et al. The role of central sensitization in shoulder pain: a systematic literature review. Seminars in arthritis and rheumatism. 2015;44:710–716.
11. Nijs J, Meeus M, Van Oosterwijck J, et al. In the mind or in the brain? Scientific evidence for central sensitisation in chronic fatigue syndrome. Eur J Clin Invest. 2012;42:203–212.
12. Meeus M, Vervisch S, De Clerck LS, et al. Central sensitization in patients with rheumatoid arthritis: a systematic literature review. Semin Arthritis Rheum. 2012;41:556–567.
13. Raphael KG, Janal MN, Anathan S, et al. Temporal summation of heat pain in temporomandibular disorder patients. J Orofac Pain. 2009;23:54–64.
14. Van Wilgen CP, Konopka KH, Keizer D, et al. Do patients with chronic patellar tendinopathy have an altered somatosensory profile? - A Quantitative Sensory Testing (QST) study. Scand J Med Sci Sports. 2013 Mar;23(2):149–155. doi: 10.1111/j.1600-0838.2011.01375.x.
15. Cornelison LE, Hawkins JL, Durham PL. Elevated levels of calcitonin gene-related peptide in upper spinal cord promotes sensitization of primary trigeminal nociceptive neurons. Neuroscience. 2016;339:491–501.
16. Coombes BK, Bisset L, Vicenzino B. Thermal hyperalgesia distinguishes those with severe pain and disability in unilateral lateral epicondylalgia. Clin J Pain. 2012;28:595–601.
17. Fernandez-Carnero J, Fernandez-de-Las-Penas C, De La Llave-Rincon AI, et al. Widespread mechanical pain hypersensitivity as sign of central sensitization in unilateral epicondylalgia: a blinded, controlled study. Clin J Pain. 2009;25:555–561.
18. Nijs J, Van Houdenhove B, Oostendorp RA. Recognition of central sensitization in patients with musculoskeletal pain: application of pain neurophysiology in manual therapy practice. Man Ther. 2010;15:135–141.
19. Baranauskas G, Nistri A. Sensitization of pain pathways in the spinal cord: cellular mechanisms. Prog Neurobiol. 1998;54:349–365.
20. Staud R, Craggs JG, Perlstein WM, et al. Brain activity associated with slow temporal summation of C-fiber evoked pain in fibromyalgia patients and healthy controls. Eur J Pain. 2008;12:1078–1089.
21. Seifert F, Maihofner C. Central mechanisms of experimental and chronic neuropathic pain: findings from functional imaging studies. Cell Mol Life Sci. 2009;66:375–390.
22. Becerra L, Veggeberg R, Prescot A, et al. A 'complex' of brain metabolites distinguish altered chemistry in the cingulate cortex of episodic migraine patients. NeuroImage Clin. 2016;11:588–594.
23. Yarnitsky D. Conditioned pain modulation (the diffuse noxious inhibitory control-like effect): its relevance for acute and chronic pain states. Curr Opin Anaesthesiol. 2010;23:611–615.
24. Silva M, Costa-Pereira JT, Martins D, et al. Pain modulation from the brain during diabetic neuropathy: uncovering the role of the rostroventromedial medulla. Neurobiol Dis. 2016;96:346–356.
25. Ma QP, Woolf CJ. Noxious stimuli induce an N-methyl-D-aspartate receptor-dependent hypersensitivity of the flexion withdrawal reflex to touch: implications for the treatment of mechanical allodynia. Pain. 1995;61:383–390.
26. Neumann S, Doubell TP, Leslie T, et al. Inflammatory pain hypersensitivity mediated by phenotypic switch in myelinated primary sensory neurons. Nature. 1996;384:360–364.
27. Nijs J, Torres-Cueco R, Van Wilgen CP, et al. Applying modern pain neuroscience in clinical practice: criteria for the classification of central sensitization pain. Pain Physician. 2014;17:447–457.
28. Neblett R, Cohen H, Choi Y, et al. The Central Sensitization Inventory (CSI): establishing clinically significant values for identifying central sensitivity syndromes in an outpatient chronic pain sample. J Pain. 2013;14:438–445.
