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# Neuroimmune activation in arthritic and neuropathic low back pain: implications for interventional therapies

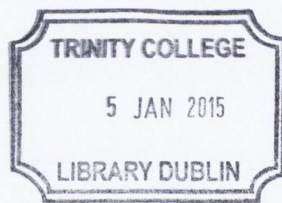
A dissertation submitted to the University of Dublin  
for the degree of Doctor of Philosophy

Kevin F McCarthy  
Trinity College Dublin, July 2013

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DEPARTMENT OF PHYSIOLOGY, SCHOOL OF MEDICINE  
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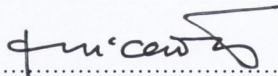
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## Summary

Chronic pain involves a progressive disturbance of mood, sleep and physical function, with significant medical, social and economic consequences. The mechanism by which acute pain may become chronic and refractory to treatment is poorly understood. Interventional pain therapies such as radiofrequency (RF) treatment and spinal cord stimulation (SCS) are directed towards interrupting the afferent nociceptive input or modulating the upward transmission of these signals within the dorsal horn at the level of the spinal cord. These technologies are efficacious in select neuropathic pain syndromes and in denervating arthritic joints although their precise mechanism of action is unclear. Recent pre-clinical research has implicated glial activation and neuroimmune mediators in the pathophysiology of chronic pain. This may account for the variable efficacy of RF and SCS in different pain syndromes and between individuals.

The aim of this study was to explore the role of neuroimmune activation in chronic low back pain and to assess if neuroablative and neuromodulatory technologies such as RF and SCS modulate this process.

We measured the levels of neuroimmune mediators in cerebrospinal fluid (CSF) and serum in patients with chronic low back pain of two different and clinically common aetiologies; low-grade inflammatory osteoarthritic in the case of facet joint arthropathy (FJA) and post-surgical neuropathic-dominant in Failed Back Surgery Syndrome (FBSS). We compared CSF and serum levels in patients with painful FJA undergoing RF treatment with those of patients with a SCS implanted for FBSS. We studied the impact of RF and SCS on the CSF

and serum concentrations of neuroimmune mediators. We examined the relationships between the concentrations of these mediators within CSF to explore possible regulatory mechanisms.

In chapter 4, we report the CSF neuroimmune mediators patients with chronic low back pain and how these messenger molecules relate to each other and with pain and quality of life. CSF IL-8 inversely correlated with pain ( $p < 0.001$ ) and positively correlated with pain relief ( $p < 0.001$ ). CSF levels of GDNF, IL-6, IL-8, MCP-1 and MMP-2 were significantly higher in patients with FBSS than patients with facet joint arthropathy ( $p < 0.01$ ). In chapter 5, we examine the effect of RF neurotomy which resulted in an acute reduction in pain ( $p = 0.007$ ), BDNF ( $p = 0.0076$ ) and MCP-1 ( $p = 0.0001$ ) at two hours post-procedure. Pain correlated with poor sleep ( $p = 0.001$ ) and poorer quality of life ( $p = 0.009$ ). Serum IL-8 and IL-6 positively correlated with baseline pain ( $p < 0.05$ ) and age ( $p < 0.01$ ). Post-procedure pain was inversely correlated with serum IL-8 ( $p = 0.047$ ) and VEGF ( $p = 0.017$ ). In chapter 6, patients with a spinal cord stimulator in situ had it turned off for at least eight hours for pre-stimulation samples and then on for five minutes for first post stimulation sampling. After five minutes of SCS, there was a decrease in CSF VEGF ( $p = 0.01$ ). There a positive correlation between CSF levels of GDNF and stimulator frequency ( $p = 0.048$ ). In chapter 7, we summarise our conclusions from our observed findings.

This thesis highlights distinct patterns of neuroimmune activation in two frequently encountered clinical pain syndromes and that these mediators are modulated in different ways by RF and SCS technologies.

“The world breaks everyone and afterward many are  
strong at the broken places.”

- Ernest Hemingway, A Farewell to Arms.

## Acknowledgements

Sincerest gratitude to my supervisors, Dr. Connail McCrory and the late Professor Tom Connor for their expertise, knowledge, and mostly for their deep wells of patience as I tried to conduct research while continuing with clinical work. Connail for thinking big and helping me keep an eye on the finish line at all times, and Tom for his enthusiasm and knack for picking signal from noise. Together, you were ‘the vertical and the horizontal’ of my trajectory through this experience. I regret that Tom did not see the final draft of this thesis before his untimely passing.

In the Neuroimmunology Research Group, thank you to Jen, Eimear, Martina, Katie, Raasay and Eadaoin for your patience with the phantom colleague who made guest appearances late in the evening and at weekends and left Post-Its promising to make up for any pipette tips I’d moved or used. In the reading room in the Department of Physiology, thank you to Catherine, Eamonn, Heather, Oscar and Dave for the random discussions that both provided a distraction and changed the course of my work; I never imagined a chain-smoking anaesthetist could find so much common ground with a bunch of exercise physiologists whose idea of a good time is a triathlon or two.

