Burke N.N., Finn DP, Roche M (2015)

Neuroinflammatory mechanisms linking Pain and Depression: *Pain in Psychiatric Disorders*

Mod Trends Pharmacopsychiatry. vol 30 DOI: 10.1159/000435931

Neuroinflammatory mechanisms linking Pain and Depression

Authors: Nikita N Burke[1,2,3], David P Finn[2,3], Michelle Roche[1,3]

[1]Physiology, [2]Pharmacology and Therapeutics, School of Medicine, [3]Galway Neuroscience Centre and Centre for Pain Research, National University of Ireland, Galway, Ireland.

Running title: Neuroinflammation in Depression-Pain

Corresponding author: Michelle Roche, Physiology, School of Medicine, National University of Ireland Galway, University Road, Galway, Ireland

Tel: 00353 91 495427

email: michelle.roche@nuigalway.ie

Abstract

Depression and chronic pain have been estimated to co-occur in up to 80% of patients suffering from these disorders, with this co-morbidity being more disabling and more expensive to both patients and society than either disorder alone. A number of neural substrates have been proposed to underlie this association, however there has been increased interest and support for a role of neuroimmune and neuroinflammatory mechanism as key players in this dyad. This chapter will provide an overview of the clinical and preclinical data supporting a role for neuroimmune alterations in depression-pain co-morbidity. We propose that such changes may impact on the functioning of key brain regions modulating emotional and nociceptive processing, thus resulting in the behavioural, psychological and physical symptoms observed in patients exhibiting depression and co-morbid pain.

Introduction to depression-pain co-morbidity

Depression is one of the most prevalent forms of psychiatric illness and is associated with significant disability, impaired health-related quality of life, and high mortality [1, 2]. Depressed patients display a constellation of psychological (depressed mood, anhedonia, feelings of worthlessness or guilt, altered concentration, and suicidality) and physical (altered appetite and weight, disturbed sleep, psychomotor agitation or retardation, and fatigue) symptoms [3]. In addition to being a debilitating disorder in its own right, depression is frequently associated with other disorders including neurological conditions, cancer, cardiovascular disease and chronic pain. For example, depression and chronic pain have been estimated to co-occur in up to 80% of patients [4] and the combination is more disabling and costlier to both patients and society than either disorder alone [5]. Thus, there is an urgent need to understand the underlying mechanisms facilitating depression-pain comorbidity in order to ensure appropriate treatment and facilitate the development of novel treatments. A number of potential common neural substrates have been implicated in the comorbidity of these conditions, including neurotransmitters, neurotrophins, inflammatory mediators and neuroendocrine alterations

[for review see 6], any or all of which may alter neural functioning in key brain regions involved in regulating emotional and nociceptive processing. While ascending and descending pain pathways are critical in the somatosensory aspects of nociception, there is a well-recognised role for cortical and limbic regions in modulating these nociceptive pathways and regulating emotional, cognitive and affective responding to painful stimuli [7-9].

Detailed discussion on the various mediators which may alter activity of supraspinal areas modulating emotional and nociceptive processing is beyond the scope of this chapter and is discussed in detail in several excellent reviews [6, 10, 11]. An abundance of evidence supports a role for altered inflammatory processes in the pathophysiology of chronic pain and psychiatric disorders independently. Increasingly, glial and neuroimmune processes are considered possible mediators in the association between these disorders. This chapter will examine the evidence for a role of neuroinflammation in the depression-pain dyad.

Peripheral Immune Alterations observed in Depression-Pain Co-Morbidity

As highlighted, a wealth of evidence supports a role for immune processes in the pathophysiology of depression and chronic pain individually, and although it may be intuitive that alterations would also be observed in co-morbid patients, few studies have examined whether chronic pain patients with depression or *vice versa*, depressed patients with comorbid pain, exhibit alterations in circulating immune mediators. One recent study examining associations between pain thresholds and peripheral levels of inflammatory mediators in outpatients with major depressive disorder demonstrated elevated plasma levels of the proinflammatory cytokine TNFα and reduced pressure pain thresholds in these patients [12]. Correlative analysis revealed that augmented levels of TNFα correlated significantly with reduced pain thresholds in depressed women, but not men [12]. To our knowledge, this is the only study to date that has examined and shown sexual dimorphic effects between immune mediators, depression, and pain responding. Further support for alterations in inflammatory mediators in

depression-pain co-occurrence arises from a primary care setting study where increased plasma levels of the proinflammatory cytokine IL-6 were associated with greater reported pain in patients exhibiting depressive symptoms, an effect not observed in patients without depressive symptomatology [13]. In addition, patients with chronic back pain and comorbid depression have been shown to exhibit higher plasma IL-6 levels compared to healthy controls [14]. Similarly, depressive symptoms and serum IL-6 levels correlated with pain in patients with burning mouth syndrome [15] and enhanced neuropathic pain was associated with higher depression symptoms and serum C-reactive protein levels in sciatica patients [16]. A recent study demonstrated that in female fibromyalgia patients, depressive symptoms positively correlate with elevated plasma C-reactive protein levels [17]. In addition, chronic lower back pain patients have been reported to have high serum levels of TNFα; and although the presence of comorbid depression did not influence TNFα levels, pain patients with depression benefited more from multidisciplinary treatment than patients without depression [18]. Thus, taken together, the data indicate that depression-pain co-morbidity is associated with enhanced peripheral proinflammatory cytokine levels, effects which may underlie, at least in part the pathophysiology of this association.

Neuroinflammation associated with Depression-Pain co-morbidity

While inflammatory mediators may modulate nociceptive processing at a peripheral level, communication with the central nervous system is required for alterations in affective and emotional processing. Cytokines communicate with the brain via several routes including circumventricular organs, saturable blood-brain barrier transporters and activation of the vagus nerve [for review see 19]. This immune-to-brain signalling influences neurotransmitter metabolism, the HPA axis and neuroendocrine function, synaptic plasticity, and neural circuits, and results in behavioural changes such as depressed mood, fatigue, cognitive dysfunction and hyperalgesia. Glial activation and the subsequent release of proinflammatory cytokines is usually temporary, restrained by anti-inflammatory mechanisms, and is an adaptive survival response. However, chronic and sustained

neuroinflammation may result in pathological changes within the brain and negatively impact on neuronal circuitries. As such, chronic neuroinflammation in key brain regions mediating emotional and nociceptive processing may underlie depression-pain co-morbidity.