29. Van Houdenhove B, Egle UT. Fibromyalgia: a stress disorder? Piecing the biopsychosocial puzzle together. Psychother Psychosom. 2004;73:267–275.
30. Austin PJ, Moalem-Taylor G. The neuro-immune balance in neuropathic pain: involvement of inflammatory immune cells, immune-like glial cells and cytokines. J Neuroimmunol. 2010;229:26–50.
31. Delpech JC, Madore C, Nadjar A, et al. Microglia in neuronal plasticity: influence of stress. Neuropharmacology. 2015;96:19–28.
32. Fields RD, Araque A, Johansen-Berg H, et al. Glial biology in learning and cognition. Neuroscientist. 2014;20:426–431.
33. Clarke LE, Barres BA. Emerging roles of astrocytes in neural circuit development. Nat Reviews Neurosci. 2013;14:311–321.
34. Agostini S, Eutamene H, Cartier C, et al. Evidence of central and peripheral sensitization in a rat model of narcotic bowel-like syndrome. Gastroenterology. 2010;139:553-63, 63 e1-5.
35. Bjurstrom MF, Giron SE, Griffis CA. Cerebrospinal fluid cytokines and neurotrophic factors in human chronic pain populations: a comprehensive review. Pain Pract. 2016;16:183–203.
36. Loggia ML, Chonde DB, Akeju O, et al. Evidence for brain glial activation in chronic pain patients. Brain: J Neurol. 2015;138:604–615.
37. Sorge RE, Mapplebeck JC, Rosen S, et al. Different immune cells mediate mechanical pain hypersensitivity in male and female mice. Nat Neurosci. 2015;18:1081–1083.
38. Wisor JP, Schmidt MA, Clegern WC. Cerebral microglia mediate sleep/wake and neuroinflammatory effects of methamphetamine. Brain Behav Immun. 2011;25:767–776.
39. Reader BF, Jarrett BL, McKim DB, et al. Peripheral and central effects of repeated social defeat stress: monocyte trafficking, microglial activation, and anxiety. Neuroscience. 2015;289:429–442.
40. Wohleb ES, McKim DB, Sheridan JF, et al. Monocyte trafficking to the brain with stress and inflammation: a novel axis of immune-to-brain communication that influences mood and behavior. Front Neurosci. 2014;8:447.
•• **Must read for everyone keen to learn more about immune–brain communication.**
41. Gritsch S, Lu J, Thilemann S, et al. Oligodendrocyte ablation triggers central pain independently of innate or adaptive immune responses in mice. Nat Commun. 2014;5:5472.
42. Spielman LJ, Little JP, Klegeris A. Physical activity and exercise attenuate neuroinflammation in neurological diseases. Brain Res Bull. 2016;125:19–29.
•• **Hallmark paper regarding the potential anti-inflammatory action of physical activity/exercise in the nervous system in health and disease.**
43. Li W, Ma L, Yang G, et al. REM sleep selectively prunes and maintains new synapses in development and learning. Nat Neurosci. 2017;20:427–437.
44. Fontainhas AM, Wang M, Liang KJ, et al. Microglial morphology and dynamic behavior is regulated by ionotropic glutamatergic and GABAergic neurotransmission. PloS One. 2011;6:e15973.
45. Svensson M, Lexell J, Deierborg T. Effects of physical exercise on neuroinflammation, neuroplasticity, neurodegeneration, and behavior: what we can learn from animal models in clinical settings. Neurorehabil Neural Repair. 2015;29:577–589.
46. Taves S, Berta T, Liu DL, et al. Spinal inhibition of p38 MAP kinase reduces inflammatory and neuropathic pain in male but not female mice: sex-dependent microglial signaling in the spinal cord. Brain Behav Immun. 2016;55:70–81.
47. Kiyomoto M, Shinoda M, Honda K, et al. p38 phosphorylation in medullary microglia mediates ectopic orofacial inflammatory pain in rats. Mol Pain. 2015;11:48.
48. Crotti A, Ransohoff RM. Microglial physiology and pathophysiology: insights from genome-wide transcriptional profiling. Immunity. 2016;44:505–515.