In St James’s, thank you to everybody on the Pain Team for their social and emotional support over the years; Anne for endless cups of tea and advice on what I should be doing with my life, Trish for the motivational pep talks, and Carmel and Dee for their kindness and encouragement. To the chronic pain patients who consented to take part. If nothing else, I may have found a biomarker for altruism.



Your willingness to give of your time and CSF “if this helps someone else” was humbling.

Thank you to my friends and family for their tolerance of my absences, both physical and figurative, over the past few years, who got used to me being an unreliable flake and learned not to ask me about it because I would tell them. In mind-numbing detail.

And the biggest thank you to my Mum and Dad, whose support was derived mainly from being blissfully unaware about what a “H Dip” actually is. Dad, your position as Chief Armchair Scientist is safe.

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## List of Publications

1. **McCarthy KF**, & McCrory C.

Cerebrospinal fluid levels of glial cell-derived neurotrophic factor correlate with spinal cord stimulation frequency: a preliminary report. *Spinal Cord*. 2014 Aug;52 Suppl 2:S8-10. doi: 10.1038/sc.2014.81.

2. **McCarthy KF**, Connor TJ, & McCrory C.

Cerebrospinal Fluid Levels of Vascular Endothelial Growth Factor Correlate With Reported Pain and Are Reduced by Spinal Cord Stimulation in Patients With Failed Back Surgery Syndrome. *Neuromodulation*. 2013 Nov-Dec;16(6):519-22; discussion 522. doi: 10.1111/j1525-1403.2012.00527.

3. **McCarthy KF**, & McCrory C.

Cerebrospinal Fluid interleukin-8 levels and poor sleep quality predict analgesic response to lumbar rhizotomy in man. Annual Scientific Meeting of the Neuromodulation Society UK and Ireland, October 4-5, 2010, Leeds, United Kingdom. Abstract published in *Neuromodulation* 2011; 14: 191-193. doi: 10.13140/2.1.2017.1529

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# 1

## Introduction

### 1.1 Chronic spinal pain

#### 1.1.1 Prevalence of back pain

While pain is universal, chronic pain represents a deeply personal journey that may arise from an single or repeated inciting event and evolves to incorporate disturbances of mood, sleep and physical function. Although pain remains difficult to objectively quantify, it frequently results in significant medical, social and economic sequelae. A survey of chronic pain prevalence in 16 European countries found that chronic pain affects 13% of the Irish population with a median duration of 4.9 years. Of the Irish survey respondents, 42% identified their back or lower back as the anatomical location of their pain [27]. The prevalence of recurrent or persistent low back pain increases with age, from a point prevalence of 13% in adolescence [35] to 27% in the elderly, which may even be an underestimate [28]. Chronic disabling back pain is on the increase across all demographic groups [80], making its effective prevention and treatment a public health concern.

## 1. INTRODUCTION

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### 1.1.2 Economic and societal impact of back pain

There is an economic impact of back pain, both for society and for the individual. In the United States, it has been estimated that back pain accounts for up to 40% of all lost work days and that the healthcare costs of individuals with back pain are about 60% higher than those without back pain [110]. In Ireland, people with a diagnosis of chronic low back pain accounted for the highest proportion (27%) of illness-related income support payments in 2002 [157]. In 2008, the total annual cost of chronic pain in Ireland for patients aged over 20 is estimated at €5.34 billion or 2.86% of Irish GDP [231]. Patients with chronic low back pain have a greater co-morbidity burden and greater use of pharmacotherapy than controls [94].

### 1.1.3 Anatomical sources of chronic low back pain

Pain in the lower back may arise from bone, muscle, ligaments, nerve roots, facet joints and intervertebral discs [152]. Intervertebral disc prolapse causing nerve root compression and sciatica accounts for 5% of low back disorders [89]. In patients with chronic low back pain who have failed to respond to conservative therapeutic modalities and do not have a confirmed radiological reason for their symptoms, eg. disc protrusion or spinal stenosis, the relative contributions of various structures have been evaluated in prospective clinical studies utilizing controlled, comparative, diagnostic blocks. The prevalence of lumbar facet joint pain is approximately 30% of patients with chronic low back pain [240] and 16% in Failed Back Surgery Syndrome (FBSS) [172]. Sacro-iliac joint pain has been reported as accounting for between 2-18% [170, 172] of patients, discogenic pain for 25%, and no identifiable source in 19% [240].

## 1.2 Rationale for this study

Pharmacological management of chronic back pain may be limited by medication side effects of commonly-used medications such as non-steroidal anti-inflammatory drugs (NSAIDs)[42] and opioids [213]. Interventional therapies such as radiofrequency (RF) and spinal cord stimulation (SCS) are safe [222][36]



and efficacious when applied to appropriately selected patients. The precise mechanism of action of either technology is not completely understood and in an age of mechanism- and evidence-based treatments, we are at a loss to explain the variation in magnitude and duration of clinical response to patients. There is compelling evidence for the role of neuroimmune activation both in the pathophysiology of chronic pain syndromes and in attenuating response to analgesia. The purpose of this study is to improve our understanding of the pathophysiological processes that are unique and common to two chronic spinal pain syndromes and how these dynamically interact with two interventional technologies and influence, or even determine, clinical response.