While post-mortem studies have indicated region-specific microglial activation and increased cytokine expression in pain- and mood-related brain regions including the thalamus and the PFC, of subjects previously diagnosed with depression [20-23], it is unknown if any of these patients also exhibited altered nociceptive responding or a chronic pain condition. Conversely, to date, post-mortem studies examining neuroinflammatory alterations associated with chronic pain have concentrated on changes within the spinal cord, demonstrating significant activation of both microglia and/or astrocytes and increased inflammatory cytokine expression in these patients [24, 25]. While post-mortem data are useful, a number of confounds can influence the data interpretation. The advent of newer imaging technologies has enabled a more detailed examination of real-time neuroinflammatory processes at a structural and functional level. For example, a recent review highlighted the use of Magnetic Resonance Spectroscopy (MRS) to assess neuroinflammation in painful conditions including low back pain, neuropathy, migraines and fibromyalgia [26]. Using this technology, researchers have found reduced N-acetylaspartate levels (suggesting neuronal injury or loss) and increased myo-inositol (glial activation) in regions such as the frontal cortex and thalamus in a number of chronic pain conditions [26], regions which have also shown neuroinflammation in post-mortem studies of depressed patients. Positron Emission Tomography (PET) and MRS have revealed increased glial activation in the thalamus of patients with limb denervation [27], and in the PFC [28] and thalamus [29] of spinal cord injury patients with neuropathic pain, regions which also play a role in modulating mood. To date, only one study has used this technology to examine microglia activity in a depressed cohort, and although alterations were not detected using currently available PET ligands [30], a number of post-mortem histological and molecular studies [24-27] have indicated that enhanced neuroinflammation is associated with this disorder.

Using this non-invasive approach, a recent study reported the first evidence for supraspinal neuroinflammation in patients exhibiting co-morbid depression and pain symptoms [31]. In this study, microglial activation was assessed in patients with myalgic encephalitis/chronic fatigue syndrome who exhibited pain and depression using PET assessment of the radiotracer 11C-(R)-PK11195. Increased binding of the radiotracer, and thus microglial activation, in the hippocampus was positively correlated with depression scores while binding in the thalamus tended to be correlated positively with pain scores in these patients, indicating that region-specific neuroinflammation may be associated with symptoms of depression and pain. It should be noted that while patients exhibiting depression-pain symptomatology exhibited robust microglial activation, only mild peripheral inflammation was observed in these patients i.e. a non-significant tendency for enahnced interferon (IFN)-gamma levels. Thus, these data indicate that neuroinflammation can occur in the absence of overt peripheral inflammation, and may underlie the changes in emotional and nociceptive processing observed in depression-pain comorbidity. Due to the heterogeneity of depressive and chronic pain disorders, the fact that inflammatory markers vary dynamically over time, and the confounding factors such as treatment or disease stage, it is difficult to achieve a clear consensus on the role of inflammation in the co-morbidity of these conditions using only clinical studies. Thus, appropriate preclinical models are essential in order to examine the neurobiological mechanisms underpinning the link between mood and pain disorders and identify new treatment strategies.

Preclinical evidence supporting a role for inflammation in emotion and pain interactions

Studies in laboratory animals support clinical data on the relationship between depression and pain and provide a means to investigate the underlying mechanisms and neural substrates mediating the association between these disorders. As a wealth of data demonstrates neuroimmune changes in models of depression and chronic pain independently [for review see 32, 33], that animal models of chronic pain display depressive- and anxiety-like behaviour [34], and that several animal models of depression exhibit altered nociceptive responding [35], the remainder of this chapter will provide an

overview of the data supporting the hypothesis that neuroimmune processes are involved in the interaction between these conditions.

Animal models of chronic pain exhibit depressive-like behaviour and neuroimmune activation

It is now well-established that depressive-like behaviour can be observed in a wide variety of preclinical chronic pain models. Rodent models of neuropathic and inflammatory pain have been shown to display depressive-like behaviours such as increased immobility in the forced swim test, anhedonia in the sucrose preference test and deficits in the social interaction [for review see 34]. Ample evidence supports a role for immune processes and glial activation in the spinal cord in mediating enhanced nociceptive processing in chronic pain [for review see 36, 37] and it has been shown that spinal microglia activation is critical in the development of neuropathic pain, while in comparison, astrocyte activation is essential for the maintenance of this pain state [38]. Furthermore, evidence indicates that chronic pain in rodents results in glial activation, cytokine and chemokine release in discrete regions including the cingulum, hippocampus and PFC [39-42], effects which may have implications for the descending control of pain, affective pain behaviour and emotional processing. In support of this, recent studies indicate that a neuroinflammatory response occurs in discrete brain regions following chronic inflammatory and neuropathic pain which is associated with the development of depressive-like behaviour.

In the complete Freund's adjuvant (CFA) model of inflammatory pain, both rats and mice have been shown to exhibit concomitant depressive-like behaviour and increased cytokine expression in the brain, specifically increased levels of the proinflammatory cytokine IL-1β in the whole brain [43] and increased IL-6 mRNA in the hippocampus [14]. Thus, inflammatory pain-induced depressive-like behaviour is associated with neuroinflammation, a consequence that is not unsurprising, given the immune cascade that occurs in a model. While this supports a possible association between neuroinflammation, pain and depression, the precise role of the individual cytokines within the brain

mediating the nociceptive and/or emotional behavioural changes in the model remain to be determined.

