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# **Neuroinflammatory mechanisms linking Pain and Depression**

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Running title: Neuroinflammation in Depression-Pain

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## **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 TNF $\alpha$  and reduced pressure pain thresholds in these patients [12]. Correlative analysis revealed that augmented levels of TNF $\alpha$  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 TNF $\alpha$ ; and although the presence of comorbid depression did not influence TNF $\alpha$  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  $^{11}\text{C}$ -(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 enhanced 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-1 $\beta$  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-1 $\beta$  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-1 $\beta$  using the IL-1 receptor antagonist attenuated pain-induced depressive-like behaviour, demonstrating a causative role of IL-1 $\beta$  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-1 $\beta$  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-1 $\beta$  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-1 $\beta$  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-1 $\beta$ , TNF $\alpha$ , 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 TNF $\alpha$  and IL-1 $\beta$  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-1 $\beta$  mRNA expression was increased in the brainstem and PFC of WKY rats following peripheral nerve injury, and IL-1 $\beta$  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 TNF $\alpha$  in the PFC with a concomitant increase in IL-6 and TNF $\alpha$  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 preclinical 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 TNF $\alpha$  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.



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<b>Chronic Pain Model</b>	<b>Depressive-like behaviour</b>	<b>Inflammatory changes/effects</b>	
<b><i>Kaolin and carrageenan-induced monoarthritis</i></b>	↓ sucrose preference ↑ immobility in FST	Not measured	[96]
<b><i>Complete Freund's Adjuvant-induced monoarthritis</i></b>	↑ immobility in FST	Not measured	[97]
<b><i>Complete Freund's Adjuvant-evoked inflammatory pain</i></b>	↓ sucrose preference ↑ immobility in FST and TST	↑ IL-1β levels in whole brain, and cortex ↑ plasma IL-6, ↑ IL-6 in hippocampus	[43] [14]
<b><i>Spinal Nerve Injury (neuropathic)</i></b>	↑ 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]
<b><i>Chronic Constriction Injury (neuropathic)</i></b>	↓ sucrose preference	↑ TNF and ↓ TNFR2 in hippocampus	[47]
<b>Model of Depression</b>	<b>Nociceptive responding/behaviour</b>	<b>Inflammatory changes/effects</b>	
<b><i>Reserpine-induced depression model</i></b>	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]
<b><i>OB rat</i></b>	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]

<b>WKY rat</b>	↑Formalin-evoked nociceptive behaviour	Not measured	[48, 85]
	↑ Mechanical hyperalgesia following CFA to the TMJ		[84]
	↑ Mechanical allodynia following nerve injury	↑ IL-1 $\beta$ mRNA in the brainstem and PFC; ↑ IL-1 $\beta$ and IL-6 in hippocampus	[40, 92, 99]
<b>Unpredictable Chronic Mild Stress</b>	↑ nociceptive responding to electrical stimulation of the dura mater	↑ plasma Substance P levels	[100]
<b>Maternal deprivation</b>	↑ Mechanical and cold allodynia following SNL in females only	↓ IL-6 mRNA in PFC; ↑ GFAP and IL-1 $\beta$ mRNA in hippocampus of MD-SNL males ↓ TNF $\alpha$ mRNA in PFC; ↑ in IL-6 and TNF $\alpha$ mRNA in hippocampus of MD-SNL females	[62]
<b>Maternal separation</b>	↑ formalin-evoked nociceptive behaviour	Not measured	[61]
<b>Chronic restraint stress</b>	↑ 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 Freund's adjuvant; CGRP calcitonin gene-related peptide; FST forced swim test; GFAP glial fibrillary acidic protein; IL-10 interleukin 10; IL-1 $\beta$  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; TNF $\alpha$  tumour necrosis factor alpha; TST tail swim test.