### 1.3 Aims of study

1. To characterise and compare the cerebrospinal fluid profile of neuroimmune mediators in Failed Back Surgery Syndrome (FBSS) and facet joint arthropathy (FJA).
2. To examine the effect of spinal cord stimulation (SCS) and radiofrequency (RF) neurotomy on CSF and serum levels of neuroimmune mediators.
3. To examine the role of neuroimmune activation in the sleep disturbance and health-related quality of life and identify how these influence pain and response to interventional techniques.

### 1.4 Outline of this thesis

In this thesis, we investigate the role of neuroimmune mediators in the pain and dysfunction of chronic arthritic and neuropathic low back pain and the effect of neuroablation and neuromodulation technologies on these mediators.

**Chapter 2:** In this chapter we provide an overview of the current knowledge pertaining to the role of glial activation and neuroimmune mediators in chronic pain and suggest a conceptual framework for integrating this knowledge with the clinical entity of chronic low back pain and known physics of the presumed mechanism of action of interventional technologies.

## 1. INTRODUCTION

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**Chapter 3:** In this chapter we briefly describe the methodology of the present work. Clinical scores, and sample and data analysis common to both patient groups, neuropathic and arthritic, are outlined. Clinical procedures and modifications of sample collection specific to the RF neurotomy in FJA patients and SCS in FBSS patients are noted.

**Chapter 4:** In this chapter we present the relationships between CSF neuroimmune mediators and each other, and with clinical measures common to chronic back pain of either aetiology. We also report the differences in CSF levels between patients with FJA and patients with FBSS. Interestingly, while there were differences in CSF levels between inflammatory and neuropathic aetiologies, the commonality was that CSF neuroimmune mediators have inverse relationships with pain and analgesia.

**Chapter 5:** In this chapter we present the results of serum mediators in patients undergoing lumbar RF neurotomy, which included a significant reduction in serum BDNF and MCP-1 post-procedure and suggested a gender difference in VEGF levels, which in turn inversely correlated with post-procedure pain scores.

**Chapter 6:** In this chapter we present the relationship between CSF levels of BDNF and VEGF with pain in FBSS and change in CSF levels of VEGF in response to spinal cord stimulation. We also found a positive correlation between CSF concentrations of GDNF and SCS frequency.

**Chapter 7:** In this chapter, we summarise the main results of the thesis, present our interpretation of our findings and discuss future avenues of investigation.

# 2

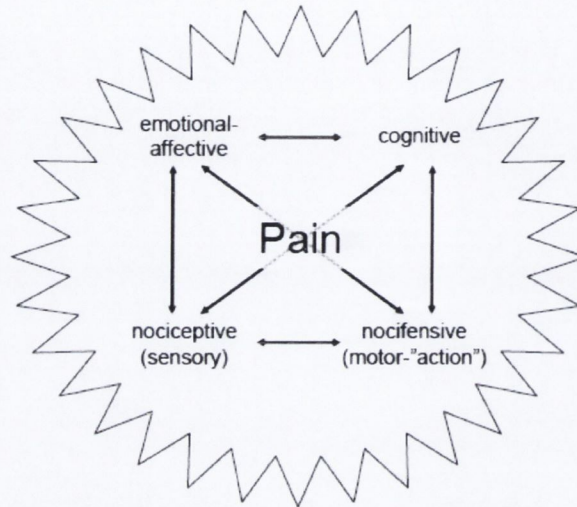
## Pain: From molecules to mind

### 2.1 Biopsychosocial Model

The current definition of pain proposed by the International Association for the Study of Pain (IASP) defines pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” [182]. As first suggested by Melzack and Casey in 1968 [139], this definition acknowledges that pain is multi-dimensional and needs to be evaluated along three components or axes: sensory-discriminative, motivational-affective, and cognitive-evaluative. The biopsychosocial model of pain management addresses the psychosocial and socioeconomic factors that may amplify the experience of pain beyond that which arises from tissue injury or inflammation. These psychosocial factors can magnify the biological component of pain at its inception, fuel its progression to chronicity, and diminish the effect of available treatments [299]. The biopsychosocial approach is also a means of understanding how pain and disease may be modulated at multiple levels of organisation, from the molecular to societal. Cognitive behavioural therapy (CBT) and mindfulness-based stress reduction (MSBR) are two psychological interventions with proven short-term efficacy in chronic low back pain [88][54]. CBT in combination with

## 2. PAIN: FROM MOLECULES TO MIND

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**Figure 2.1:** The emotional-affective, cognitive, nociceptive and nocifensive components may interact dynamically to form the multidimensional experience of pain. The reciprocal relationships are indicated by arrows. Reprinted from *Brain Research Reviews*, Vol. 60, Neugebauer et al., Forebrain pain mechanisms, 226-42, ©2009, with permission from Elsevier.

an interventional procedure can result in significant long-term improvements in disability, self-efficacy and affective distress [196]. One of possible explanations for the heterogeneity of response encountered is that ‘CBT’ is used as an umbrella term to cover reframing of thoughts and also elements of distraction, relaxation and biofeedback, so it is not always clear which component is providing the beneficial effect observed. CBT has been shown to improve sleep quality and reduce serum proinflammatory cytokine levels [41] in one small study of dialysis patients. If CBT is able to modulate pain experience via an attenuation of neuroimmune activation, it may prime microglia towards a neuroprotective, analgesic phenotype and augment other treatment modalities. In parallel with technological interventions, there is no question of efficacy for individual responders, it is one of extending the duration of that response and increasing the number of responders. The ‘psychology of pain’ is that of a normal psychological response to a disruptive signal that demands attention [71]. Our definitions of pain, and the descriptors that we use, carry the assumption or implication of tissue damage, which we are hardwired not to ignore.