Peripheral nerve injury (spared nerve injury, SNI) in mice induces a pronounced mechanical allodynia which is accompanied by the development of depressive-like behaviour as determined by enhanced immobility in the forced swim test, increased IL-1β expression in the frontal cortex, and increased astrocyte activation in the periaqueductal grey, a key brain region for the descending modulation of pain [44]. Blocking central IL-1β using the IL-1 receptor antagonist attenuated pain-induced depressive-like behaviour, demonstrating a causative role of IL-1β in nerve injury-induced depression. Interestingly, chronic stress or social isolation exacerbated SNI-induced mechanical allodynia and depressive-like behaviour, and resulted in a greater enhancement of IL-1β mRNA in the frontal cortex, indicating that stressful events may potentiate pain and precipitate depression by promoting neuroinflammation [44, 45]. Extending these findings, Zhou et al showed that SNI-induced mechanical hyperalgesia and depressive-like behaviour was accompanied by increased IL-1β mRNA in the liver, ipsilateral lumbar dorsal horn and contralateral frontal cortex [46], highlighting concomitant peripheral, spinal and supraspinal neuroinflammation in a model exhibiting depression and pain behaviour.

The chronic constriction injury model of neuropathic pain has been shown to exhibit anhedonia (a core depressive behaviour) and impaired hippocampal neurogenesis/plasticity in a time-dependent manner. Furthermore, TNF protein levels were increased and TNFR2 expression, the neuroprotective receptor subtype, was decreased, in the hippocampus. The aforementioned behavioural and neurogenesis effects were not seen in TNFR1 knockout mice, indicating that neuropathic pain-related depression and hippocampal plasticity are dependent on TNF signalling through TNFR1 [47].

Taken together, these studies show that the development of depressive-like behaviour following peripheral nerve injury is accompanied by enhanced IL-1β and TNF signalling in the frontal cortex

and hippocampus, respectively, suggesting that neuroimmune alterations induced by persistent nociceptive input to brain regions that process mood may account, at least in part, for behavioural deficits such as depression. Moreover, these studies demonstrate a causal role of these proinflammatory cytokines in neuropathic pain-induced depressive-like behaviour.

Animal models of depression exhibit altered nociceptive thresholds, possible role for inflammatory mediators?

While depressive-like behaviour has been demonstrated in a wide variety of animal models of chronic pain [for review see 34], until relatively recently, less was known about whether animal models of depression exhibited altered nociceptive responding. However, several studies have now demonstrated that a variety of well-validated models of depression based on stress, lesion, genetics, and pharmacological manipulation display altered nociceptive responding, mimicking the clinical picture. For example, the Wistar-Kyoto (WKY) rat, a stress hyperresponsive rat strain with a depressive-like phenotype, exhibits thermal hyperalgesia in the hot plate test [48], visceral hyperalgesia to colorectal distension [49-51], and hyperalgesia to intra-plantar injection of formalin [48]. The reserpine-induced depression model results in pronounced and long-lasting mechanical hyperalgesia and allodynia, and cold allodynia [52, 53]. The chronic mild stress model has been shown to display cold allodynia [54, 55], mechanical allodynia and hyperalgesia [54, 56] and thermal hypoalgesia [57, 58]. Early life stress is known to result in depressive-like behaviour in adulthood and results in altered sensitivity to thermal stimuli [59-62], increased sensitivity to mechanical stimuli [62, 63], and visceral hypersensitivity [50, 51, 59, 64]. Recent work from our laboratory and others has shown that the olfactory bulbectomised (OB) rat, a lesion model of depression, exhibits increased sensitivity to mechanical and thermal stimuli in the von Frey, acetone drop test, hot plate and tail flick tests [48, 65-68] while exhibiting reduced thermal and mechanical sensitivity in the paw pressure and Hargreaves test [69-71], indicating that effects that may be dependent on the type, intensity and

duration of stimuli applied. Thus, a variety of animal models of depression exhibit altered nociceptive thresholds, mimicking the changes in sensitivity to noxious stimuli as seen in depressed patients.

Animal models of depression are well known to exhibit altered peripheral and central inflammatory processing. For example, early life stress results in increased astrocyte density in the hippocampus and cerebellum [72-74]. WKY rats exhibit increased astrocyte expression in the dorsal raphe nuclei [75] and reductions in astrocyte expression in the PFC and amygdala [76]. Microglial activation and increased protein levels of proinflammatory cytokines has been reported following chronic/social stress in the PFC, PAG, amygdala and hippocampus [77-79] and OB rats exhibit increased IL-1β, TNFα, prostaglandins and astrocyte activation in the brain [80-83]. Therefore, models of depression exhibit region-specific alterations in glial activation in sites implicated in emotional and pain processing, effects which may contribute to the altered nociceptive responding in these models of depression.

In addition to examining nociceptive thresholds, research has also examined if models of depression exhibit altered nociceptive responding in a chronic persistent pain state. An abundance of data now shows that the induction of inflammatory pain consistently results in enhanced nociceptive responding across a range of models of depression. Specifically, inflammatory nociceptive responding has been reported to be enhanced in the WKY rat [48, 84, 85], the OB rat [48, 57, 69], following chronic stress [14, 54, 86, 87] and in the early life stress [61] models of depression (See Table 1). Several of these studies have demonstrated altered levels of monoamines in key brain sites in models of depression following inflammatory pain, indicating that the inflammatory stimulus results in alterations in neuronal functioning that may underlie the hyperalgesia observed. For example, research from our group has demonstrated that both OB and WKY rats exhibit enhanced nociceptive behaviour in response to intraplantar administration of formalin, effects correlating with alterations in monoamine levels in key brain site regulating emotional and nociceptive processing [48]. It is possible that the heightened inflammatory processes observed in models of depression may

prime the system such that when challenged with an exogenous inflammatory stimulus, pain behaviour is enhanced. Few studies have examined this directly, however rats subjected to chronic social defeat exhibit spinal neuroinflammation, specifically, increased iNOS and COX-2 mRNA in the dorsal lumbar spinal cord, which was associated with mechanical hyperalgesia [87], and repeated reserpine treatment results in increased TNFα and IL-1β in the cortex and hippocampus of rats and mice, effects accompanied increased nociceptive responding and depressive-like behaviour [88-90]. Thus, while peripheral mechanisms cannot be ruled out, it is possible that neuroinflammatory processing may underlie, at least in part, the altered nociceptive thresholds observed in rodent models of depression.

Animal models of depression exhibit altered neuropathic pain responding and accompanying neuroimmune alterations

A number of studies have examined if the development and expression of neuropathic pain is altered in animal models of depression (see Table 1). Pain-related behaviour is altered in the majority of these studies; however, the effects vary depending on the model of depression and means of neuropathic pain induction. A thorough overview of all the data in this area is beyond the scope of this chapter, however, presented herein are studies that support a role for neuroinflammation in the altered behavioural responding in these models.