49. Wang ML, Li WB. Cognitive impairment after traumatic brain injury: the role of MRI and possible pathological basis. J Neurol Sci. 2016;370:244–250.

50. Liu C, Li CP, Wang JJ, et al. RNCR3 knockdown inhibits diabetes mellitus-induced retinal reactive gliosis. Biochem Biophys Res Commun. 2016;479:198–203.
51. Campbell JG, Miller DC, Cundiff DD, et al. Neural stem/progenitor cells react to non-glial cns neoplasms. SpringerPlus. 2015;4:53.
52. Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? Pain. 2013;154(Suppl 1):S10–28.
53. Guida F, Luongo L, Aviello G, et al. Salvinorin A reduces mechanical allodynia and spinal neuronal hyperexcitability induced by peripheral formalin injection. Mol Pain. 2012;8:60.
54. Luongo L, Guida F, Boccella S, et al. Palmitoylethanolamide reduces formalin-induced neuropathic-like behaviour through spinal glial/microglial phenotypical changes in mice. CNS Neurol Disord Drug Targets. 2013;12:45–54.
55. Clark AK, Staniland AA, Malcangio M. Fractalkine/CX3CR1 signalling in chronic pain and inflammation. Curr Pharm Biotechnol. 2011;12:1707–1714.
56. Smith PA. BDNF: no gain without pain?. Neuroscience. 2014 Dec 26;283:107–123. doi: 10.1016/j.neuroscience.2014.05.044.
57. Ferrini F, De Koninck Y. Microglia control neuronal network excitability via BDNF signalling. Neural Plast. 2013;2013:429815.
58. Nijs J, Meeus M, Versijpt J, et al. Brain-derived neurotrophic factor as a driving force behind neuroplasticity in neuropathic and central sensitization pain: a new therapeutic target? Expert Opin Ther Targets. 2015;19:565–576.
59. Gao YJ, Ji RR. Activation of JNK pathway in persistent pain. Neurosci Lett. 2008;437:180–183.
60. Ji RR, Kohno T, Moore KA, et al. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci. 2003;26:696–705.
61. Zhou LJ, Yang T, Wei X, et al. Brain-derived neurotrophic factor contributes to spinal long-term potentiation and mechanical hypersensitivity by activation of spinal microglia in rat. Brain Behav Immun. 2011;25:322–334.
62. Zhuo M. A synaptic model for pain: long-term potentiation in the anterior cingulate cortex. Mol Cells. 2007;23:259–271.
63. Apkarian AV, Mutso AA, Centeno MV, et al. Role of adult hippocampal neurogenesis in persistent pain. Pain. 2016;157:418–428.
64. Nijs J, Lluch Girbes E, Lundberg M, et al. Exercise therapy for chronic musculoskeletal pain: innovation by altering pain memories. Man Ther. 2015;20:216–220.
65. Ji RR, Berta T, Nedergaard M. Glia and pain: is chronic pain a gliopathy? Pain. 2013;154(Suppl):1.
66. Ji RR, Kawasaki Y, Zhuang ZY, et al. Possible role of spinal astrocytes in maintaining chronic pain sensitization: review of current evidence with focus on bFGF/JNK pathway. Neuron Glia Biol. 2006;2:259–269.
67. Ren K, Dubner R. Interactions between the immune and nervous systems in pain. Nat Med. 2010;16:1267–1276.
68. Guo W, Wang H, Watanabe M, et al. Glial-cytokine-neuronal interactions underlying the mechanisms of persistent pain. J Neurosci. 2007;27:6006–6018.
69. Okada-Ogawa A, Suzuki I, Sessle BJ, et al. Astroglia in medullary dorsal horn (trigeminal spinal subnucleus caudalis) are involved in trigeminal neuropathic pain mechanisms. J Neurosci. 2009;29:11161–11171.
70. Meller ST, Dykstra C, Grzybycki D, et al. The possible role of glia in nociceptive processing and hyperalgesia in the spinal cord of the rat. Neuropharmacology. 1994;33:1471–1478.
71. Chen G, Park CK, Xie RG, et al. Connexin-43 induces chemokine release from spinal cord astrocytes to maintain late-phase neuropathic pain in mice. Brain. 2014;137:2193–2209.