## 2.2 Function of pain

Pain is not a homogenous entity and can be qualitatively quite different depending on the tissue site, stimulus and biochemical substrates involved. This heterogeneity of sensation is part of the universal experience of acute pain; sunburn may feel very different from a toothache. Ultimately, pain has evolved to promote survival either by protecting injured tissue or avoiding dangerous behaviour. We see evidence for this in that the familial syndromes associated with a congenital insensitivity to pain are typically associated with a shortened lifespan [53, 121], therefore one method of classifying pain is by whether or not it serves a protective biological function that ultimately promotes survival.

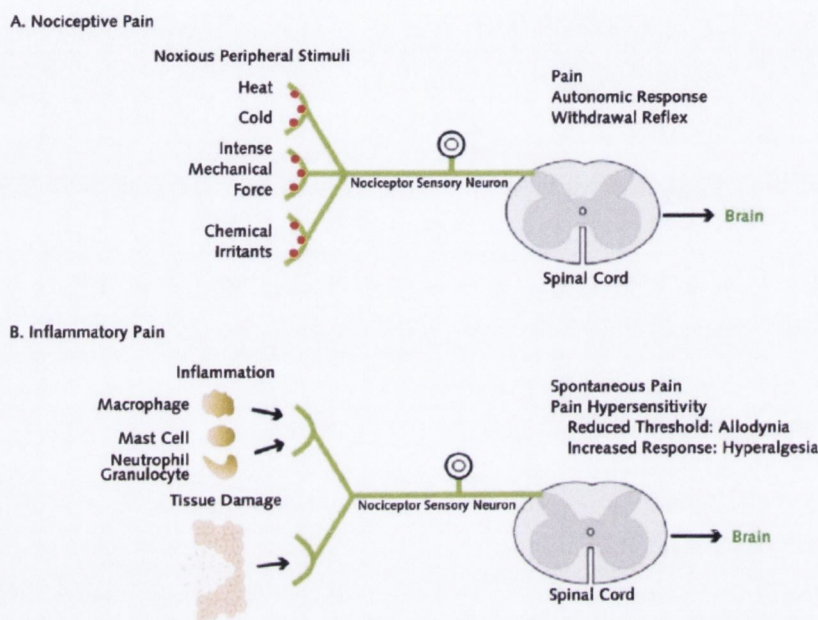
### 2.2.1 Adaptive pain

Nociceptive pain is a transient protective response to a noxious stimulus that prevents potential injury [132]. It is designed as a rapid response system. Once the perceived threat is dealt with, attention turns to drowning out any residual signal, usually by recruiting equally fast  $A\beta$  fibres. Inflammatory pain arises as a consequence of the body's attempt to contain and clear more insidious assailants, such as infectious organisms, or cellular debris where tissue integrity has been compromised. Pain is directed towards allowing containment or repair of tissue damage by limiting function until healing is complete. Again this process is intended to be adaptive, to protect the organism and ensure its ongoing survival. It is also intended to be relatively short-lived; a pro-inflammatory response is followed and counterbalanced by an attenuating anti-inflammatory reaction that acts to preserve epithelial integrity and tissue homeostasis [217]. However good the intention, as noted as far back as 1794, "when inflammation cannot accomplish that salutatory purpose, it does mischief" [114]. The mischief in this context is a sensory nervous system that is changed by this afferent input, a process of both peripheral and central sensitisation [320]. The functional state of the three-order-neuron nociceptive circuit is altered, with lower firing thresholds which may result in spontaneous pain, hyperalgesia and allodynia through the actions of prostaglandins (PGs), bradykinin, nerve growth factor (NGF) and

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cytokines [178]. Over time, this may become indistinguishable from the clinical features that are typically associated with maladaptive pain states.



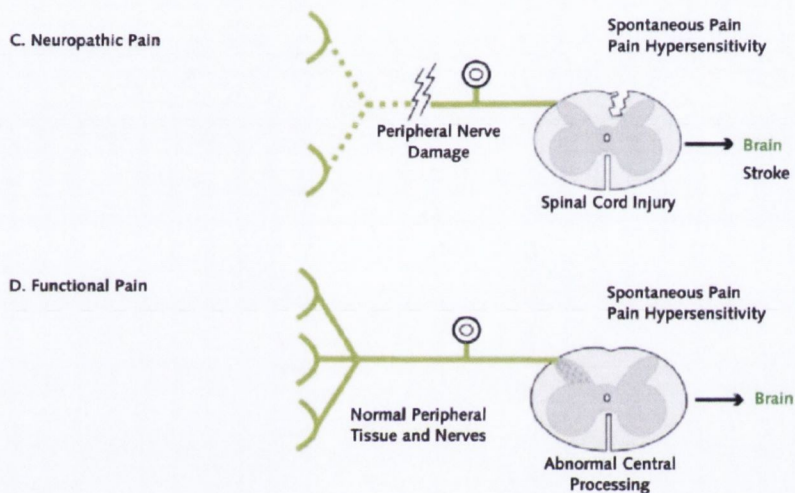
**Figure 2.2:** Adaptive Pain: Nociceptive and inflammatory. Reprinted from *Annals of Internal Medicine*, Vol. 140, C Woolf, Pain: Moving from Symptom Control toward Mechanism-Specific Pharmacologic Management, ©2004, with permission from the American College of Physicians.