Recent data from our laboratory reported that following L5-L6 spinal nerve ligation (SNL), the OB rat model of depression exhibits bilateral mechanical allodynia and altered nociceptive responding to an innocuous cold stimulus when compared to sham controls [66, 67], an effect accompanied by bilateral astrocyte activation in the dorsal horn of the spinal cord (unpublished). Spinal astrocytes have been implicated in the development of mirror-image mechanical allodynia following nerve injury [91], and thus these data suggest that astrocyte activation at the level of the spinal cord may contribute to the bilateral mechanical allodynia observed in OB rats following nerve injury.

Interestingly, supraspinal neuroinflammation is also associated with altered pain responding in the OB-SNL rat. For instance, OB rats exhibited reduced latency and number of responses to an innocuous cold stimulus following SNL, an effect positively correlated with IL-6 and IL-10 mRNA expression in the amygdala [66]. Moreover, OB-SNL rats exhibited heightened expression of markers of microglia and astrocyte activation, anti-inflammatory cytokines and proinflammatory chemokines in the PFC, when compared to either OB or SNL alone [65, 67]. The functional role of these inflammatory mediators in the PFC on affective and nociceptive behaviour in the present model remains to be determined, yet these data clearly indicate that the combination of depression and pain leads to altered inflammatory gene expression in critical brain regions.

The WKY rat model of depression also exhibits exacerbated mechanical allodynia following peripheral nerve injury (CCI) compared to non-depressed Wistar controls [92]. IL-1β mRNA expression was increased in the brainstem and PFC of WKY rats following peripheral nerve injury, and IL-1β levels in the PFC correlated with mechanical sensitivity [40]. Thus, the proinflammatory profile in the PFC may underlie the heightened neuropathic-pain related behaviour associated with the depressive-like phenotype of WKY rats.

Gender is an important and often overlooked factor in research - females are twice as likely to suffer depression compared to males, have greater pain sensitivity, and are more likely to experience chronic pain [93, 94]. We have recently shown that following peripheral nerve injury (SNL), females, but not males, exposed to early life stress exhibited exacerbated nerve-injury induced mechanical and cold allodynia compared with non-stressed counterparts [62]. This behavioural change was accompanied by reduced TNFα in the PFC with a concomitant increase in IL-6 and TNFα expression in the hippocampus, compared with either MD or SNL alone. As such, the combination of early life stress and peripheral nerve injury resulted in sexually dimorphic alterations in neuropathic-pain related behaviour and the expression of genes encoding inflammatory mediators depending on the

brain region. Sex-dependent changes in neuroimmune functioning in these regions may underlie the higher incidence of depression and pain disorders in women.

Taken together, these studies show that the induction of neuropathic pain in an animal model with a pre-existing depressive-like phenotype results in exacerbated pain-related responding accompanied by differential inflammatory gene expression in brain regions responsible for the regulation of emotion and pain. These data corroborate limited clinical data that show that increased depressive symptoms in patients with neuropathic pain is associated with increased inflammation, at least at a peripheral level and expand it to provide evidence for supraspinal inflammation. Further studies are required to determine the precise mechanisms by which microglia and astrocytes modulate neuronal activity and influence emotional and nociceptive processing.

Modulating (neuro)inflammation reduces depression-like and pain-related behaviour in preclinial models

If neuroinflammatory mechanisms truly underlie the co-occurrence of depression and pain, then modulation of such processes should be of therapeutic benefit to patients. Studies have examined effects of systemic, intrathecal and central administration of anti-inflammatory compounds on emotional and nociceptive processing. For example, oral treatment with resveratrol, an anti-inflammatory antioxidant, has been shown to reduce depressive-like behaviour and thermal hyperalgesia in a model of neuropathic pain (CCI), effects which were abolished by depletion of central serotonin [95], highlighting a possible interaction between inflammation and monoamine neurotransmission that may underlie the association between depression and pain. Indeed, while antidepressants are known to modulate monoaminergic neurotransmission, these pharmacological agents are also known to exhibit anti-inflammatory effects; and amitriptyline, a tricyclic antidepressant, is a first-line treatment for neuropathic pain. We have recently reported that chronic systemic amitriptyline administration to OB rats that have undergone nerve injury (SNL), results in

an attenuation of depressive-like behaviour and SNL-induced mechanical allodynia [65]. These behavioural changes were accompanied by an attenuation of astrocyte activation, the expression of the anti-inflammatory cytokine IL-10 and the chemokine CCL5, and enhanced the expression of TNFα in the PFC. Thus, chronic antidepressant treatment can alleviate depressive-like and neuropathic pain responding possibly by normalising neuroinflammatory processes in brain regions that influence emotional and nociceptive responding such as the PFC.

Further evidence supporting a role for neuroimmune processing in altered neuropathic pain responding associated with a depressive phenotype arises from a recent study where we demonstrated that chronic administration of minocycline, a microglial inhibitor, attenuated OB-induced depressive-like behaviour and prevented the development of SNL-induced mechanical and cold allodynia in OB rats. Strikingly, chronic minocycline treatment enhanced the anti-inflammatory M2 microglial phenotype in the PFC of OB-SNL animals, in contrast to sham-SNL animals, where minocycline rather modulated the M1 proinflammatory phenotype. Evidence suggests that the phenotype of activated microglia (M1 vs. M2) governs the repair and regeneration response following nerve injury and these data suggest a differential mechanism of action of minocycline in the presence of a depressive-like phenotype, which may have important implications for clinical therapy.

Conclusion and Future directions

The current data indicate that, regardless of the primary disorder, the combination of depression and pain is associated with dysregulated inflammation in the central nervous system. The behavioural outputs may represent molecular, cellular, chemical, structural and functional alterations in the brain triggered by neuroinflammatory changes in specific brain areas responsible for parallel processing of emotion and nociception. However, further clinical studies are warranted to confirm and extend the preclinical data, and the precise mechanisms and sites by which neuroimmune processes influence emotion and nociception remain to be determined.