72. Luongo L, Guida F, Imperatore R, et al. The A1 adenosine receptor as a new player in microglia physiology. Glia. 2014;62:122–132.
73. Liu S, Liu YP, Yue DM, et al. Protease-activated receptor 2 in dorsal root ganglion contributes to peripheral sensitization of bone cancer pain. Eur J Pain. 2014 Mar;18(3):326–337. doi: 10.1002/j.1532-2149.2013.00372.x..
74. Grant AD, Cottrell GS, Amadesi S, et al. Protease-activated receptor 2 sensitizes the transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice. J Physiol. 2007;578:715–733.
75. Amadesi S, Nie J, Vergnolle N, et al. Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. J Neuroscience: Official Journal Soc Neurosci. 2004;24:4300–4312.
76. Rothmeier AS, Ruf W. Protease-activated receptor 2 signaling in inflammation. Semin Immunopathol. 2012;34:133–149.
77. Bao Y, Hou W, Liu R, et al. PAR2-mediated upregulation of BDNF contributes to central sensitization in bone cancer pain. Mol Pain. 2014;10:28.
78. Watkins LR, Martin D, Ulrich P, et al. Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the rat. Pain. 1997;71:225–235.
79. Banati RB, Cagnin A, Brooks DJ, et al. Long-term trans-synaptic glial responses in the human thalamus after peripheral nerve injury. Neuroreport. 2001;12:3439–3442.
80. Schouten M, Aschrafi A, Bielefeld P, et al. microRNAs and the regulation of neuronal plasticity under stress conditions. Neuroscience. 2013;241:188–205.
81. Rosenbloom BN, Katz J, Chin KY, et al. Predicting pain outcomes after traumatic musculoskeletal injury. Pain. 2016;157:1733–1743.
82. Tesarz J, Eich W, Treede RD, et al. Altered pressure pain thresholds and increased wind-up in adult patients with chronic back pain with a history of childhood maltreatment: a quantitative sensory testing study. Pain. 2016;157:1799–1809.
83. Anderson G, Maes M. Schizophrenia: linking prenatal infection to cytokines, the tryptophan catabolite (TRYCAT) pathway, NMDA receptor hypofunction, neurodevelopment and neuroprogression. Prog Neuropsychopharmacol Biol Psychiatry. 2013;42:5–19.

•• **Key paper for understanding the influence of stress on brain neuroplasticity.**

84. Radley J, Morilak D, Viau V, et al. Chronic stress and brain plasticity: mechanisms underlying adaptive and maladaptive changes and implications for stress-related CNS disorders. Neurosci Biobehav Rev. 2015;58:79–91.
85. Vyas A, Mitra R, Shankaranarayana Rao BS, et al. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neuroscience: Official Journal Soc Neurosci. 2002;22:6810–6818.

•• **Key for those keen to learn more about how severe stress affects various brain areas differently.**

86. Zandieh S, Bernt R, Knoll P, et al. Analysis of the metabolic and structural brain changes in patients with torture-related post-traumatic stress disorder (TR-PTSD) Using (1, 8)F-FDG PET and MRI. Medicine. 2016;95:e3387.
87. Gracely RH, Geisser ME, Giesecke T, et al. Pain catastrophizing and neural responses to pain among persons with fibromyalgia. Brain: J Neurol. 2004;127:835–843.
88. Li Z, Wang J, Chen L, et al. Basolateral amygdala lesion inhibits the development of pain chronicity in neuropathic pain rats. PloS One. 2013 Aug 5;8(8):e70921. doi: 10.1371/journal.pone.0070921. Print 2013.
89. Lutz J, Jager L, De Quervain D, et al. White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. Arthritis Rheum. 2008;58:3960–3969.
90. McEwen BS, Eiland L, Hunter RG, et al. Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. Neuropharmacology. 2012;62:3–12.

• **Important piece of work regarding epigenetic changes in the brain in response to stress.**

91. Leuner B, Shors TJ. Stress, anxiety, and dendritic spines: what are the connections? Neuroscience. 2013;251:108–119.
92. Jungquist CR, O'Brien C, Matteson-Rusby S, et al. The efficacy of cognitive-behavioral therapy for insomnia in patients with chronic pain. Sleep Med. 2010;11:302–309.