### 2.2.2 Maladaptive pain

Neuropathic pain arises where the injury or inflammation is within the somatosensory nervous system itself and may be spontaneous or constant in nature. The classic definition of neuropathic pain ties it to a discreet neurological lesion, the anatomical location of which has been localised by clinical examination or with confirmatory testing [287]. While this distinction is important for research and therapeutic purposes, pain syndromes that include features of central sensitisation or maladaptive plasticity probably outnumber those with such well-defined pathologies. In population-based studies using neuropathic pain screening tools, with a neuropathic component is at least as prevalent as pain with a predominantly neuropathic aetiology [26][285]. In patients with chronic musculoskeletal

## 2.3 Gate Control Theory of Pain

pain, neuropathic symptoms are a prominent feature and are stable over time [92]. The other category of pain that does not serve a protective biological function is functional pain is an amplification of normal sensory input within the central nervous system to the point where it becomes noxious [319]. Fibromyalgia and irritable bowel syndrome are two examples of abnormal central processing of sensory input that is amplified to a degree that renders it noxious.



**Figure 2.3:** Maladaptive pain: Neuropathic and functional. Reprinted from *Annals of Internal Medicine*, Vol. 140, C Woolf, Pain: Moving from Symptom Control toward Mechanism-Specific Pharmacologic Management, ©2004, with permission from the American College of Physicians

## 2.3 Gate Control Theory of Pain

The Gate Control Theory of pain as proposed by Melzack and Wall in 1965 [180] has been the most widely-embraced theories of pain of the 20th century and was not without controversy when first proposed. Since publication, it facilitated a major conceptual shift in pain research and treatment by proposing the existence of a spinal gating mechanism within the dorsal horn of the spinal cord. This ‘gate’ is dependent on the relative activity of large-diameter fibres, which modulate the upward transmission of nociceptive signals from small-diameter fibres. The idea that the brain could send downward inhibition and that the spinal cord was more

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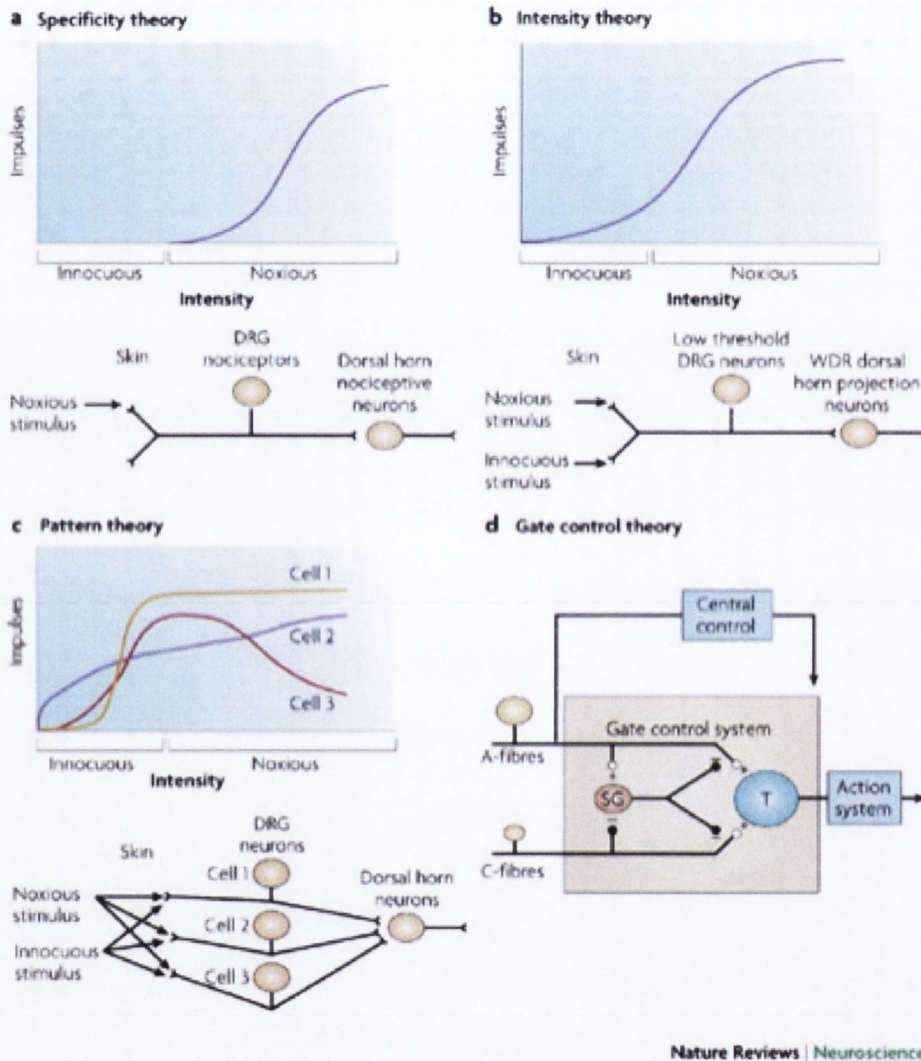
than a passive conduit and was dynamically involved in the modulation of pain signals was a novel one and placed the central nervous system at the centre of pain research and drug development. In terms of technological innovations, the concept of stimulating large fibres at a non-painful level and therefore closing the gate was successfully tested by Wall and Sweet in 1967 [301], heralding the modern era of neuromodulation for pain relief.