Acknowledgements

This work was funded by grants from Science Foundation Ireland Research Frontiers Project (*11/RFP/NES/3175)*, the Discipline of Physiology and a PhD Scholarship from the College of Medicine, National University of Ireland Galway.

References

1. Ormel, J., et al., *Common mental disorders and disability across cultures. Results from the WHO Collaborative Study on Psychological Problems in General Health Care.* JAMA, 1994. **272**(22): p. 1741-8.
2. Spitzer, R.L., et al., *Health-related quality of life in primary care patients with mental disorders. Results from the PRIME-MD 1000 Study.* JAMA, 1995. **274**(19): p. 1511-7.
3. DSM-5, *Diagnostic and Statistical Manual of Mental Disorders*. Fifth ed. ed2013, Arlington, VA: American Psychiartic Publishing.
4. Poole, H., et al., *Depression in chronic pain patients: prevalence and measurement.* Pain Pract, 2009. **9**(3): p. 173-80.
5. Gameroff, M.J. and M. Olfson, *Major depressive disorder, somatic pain, and health care costs in an urban primary care practice.* J Clin Psychiatry, 2006. **67**(8): p. 1232-9.
6. Maletic, V. and C.L. Raison, *Neurobiology of depression, fibromyalgia and neuropathic pain.* Front Biosci (Landmark Ed), 2009. **14**: p. 5291-338.
7. Graff-Guerrero, A., et al., *Cerebral blood flow changes associated with experimental pain stimulation in patients with major depression.* J Affect Disord, 2008. **107**(1-3): p. 161-8.
8. Lopez-Sola, M., et al., *Effects of duloxetine treatment on brain response to painful stimulation in major depressive disorder.* Neuropsychopharmacology, 2010. **35**(11): p. 2305-17.
9. Bar, K.J., et al., *Increased prefrontal activation during pain perception in major depression.* Biol Psychiatry, 2007. **62**(11): p. 1281-7.

10. Walker, A.K., et al., *Neuroinflammation and comorbidity of pain and depression.* Pharmacol Rev, 2014. **66**(1): p. 80-101.

11. Bair, M.J., et al., *Depression and pain comorbidity: a literature review.* Arch Intern Med, 2003. **163**(20): p. 2433-45.

12. Euteneuer, F., et al., *Depression, cytokines and experimental pain: evidence for sex-related association patterns.* J Affect Disord, 2010. **131**(1-3): p. 143-9.

13. Poleshuck, E.L., et al., *Depressive Symptoms, Pain, Chronic Medical Morbidity, and Interleukin-6 among Primary Care Patients.* Pain Med, 2013. **14**(5): p. 686-91.

14. Kim, H., et al., *Brain indoleamine 2,3-dioxygenase contributes to the comorbidity of pain and depression.* J Clin Invest, 2012. **122**(8): p. 2940-54.

15. Chen, Q., et al., *Serum interleukin-6 in patients with burning mouth syndrome and relationship with depression and perceived pain.* Mediators Inflamm, 2007. **2007**: p. 45327.

16. Uher, T. and P. Bob, *Neuropathic pain, depressive symptoms, and C-reactive protein in sciatica patients.* Int J Neurosci, 2013. **123**(3): p. 204-8.

17. Menzies, V., et al., *Psychoneuroimmunological Relationships in Women With Fibromyalgia.* Biol Res Nurs, 2011.

18. Wang, H., et al., *Influence of comorbidity with depression on interdisciplinary therapy: outcomes in patients with chronic low back pain.* Arthritis Res Ther, 2010. **12**(5): p. R185.

19. Raison, C.L., L. Capuron, and A.H. Miller, *Cytokines sing the blues: inflammation and the pathogenesis of depression.* Trends in Immunology, 2006. **27**(1): p. 24-31.

20. Steiner, J., et al., *Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide.* J Psychiatr Res, 2008. **42**(2): p. 151-7.

21. Shelton, R.C., et al., *Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression.* Mol Psychiatry, 2011. **16**(7): p. 751-62.

22. Pandey, G.N., et al., *Proinflammatory cytokines in the prefrontal cortex of teenage suicide victims.* J Psychiatr Res, 2012. **46**(1): p. 57-63.

23. Tonelli, L.H., et al., *Elevated cytokine expression in the orbitofrontal cortex of victims of suicide.* Acta Psychiatr Scand, 2008. **117**(3): p. 198-206.

24. Del Valle, L., R.J. Schwartzman, and G. Alexander, *Spinal cord histopathological alterations in a patient with longstanding complex regional pain syndrome.* Brain Behav Immun, 2009. **23**(1): p. 85-91.

25. Shi, Y., et al., *Chronic-pain-associated astrocytic reaction in the spinal cord dorsal horn of human immunodeficiency virus-infected patients.* J Neurosci, 2012. **32**(32): p. 10833-40.

26. Chang, L., et al., *Magnetic resonance spectroscopy to assess neuroinflammation and neuropathic pain.* J Neuroimmune Pharmacol, 2013. **8**(3): p. 576-93.

27. Banati, R.B., et al., *Long-term trans-synaptic glial responses in the human thalamus after peripheral nerve injury.* Neuroreport, 2001. **12**(16): p. 3439-42.

28. Widerstrom-Noga, E., et al., *Metabolite concentrations in the anterior cingulate cortex predict high neuropathic pain impact after spinal cord injury.* Pain, 2013. **154**(2): p. 204-12.

29. Pattany, P.M., et al., *Proton magnetic resonance spectroscopy of the thalamus in patients with chronic neuropathic pain after spinal cord injury.* AJNR Am J Neuroradiol, 2002. **23**(6): p. 901-5.

30. Hannestad, J., et al., *The neuroinflammation marker translocator protein is not elevated in individuals with mild-to-moderate depression: a [(1)(1)C]PBR28 PET study.* Brain Behav Immun, 2013. **33**: p. 131-8.

31. Nakatomi, Y., et al., *Neuroinflammation in Patients with Chronic Fatigue Syndrome/Myalgic Encephalomyelitis: An 11C-(R)-PK11195 PET Study.* J Nucl Med, 2014. **55**(6): p. 945-950.