93. Pigeon WR, Moynihan J, Matteson-Rusby S, et al. Comparative effectiveness of CBT interventions for co-morbid chronic pain & insomnia: a pilot study. Behav Res Ther. 2012;50:685–689.
94. Becker N, Bondegaard Thomsen A, Olsen AK, et al. Pain epidemiology and health related quality of life in chronic non-malignant pain patients referred to a Danish multidisciplinary pain center. Pain. 1997;73:393–400.
95. McCracken LM, Iverson GL. Disrupted sleep patterns and daily functioning in patients with chronic pain. Pain Res & Manage. 2002;7:75–79.
96. Tang NK, Wright KJ, Salkovskis PM. Prevalence and correlates of clinical insomnia co-occurring with chronic back pain. J Sleep Res. 2007;16:85–95.
97. Daly-Eichenhardt A, Scott W, Howard-Jones M, et al. Changes in sleep problems and psychological flexibility following interdisciplinary acceptance and commitment therapy for chronic pain: an observational cohort study. Front Psychol. 2016;7:1326.
98. Kim EJ, Dimsdale JE. The effect of psychosocial stress on sleep: a review of polysomnographic evidence. Behav Sleep Med. 2007;5:256–278.
99. Onen SH, Alloui A, Gross A, et al. The effects of total sleep deprivation, selective sleep interruption and sleep recovery on pain tolerance thresholds in healthy subjects. J Sleep Res. 2001;10:35–42.
100. Schuh-Hofer S, Wodarski R, Pfau DB, et al. One night of total sleep deprivation promotes a state of generalized hyperalgesia: a surrogate pain model to study the relationship of insomnia and pain. Pain. 2013;154:1613–1621.
101. Lopez-Canul M, Palazzo E, Dominguez-Lopez S, et al. Selective melatonin MT2 receptor ligands relieve neuropathic pain through modulation of brainstem descending antinociceptive pathways. Pain. 2015;156:305–317.
102. Lopez-Canul M, Comai S, Dominguez-Lopez S, et al. Antinociceptive properties of selective MT(2) melatonin receptor partial agonists. Eur J Pharmacol. 2015;764:424–432.
103. Mullington JM, Simpson NS, Meier-Ewert HK, et al. Sleep loss and inflammation. Best Pract Res Clin Endocrinol Metab. 2010;24:775–784.
104. Haack M, Lee E, Cohen DA, et al. Activation of the prostaglandin system in response to sleep loss in healthy humans: potential mediator of increased spontaneous pain. Pain. 2009;145:136–141.
105. Haack M, Sanchez E, Mullington JM. Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers. Sleep. 2007;30:1145–1152.
106. Pollmacher T, Haack M, Schuld A, et al. Low levels of circulating inflammatory cytokines–do they affect human brain functions? Brain Behav Immun. 2002;16:525–532.
107. Wodarski R, Schuh-Hofer S, Yurek DA, et al. Development and pharmacological characterization of a model of sleep disruption-induced hypersensitivity in the rat. Eur J Pain. 2015;19:554–566.
108. Kiernan EA, Smith SM, Mitchell GS, et al. Mechanisms of microglial activation in models of inflammation and hypoxia: implications for chronic intermittent hypoxia. J Physiol. 2016;594:1563–1577.
109. Hou Y, Xie G, Liu X, et al. Minocycline protects against lipopolysaccharide-induced cognitive impairment in mice. Psychopharmacology. 2016;233:905–916.
110. Jin WJ, Feng SW, Feng Z, et al. Minocycline improves postoperative cognitive impairment in aged mice by inhibiting astrocytic activation. Neuroreport. 2014;25:1–6.
111. Sun JS, Yang YJ, Zhang YZ, et al. Minocycline attenuates pain by inhibiting spinal microglia activation in diabetic rats. Mol Med Rep. 2015;12:2677–2682.
112. Vanelderen P, Van Zundert J, Kozicz T, et al. Effect of minocycline on lumbar radicular neuropathic pain: a randomized, placebo-controlled, double-blind clinical trial with amitriptyline as a comparator. Anesthesiology. 2015;122:399–406.