The gate control theory is not universally applicable to all pain phenomena and there are several clinical pain syndromes, such as phantom limb pain, that cannot be explained in terms of spinal gating mechanisms [179]. Where the theory of gate control retains great utility is as an educational tool for discussing the likely mechanism of action of various treatment modalities with patients. For example, at their most simplistic and reductionistic, the mechanism of interventional procedures such as neuroablation or neuromodulation can be categorised as closing the gate by either disrupting small fibre input or by depolarising large fibre afferents respectively.

The progression of proposed pain theories that culminated in the gate control theory [194] forms an interesting parallel with our current understanding of the role of glial activation and neuroimmune mediators in both chronic inflammatory and neuropathic pain. Initially it was assumed that glial cells are the “bad guys” [307] with an ‘on/off’ paradigm that produces pain when activated (specificity theory), which developed into an appreciation that glia may have either protective and/or pathological roles [187] depending on the magnitude or degree of activation (intensity theory) and that astrocytes and microglia exhibit different temporal [311], and possibly hierarchical, profiles of activation (pattern theory). These theories, and the gate control theory, offer a conceptual framework for incorporating new information into existing models and at various levels of organisation. Nervous and immune systems; astrocytes, microglia and neurons; cytokines, neurotrophins, proteases and growth factors; each may be thought of as gating and modulating the others depending on how it has been primed by prior exposure .



## 2.4 Gate Control Theory 2.0: Glia and pain



Nature Reviews | Neuroscience

**Figure 2.4:** Prevailing theories about pain in mid-20th century. Reprinted from Nature Reviews Neuroscience, Vol. 8, ER Perl, Ideas about pain, a historical view, ©2007, with permission from Nature Publishing Group

## 2.4 Gate Control Theory 2.0: Glia and pain

Even though they make up approximately 90% of all cells within the central nervous system, glial cells were largely ascribed a supportive nutritive role until it was noted that peripheral nerve injury that produced pain behaviours also caused

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glial activation [87]. The two main types of glial cells in the central nervous system (CNS), astrocytes and microglia, each have distinct roles in the homeostasis and defence of the neuronal microenvironment. The discovery that glial cells both produce and possess receptors for neurotransmitters, neurotrophins and cytokines has placed the dynamics of glial-neuronal signalling at the nexus of the cross-talk between the nervous and immune systems.

Astrocytes occupy a pivotal role, in terms of their number and physical proximity to neurons. They function in ensuring focal signal transfer between neurons as part of a tripartite synapse [11] and by providing an alternative extra-synaptic signalling pathway via the propagation of calcium waves [250]. Microglia on the other hand are mobile and depending on how they are primed by the signals they receive, will change their phenotype and rate of turnover accordingly [142]. Priming may occur in response to variety of stimuli from trauma to environmental stressors [79].

Astrocytes and microglia may be the ‘dark matter’ of the central nervous system that account for some of the epiphenomena encountered in the clinical treatment of chronic pain; we know they are there, we suspect they are up to something and yet we have no direct evidence in human subjects of their role. Therefore we are left with looking for indirect evidence and turning our attention towards levels of neuroimmune mediators to try and intercept and decipher the conversations that these cells are having.

### 2.4.1 Neuroimmune mediators: cytokines

Cytokines are small proteins that were first characterised as part of the immune response but have now been found to play a much broader role in diverse aspects of physiology. Spinal pro-inflammatory cytokine release parallels microglial activation following peripheral inflammation [234], in peripheral nerve injury models [65, 103] and following the intrathecal injection of HIV-1 envelope glycoprotein gp120 [192]. Release typically follows a tightly-regulated sequential cascade initiated by tumour necrosis factor-alpha (TNF- $\alpha$ ). Pro-inflammatory cytokines may modulate spinal pain facilitation by several possible mechanisms, either directly affecting neuronal excitability [209] or, over time, altering gene expression [308].

## 2.4 Gate Control Theory 2.0: Glia and pain

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There may be a reciprocal action between cytokines and other mediators such as nitric oxide (NO) [111], adenosine triphosphate (ATP)[123] or they may exert effects indirectly via other substances known to modify neuronal nociceptive processing, such as substance P, prostaglandin E<sub>2</sub>(PGE<sub>2</sub>) [247, 322] or nerve growth factor (NGF) [318]. Intrathecal injection of pro-inflammatory cytokines enhances pain behaviour [65, 76, 153, 237, 277] and glial inhibitors and cytokine antagonists prevent or reverse these pain states [178, 308] as does disrupting normal cytokine signalling [113, 317].