32. Song, C. and H. Wang, *Cytokines mediated inflammation and decreased neurogenesis in animal models of depression.* Prog Neuropsychopharmacol Biol Psychiatry, 2011. **35**(3): p. 760-8.

33. Austin, P.J. and G. Moalem-Taylor, *The neuro-immune balance in neuropathic pain: involvement of inflammatory immune cells, immune-like glial cells and cytokines.* J Neuroimmunol, 2010. **229**(1-2): p. 26-50.

34. Yalcin, I., F. Barthas, and M. Barrot, *Emotional consequences of neuropathic pain: Insight from preclinical studies.* Neurosci Biobehav Rev, 2014. **47C**: p. 154-164.

35. Li, J.X., *Pain and depression comorbidity: A preclinical perspective.* Behav Brain Res, 2014.

36. Vallejo, R., et al., *The role of glia and the immune system in the development and maintenance of neuropathic pain.* Pain Pract, 2010. **10**(3): p. 167-84.

37. Bradesi, S., *Role of spinal cord glia in the central processing of peripheral pain perception.* Neurogastroenterol Motil, 2010. **22**(5): p. 499-511.

38. Raghavendra, V., F. Tanga, and J.A. DeLeo, *Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy.* J Pharmacol Exp Ther, 2003. **306**(2): p. 624-30.

39. Zhu, J., et al., *Interaction of glia activation and neurotransmission in hippocampus of neuropathic rats treated with mirtazapine.* Exp Clin Psychopharmacol, 2009. **17**(3): p. 198-203.

40. Apkarian, A.V., et al., *Expression of IL-1beta in supraspinal brain regions in rats with neuropathic pain.* Neurosci Lett, 2006. **407**(2): p. 176-81.

41. Ignatowski, T.A., et al., *Brain-derived TNFalpha mediates neuropathic pain.* Brain Res, 1999. **841**(1-2): p. 70-7.

42. Wei, F., et al., *Supraspinal glial-neuronal interactions contribute to descending pain facilitation.* J Neurosci, 2008. **28**(42): p. 10482-95.

43. Maciel, I.S., et al., *Synergistic effects of celecoxib and bupropion in a model of chronic inflammation-related depression in mice.* PLoS One, 2013. **8**(9): p. e77227.

44. Norman, G.J., et al., *Stress and IL-1beta contribute to the development of depressive-like behavior following peripheral nerve injury.* Mol Psychiatry, 2010. **15**(4): p. 404-14.

45. Norman, G.J., et al., *Social interaction prevents the development of depressive-like behavior post nerve injury in mice: a potential role for oxytocin.* Psychosom Med, 2010. **72**(6): p. 519-26.

46. Zhou, W., et al., *118. Indoleamine 2,3 dioxygenase (IDO1) is a key regulator of depressive-like behavior but not of mechanical hyperalgesia in the spared nerve injury model of neuropathic pain.* Brain Behav Immun, 2013. **32, Supplement**(0): p. e34.

47. Dellarole, A., et al., *Neuropathic pain-induced depressive-like behavior and hippocampal neurogenesis and plasticity are dependent on TNFR1 signaling.* Brain Behav Immun, 2014. **41**: p. 65-81.

48. Burke, N.N., et al., *Enhanced nociceptive responding in two rat models of depression is associated with alterations in monoamine levels in discrete brain regions.* Neuroscience, 2010. **171**(4): p. 1300-13.

49. Gibney, S.M., et al., *Colorectal distension-induced prefrontal cortex activation in the Wistar-Kyoto rat: implications for irritable bowel syndrome.* Neuroscience, 2009. **165**(3): p. 675-83.

50. Gosselin, R.D., et al., *Riluzole normalizes early-life stress-induced visceral hypersensitivity in rats: role of spinal glutamate reuptake mechanisms.* Gastroenterology, 2010. **138**(7): p. 2418-25.

51. O'Malley, D., et al., *Distinct alterations in colonic morphology and physiology in two rat models of enhanced stress-induced anxiety and depression-like behaviour.* Stress-the International Journal on the Biology of Stress, 2010. **13**(2): p. 114-122.

52. Ogino, S., et al., *Systemic administration of 5-HT(2C) receptor agonists attenuates muscular hyperalgesia in reserpine-induced myalgia model.* Pharmacol Biochem Behav, 2013. **108**: p. 8-15.

53. Nagakura, Y., et al., *Different pathophysiology underlying animal models of fibromyalgia and neuropathic pain: comparison of reserpine-induced myalgia and chronic constriction injury rats.* Behav Brain Res, 2012. **226**(1): p. 242-9.

54. Bardin, L., et al., *Chronic restraint stress induces mechanical and cold allodynia, and enhances inflammatory pain in rat: Relevance to human stress-associated painful pathologies.* Behav Brain Res, 2009. **205**(2): p. 360-6.

55. Bravo, L., et al., *Depressive-like states heighten the aversion to painful stimuli in a rat model of comorbid chronic pain and depression.* Anesthesiology, 2012. **117**(3): p. 613-25.

56. Imbe, H., et al., *Chronic restraint stress decreases glial fibrillary acidic protein and glutamate transporter in the periaqueductal gray matter.* Neuroscience, 2012. **223**: p. 209-18.

57. Shi, M., J.Y. Wang, and F. Luo, *Depression shows divergent effects on evoked and spontaneous pain behaviors in rats.* J Pain, 2010. **11**(3): p. 219-29.

58. Qi, Q., et al., *Depressive-like history alters persistent pain behavior in rats: Opposite contribution of frontal cortex and amygdala implied.* PsyCh Journal, 2013. **2**(2): p. 133-145.

59. Coutinho, S.V., et al., *Neonatal maternal separation alters stress-induced responses to viscerosomatic nociceptive stimuli in rat.* Am J Physiol Gastrointest Liver Physiol, 2002. **282**(2): p. G307-16.

60. Weaver, S.A., J. Diorio, and M.J. Meaney, *Maternal separation leads to persistent reductions in pain sensitivity in female rats.* J Pain, 2007. **8**(12): p. 962-9.

61. Uhelski, M.L. and P.N. Fuchs, *Maternal separation stress leads to enhanced emotional responses to noxious stimuli in adult rats.* Behav Brain Res, 2010. **212**(2): p. 208-12.