113. Wohleb ES, Hanke ML, Corona AW, et al. beta-Adrenergic receptor antagonism prevents anxiety-like behavior and microglial reactivity induced by repeated social defeat. J Neuroscience: Official Journal Soc Neurosci. 2011;31:6277–6288.
114. Shibakawa YS, Sasaki Y, Goshima Y, et al. Effects of ketamine and propofol on inflammatory responses of primary glial cell cultures stimulated with lipopolysaccharide. Br J Anaesth. 2005;95:803–810.
115. Gomes FV, Llorente R, Del Bel EA, et al. Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol. Schizophr Res. 2015;164:155–163.
116. Landry RP, Jacobs VL, Romero-Sandoval EA, et al. Propentofylline, a CNS glial modulator does not decrease pain in post-herpetic neuralgia patients: *in vitro* evidence for differential responses in human and rodent microglia and macrophages. Exp Neurol. 2012;234:340–350.
117. Almeida C, DeMaman A, Kusuda R, et al. Exercise therapy normalizes BDNF upregulation and glial hyperactivity in a mouse model of neuropathic pain. Pain. 2015;156:504–513.
•• **Inspiring paper that potentially explains why and how exercise can diminish central sensitization in chronic pain.**
118. Commentary on Landry; Watkins LR, Hutchinson MR, Johnson KW, et al. "Propentofylline, a CNS glial modulator, does not decrease pain in post-herpetic neuralgia patients: *in vitro* evidence for differential responses in human and rodent microglia and macrophages". Exp Neurol. 2012;234:351–353.
119. Zhuang ZY, Gerner P, Woolf CJ, et al. ERK is sequentially activated in neurons, microglia, and astrocytes by spinal nerve ligation and contributes to mechanical allodynia in this neuropathic pain model. Pain. 2005;114:149–159.
120. Wilhelmsson U, Bushong EA, Price DL, et al. Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury. Proceedings of the National Academy of Sciences of the United States of America. 2006;103:17513–17518.
121. Sorge RE, LaCroix-Fralish ML, Tuttle AH, et al. Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. J Neuroscience: Official Journal Soc Neurosci. 2011;31:15450–15454.
122. Rosen S, Ham B, Mogil JS. Sex differences in neuroimmunity and pain. J Neurosci Res. 2017;95:500–508.
123. Saur L, Baptista PP, De Senna PN, et al. Physical exercise increases GFAP expression and induces morphological changes in hippocampal astrocytes. Brain Struct Funct. 2014;219:293–302.
124. Coelho FG, Gobbi S, Andreatto CA, et al. Physical exercise modulates peripheral levels of brain-derived neurotrophic factor (BDNF): a systematic review of experimental studies in the elderly. Arch Gerontol Geriatr. 2013;56:10–15.
125. Knaepen K, Goekint M, Heyman EM, et al. Neuroplasticity - exercise-induced response of peripheral brain-derived neurotrophic factor: a systematic review of experimental studies in human subjects. Sports Med. 2010;40:765–801.
126. Rinaldi B, Guida F, Furiano A, et al. Effect of prolonged moderate exercise on the changes of nonneuronal cells in early myocardial infarction. Neural Plast. 2015;2015:265967.
127. Henriksen M, Klokker L, Graven-Nielsen T, et al. Association of exercise therapy and reduction of pain sensitivity in patients with knee osteoarthritis: a randomized controlled trial. Arthritis Care Res (Hoboken). 2014;66:1836–1843.
128. Andersen LL, Andersen CH, Sundstrup E, et al. Central adaptation of pain perception in response to rehabilitation of musculoskeletal pain: randomized controlled trial. Pain Physician. 2012;15:385–394.
129. Chang CK, Chou W, Lin HJ, et al. Exercise preconditioning protects against spinal cord injury in rats by upregulating neuronal and astroglial heat shock protein 72. Int J Mol Sci. 2014;15:19018–19036.
130. Sheppard PW, Sun X, Khammash M, et al. Overexpression of heat shock protein 72 attenuates NF-kappaB activation using a combination of regulatory mechanisms in microglia. PLoS Comput Biol. 2014;10:e1003471.