### 2.4.1.1 Neuroimmune mediators: TNF $\alpha$

Sensory neurons express receptor components capable of transducing extracellular tumour necrosis factor- $\alpha$ (TNF- $\alpha$ )[226], interleukin-1 $\beta$  (IL-1 $\beta$ )[85] and interleukin-6 (IL-6) [124]. TNF- $\alpha$  and IL-1 $\beta$  increase neuronal cell surface expression of both  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-Methyl-D-aspartate (NMDA) receptors, while decreasing the cell surface expression of  $\gamma$ -aminobutyric acid (GABA) [270]. The mechanical [58] and thermal [220] hyperalgesic responses produced by the intra-neuronal injection of TNF- are produced via the TNF-1 receptor [265]. The local release of TNF- from the lumbar dorsal spinal cord into the cerebrospinal fluid (CSF) has been documented upon induction of pain facilitation, the effects of which were reduced by intrathecal administration of a TNF functional antagonist [192].

### 2.4.1.2 Neuroimmune mediators: IL-1 $\beta$

Central IL-1 $\beta$  is normally lowly expressed and involved in neural functions such as sleep [298]and basal pain sensitivity [316]. Spinal cord levels of IL-1 are elevated in neuropathic pain models [64, 188, 302] and following acute peripheral inflammation [247]. Intrathecal IL-1 produces mechanical hyperalgesia and allodynia [76, 237]. These pain states are attenuated to greater degree by blocking the central IL-1 $\beta$  elevation than that in serum [248]. Larger doses of intrathecal IL-1 $\beta$  appear to be antinociceptive in the setting of previous sensitisation or ongoing inflammation, and this antinociception is not an opioid-mediated phenomenon

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[267]. Intrathecal IL-1 $\beta$  increases central PGE2 levels in a dose-dependent fashion and this PGE2 production is mediated primarily by cyclo-oxygenase-2 (COX-2) [258]. This spinal COX-2 upregulation is in turn attenuated by interleukin-1 receptor antagonist (IL-1ra) [119]. IL-1 $\beta$  was found to be elevated in the cerebrospinal fluid of patients with Complex Regional Pain Syndrome (CRPS) compared to controls [5].

### 2.4.1.3 Neuroimmune mediators: IL-6

Interleukin-6 is the prototypical member of what is variously termed the IL-6, gp 130 or neuroipoietic cytokine family [19]. All members signal through the gp 130 receptor, which is present on all cells, and IL-6 forms complexes with neuronally-expressed soluble IL-6 receptor (IL-6R) which then bind to gp 130. Studies of whether IL-6 is primarily neuroprotective or neurodestructive, and in the context of pain, nociceptive or anti-nociceptive, have yielded conflicting results. Virtually absent from the normal adult nervous system, increased levels of CSF IL-6 have been found in neuroinflammatory conditions, traumatic neurological injuries and experimental pain models [62]. In addition, IL-6 modulates both classic and novel mediators of pain transmission and facilitation, such as nitric oxide (NO) [111], NMDA [286], substance P [81], galanin [84], and nerve growth factor [286] (which in turn upregulates IL-6R [271]).

Intrathecal administration of IL-6 appears to have a direct nociceptive effect at the spinal level in a previously sensitised spinal cord versus a non-specific effect that could be related to illness-induced hyperalgesia [65], an effect that was not dependent on the dose of exogenous IL-6 administered [297]. However, antinociceptive effects of IL-6 have also been demonstrated [78]. Generally, there has been a trend of endogenous IL-6 producing pro-nociceptive effects [12, 39, 64, 65], and knockout mice show attenuated neuropathic pain behaviours after nerve injury [199, 235] indicating a pro-nociceptive role overall. Although, IL-6 may not produce sickness behaviour of the same magnitude as TNF- $\alpha$  or IL-1 $\beta$ , it has been shown to mediate the release of TNF- $\alpha$  and IL-1 $\beta$  via an alternative pro-inflammatory cytokine pathway to that found in the periphery [253] so may be part of a feed-forward loop. In patients with CRPS, cerebrospinal fluid levels

of IL-6 were elevated relative to controls with pain and this increase was greater in patients with CRPS Type II than CRPS Type I [5].

### 2.4.1.4 Neuroimmune mediators: IL-10

Intrathecal IL-10 can both prevent[131] and reverse [191] pain facilitation associated with spinal glial activation and release of pro-inflammatory cytokines and blocks the IL-1-mediated mechanical allodynia produced by intrathecal dynorphin [155]. IL-10 reduces the transcription of pro-inflammatory cytokine (PIC) gene expression [197], however the rapid reversal of pain behaviours by intrathecal IL-10 in under one hour suggests gene suppression alone cannot account for its anti-nociceptive effects [190]. Non-genomic effects of IL-10 include inhibition[144] and destabilisation [243] of PIC mRNAs, in part by inhibition of p38 mitogen-activated protein kinase (MAPK). Due to the negligible passage across the intact blood-brain barrier of IL-10 [17] and its short systemic half-life [230] necessitating sustained delivery, targeted delivery to the spine is required. Intrathecal IL-10 administered to the lumbar spine is not degraded and is removed from the CSF by bulk flow. However, only 20% reaches the cervical region suggesting uneven distribution or rapid clearance [186]. Therefore, the clinical utilisation of IL-10 therapy for chronic pain states remains some time away.