62. Burke, N.N., et al., *Maternal Deprivation Is Associated With Sex-Dependent Alterations in Nociceptive Behavior and Neuroinflammatory Mediators in the Rat Following Peripheral Nerve Injury*. J Pain, 2013.

63. Alvarez, P., P.G. Green, and J.D. Levine, *Stress in the Adult Rat Exacerbates Muscle Pain Induced by Early-Life Stress.* Biol Psychiatry, 2013.

64. Moloney, R.D., et al., *Early-life stress induces visceral hypersensitivity in mice.* Neurosci Lett, 2012. **512**(2): p. 99-102.

65. Burke, N.N., D.P. Finn, and M. Roche, *Chronic administration of amitriptyline differentially alters neuropathic pain-related behaviour in the presence and absence of a depressive-like phenotype.* Behav Brain Res, 2014.

66. Burke, N.N., et al., *Altered neuropathic pain behaviour in a rat model of depression is associated with changes in inflammatory gene expression in the amygdala.* Genes Brain Behav, 2013.

67. Burke, N.N., et al., *Minocycline modulates neuropathic pain behaviour and cortical M1-M2 microglial gene expression in a rat model of depression.* Brain Behav Immun, 2014.

68. Rodriguez-Gaztelumendi, A., et al., *An altered spinal serotonergic system contributes to increased thermal nociception in an animal model of depression.* Exp Brain Res, 2014. **232**(6): p. 1793-803.

69. Wang, W., et al., *The differential effects of depression on evoked and spontaneous pain behaviors in olfactory bulbectomized rats.* Neuroscience Letters, 2010. **472**(2): p. 143-147.

70. Belcheva, I., et al., *Differential involvement of hippocampal vasoactive intestinal peptide in nociception of rats with a model of depression.* Peptides, 2009. **30**(8): p. 1497-501.

71. Su, Y.L., et al., *The effect of depression on the thermal nociceptive thresholds in rats with spontaneous pain.* Neurosci Bull, 2010. **26**(6): p. 429-36.

72. Llorente, R., et al., *Early maternal deprivation in rats induces gender-dependent effects on developing hippocampal and cerebellar cells.* Int J Dev Neurosci, 2009. **27**(3): p. 233-41.

73. Marco, E.M., et al., *Maternal deprivation effects on brain plasticity and recognition memory in adolescent male and female rats.* Neuropharmacology, 2012.

74. Lopez-Gallardo, M., et al., *Maternal Deprivation and Adolescent Cannabinoid Exposure Impact Hippocampal Astrocytes, Cbi Receptors and Brain-Derived Neurotrophic Factor in a Sexually Dimorphic Fashion.* Neuroscience, 2012. **204**: p. 90-103.

75. Pearson, K.A., et al., *Identifying genes in monoamine nuclei that may determine stress vulnerability and depressive behavior in Wistar-Kyoto rats.* Neuropsychopharmacology, 2006. **31**(11): p. 2449-61.

76. Gosselin, R.D., et al., *Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression.* Neuroscience, 2009. **159**(2): p. 915-25.

77. Farooq, R.K., et al., *Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation?* Behav Brain Res, 2012. **231**(1): p. 130-137.

78. Tynan, R.J., et al., *Chronic stress alters the density and morphology of microglia in a subset of stress-responsive brain regions.* Brain Behav Immun, 2010. **24**(7): p. 1058-68.

79. Mormede, C., et al., *Chronic mild stress in mice decreases peripheral cytokine and increases central cytokine expression independently of IL-10 regulation of the cytokine network.* Neuroimmunomodulation, 2002. **10**(6): p. 359-66.

80. Rinwa, P., A. Kumar, and S. Garg, *Suppression of neuroinflammatory and apoptotic signaling cascade by curcumin alone and in combination with piperine in rat model of olfactory bulbectomy induced depression.* PLoS One, 2013. **8**(4): p. e61052.

81. Borre, Y., et al., *Minocycline restores spatial but not fear memory in olfactory bulbectomized rats.* Eur J Pharmacol, 2012. **697**(1-3): p. 59-64.

82. Song, C., X.Y. Zhang, and M. Manku, *Increased phospholipase A2 activity and inflammatory response but decreased nerve growth factor expression in the olfactory bulbectomized rat model of depression: effects of chronic ethyl-eicosapentaenoate treatment.* J Neurosci, 2009. **29**(1): p. 14-22.

83. Cizkova, D., E. Racekova, and I. Vanicky, *The expression of B-50/GAP-43 and GFAP after bilateral olfactory bulbectomy in rats.* Physiol Res, 1997. **46**(6): p. 487-95.

84. Wang, S., et al., *Exacerbated mechanical hyperalgesia in rats with genetically predisposed depressive behavior: role of melatonin and NMDA receptors.* Pain, 2012. **153**(12): p. 2448-57.

85. Rea, K., et al., *Impaired endocannabinoid signalling in the rostral ventromedial medulla underpins genotype-dependent hyper-responsivity to noxious stimuli.* Pain, 2014. **155**(1): p. 69-79.

86. Gameiro, G.H., et al., *The effects of restraint stress on nociceptive responses induced by formalin injected in rat's TMJ.* Pharmacol Biochem Behav, 2005. **82**(2): p. 338-44.

87. Rivat, C., et al., *Chronic stress induces transient spinal neuroinflammation, triggering sensory hypersensitivity and long-lasting anxiety-induced hyperalgesia.* Pain, 2010. **150**(2): p. 358-68.

88. Arora, V., et al., *Curcumin ameliorates reserpine-induced pain-depression dyad: Behavioural, biochemical, neurochemical and molecular evidences.* Psychoneuroendocrinology, 2011.

89. Xu, Y., et al., *Ferulic acid increases pain threshold and ameliorates depression-like behaviors in reserpine-treated mice: behavioral and neurobiological analyses.* Metab Brain Dis, 2013.

90. Arora, V. and K. Chopra, *Possible involvement of oxido-nitrosative stress induced neuro-inflammatory cascade and monoaminergic pathway: Underpinning the correlation between*

nociceptive and depressive behaviour in a rodent model. Journal of Affective Disorders, 2013. **151**(3): p. 1041-1052.

91. Obata, H., et al., *Activation of astrocytes in the spinal cord contributes to the development of bilateral allodynia after peripheral nerve injury in rats.* Brain Res, 2010. **1363**: p. 72-80.
92. Zeng, Q., et al., *Exacerbated mechanical allodynia in rats with depression-like behavior.* Brain Res, 2008. **1200**: p. 27-38.
93. Tsang, A., et al., *Common chronic pain conditions in developed and developing countries: gender and age differences and comorbidity with depression-anxiety disorders.* J Pain, 2008. **9**(10): p. 883-91.
94. Munce, S.E. and D.E. Stewart, *Gender differences in depression and chronic pain conditions in a national epidemiologic survey.* Psychosomatics, 2007. **48**(5): p. 394-9.
95. Zhao, X., et al., *Chronic resveratrol treatment exerts antihyperalgesic effect and corrects co-morbid depressive like behaviors in mice with mononeuropathy: involvement of serotonergic system.* Neuropharmacology, 2014. **85**: p. 131-41.
96. Amorim, D., et al., *Amitriptyline reverses hyperalgesia and improves associated mood-like disorders in a model of experimental monoarthritis.* Behavioural Brain Research, 2014. **265**: p. 12-21.
97. Borges, G., et al., *Reversal of monoarthritis-induced affective disorders by diclofenac in rats.* Anesthesiology, 2014. **120**(6): p. 1476-90.
98. Liang, J., et al., *The effects of OB-induced depression on nociceptive behaviors induced by electrical stimulation of the dura mater surrounding the superior sagittal sinus.* Brain Res, 2011. **1424**: p. 9-19.
99. del Rey, A., et al., *Chronic neuropathic pain-like behavior correlates with IL-1beta expression and disrupts cytokine interactions in the hippocampus.* Pain, 2011. **152**(12): p. 2827-35.

100. Zhang, M., et al., *Effects of UCMS-induced depression on nociceptive behaviors induced by electrical stimulation of the dura mater.* Neurosci Lett, 2013.

Chronic Pain Model	*Depressive-like behaviour*	*Inflammatory changes/effects*	
Kaolin and carrageenan-induced monoarthritis	↓ sucrose preference ↑ immobility in FST	Not measured	[96]
Complete Freunds Adjuvant-induced monoarthritis	↑ immobility in FST	Not measured	[97]
Complete Freunds Adjuvant-evoked inflammatory pain	↓ sucrose preference ↑ immobility in FST and TST	↑ IL-1β levels in whole brain, and cortex ↑ plasma IL-6, ↑ IL-6 in hippocampus	[43] [14]
Spinal Nerve Injury (neuropathic)	↑ immobility in FST ↓ social interaction	↑ IL-1β mRNA in the frontal cortex ↑ GFAP mRNA in the periaqueductal grey ↑ IL-1β mRNA liver ↑ IL-1β mRNA dorsal horn of spinal cord	[44-46]
Chronic Constriction Injury (neuropathic)	↓ sucrose preference	↑TNF and ↓TNFR2 in hippocampus	[47]
Model of Depression	***Nociceptive responding/ behaviour***	***Inflammatory changes/effects***	
Reserpine-induced depression model	Mechanical allodynia in von Frey test Mechanical hyperalgesia in paw pressure test Thermal hyperalgesia in tail immersion test	↑ TNFα, IL-1β and substance P in cortex and hippocampus	[88-90]
OB rat	Mechanical allodynia in von Frey test Cold allodynia in acetone drop test	↑ GFAP (astrocyte activation), CD11b (microglial activation) and IL-1β mRNA in the amygdala	[66]
	↑ formalin-evoked nociceptive behaviour	Not measured	[48, 69]
	↑ nociceptive responding to electrical stimulation of the dura mater (model of trigeminal nociception)	↑ plasma Substance P and CGRP levels	[98]
	Bilateral mechanical allodynia following SNL Increased cold allodynia following SNL	↓ IL-6 and IL-10 mRNA in amygdala, ↑ microglia (CD68, CD40, CD11b) and astrocyte (GFAP) activation, anti-inflammatory (IL-10) cytokines and pro-inflammatory chemokines (CCL2, CCL5) in mRNA in PFC	[65, 66]

WKY rat	↑Formalin-evoked nociceptive behaviour ↑ Mechanical hyperalgesia following CFA to the TMJ	Not measured	[48, 85] [84]
	↑ Mechanical allodynia following nerve injury	↑ IL-1β mRNA in the brainstem and PFC; ↑ IL-1β and IL-6 in hippocampus	[40, 92, 99]
Unpredictable Chronic Mild Stress	↑ nociceptive responding to electrical stimulation of the dura mater	↑ plasma Substance P levels	[100]
Maternal deprivation	↑ Mechanical and cold allodynia following SNL in females only	↓ IL-6 mRNA in PFC; ↑ GFAP and IL-1β mRNA in hippocampus of MD-SNL males ↓ TNFα mRNA in PFC; ↑ in IL-6 and TNFα mRNA in hippocampus of MD-SNL females	[62]
Maternal separation	↑ formalin-evoked nociceptive behaviour	Not measured	[61]
Chronic restraint stress	↑ formalin-evoked nociceptive behaviour	Not measured	[54, 86]

Table 1: Preclinical animal studies demonstrating relationship between inflammation-depression and pain

Abbreviations: CCL2 chemokine C-C motif ligand 2; CCL5 chemokine C-C motif ligand 5 ;CD11b cluster of differentiation 11b; CD40 cluster of differentiation 40; CD68 cluster of differentiation 68; CFA Complete Freunds adjuvant; CGRP calcitonin gene-related peptide; FST forced swim test; GFAP glial fibrillary acidic protein; IL-10 interleukin 10; IL-1β interleukin 1 beta; IL-6 interleukin 6; MD maternal deprivation; mRNA messenger RNA; OB olfactory bulbectomy; PFC prefrontal cortex; SNL spinal nerve ligation; TMJ temporomandibular joint; TNF tumour necrosis factor; TNFR2 tumour necrosis factor receptor subtype 2; TNFα tumour necrosis factor alpha; TST tail swim test.