### 2.4.1.5 Neuroimmune mediators: IL-2

Interleukin-2 (IL-2) is one of the most actively studied cytokines. In addition to being an important immunoregulatory molecule enhancing T-lymphocyte proliferation after antigenic stimulation, it also has several neuroregulatory functions [102] and has been shown to have an antinociceptive effect with intrathecal [325] and systemic[266] administration. The short half-life of systemic IL-2 protein [143] makes it an unlikely clinical analgesic. However, there is a case report [242] of immediate and sustained resolution of intractable postherpetic neuralgia (PHN) in a patient with human immunodeficiency virus (HIV) infection, who was enrolled in a clinical trial evaluating the effect of IL-2 in combination with antiretroviral therapy [73]. Intrathecal IL-2 gene therapy has produced a sustained antinociceptive effect in neuropathic [324] and inflammatory pain models [325], an effect that is at least partly mediated by opioid receptors.

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### 2.4.2 Neuroimmune mediators: chemokines

Chemokines are a group of about forty structurally-related cytokines consisting of small peptides of about 8 kDa that facilitate the passage of leucocytes from the circulation into tissues [95]. Chemokines are divided into four subfamilies according to the spacing of their cysteine residues: CXC, CC, C, and CX3C[20].

#### 2.4.2.1 Neuroimmune mediators: IL-8

The prototypical chemokine is interleukin-8 (IL-8) or CXCL8, which functions primarily as a neutrophil chemoattractant. IL-8 appears to mediate the effects of several neurodestructive disorders [140, 312] and recombinant neutrophil inhibitory factor (NIF), pharmacologically an IL-8 antagonist, has improved neurological outcomes in animal models of traumatic brain injury [129]. IL-8 stimulated the production of nerve growth factor (NGF) in cultured astrocytes [145] and correlates closely with NGF concentration in the spinal cord following brain injury. In one study of 170 patients [147], CSF IL-8 levels at the time of crusting of herpes zoster lesions were predictive of subsequently developing postherpetic neuralgia (PHN). A positive response to intrathecal steroids for the treatment of PHN [146] and cancer pain from vertebral metastases [120] has also been correlated to a reduction in CSF IL-8 levels. In a study of CSF levels of pro-inflammatory cytokines in intervertebral disc herniation and sciatica, CSF IL-8 levels correlated with the degree of intervertebral disc herniation but not with pain intensity or neurological findings [29].

#### 2.4.2.2 Neuroimmune mediators: CX<sub>3</sub>CL1

Fractalkine (CX<sub>3</sub>CL1) was the first chemokine discovered to have a role in neuronal to glial signalling and, to date, is the only known ligand for the receptor CX<sub>3</sub>CR1 [13]. Fractalkine is only expressed by neurons and CX<sub>3</sub>CR1 only by microglia under normal conditions; astrocytic upregulation occurs in the gliosis associated with spinal nerve injury [166] and neuroinflammatory conditions [275]. Expression is increased in pain modulatory regions of the spinal cord in both neuropathic and monoarthritic pain models [295]. Exogenous fractalkine induces pain facilitation [186] and blockade of fractalkine receptors delays [189]

and reverses [256] neuropathic pain behaviours, implicating neuronally-derived fractalkine in the initiation and maintenance of neuropathic pain. The pronociceptive mechanisms of fractalkine are varied and include the release of IL-1, IL-6 and nitric oxide [186], and co-administration of anti-CX<sub>3</sub>CL1 antibody with intrathecal morphine attenuates the subsequent development of allodynia and hyperalgesia [131].

### 2.4.2.3 Neuroimmune mediators: MCP-1

Monocyte chemoattractant factor (MCP-1), or CCL2, is expressed by damaged dorsal root ganglion neurons [313] and mediates macrophage recruitment to the site of injury. However, while MCP-1 may cause proliferation of microglia, it does not seem to affect neuronal viability [108]. Intrathecal CCL2 produces pain facilitation [279] and administration of neutralising antibodies suppresses neuropathic pain behaviour [1]. MCP-1 depolarises or increases the excitability of small-fibre nociceptive neurons in the dorsal root ganglion (DRG) after a chronic constriction injury [274] and increases the permeability of the blood-spinal cord barrier after peripheral nerve injury [72]. Recently upregulation of MCP-1 in the dorsal root ganglion (DRG) has also been implicated in pain behaviour in an osteoarthritis model [185].

## 2.4.3 Neuroimmune mediators: neurotrophins

### 2.4.3.1 Neuroimmune mediators: NGF

Interest in the role of the neurotrophin family of proteins in pain facilitation arose from the determination of the genetic basis of the congenital insensitivity to pain in a single family [122]. The mutation identified coded for a tyrosine kinase receptor (trkA) which is a high affinity receptor for a single trophic factor, nerve growth factor (NGF). Neurotrophins that appear to have a role in nociception and neuropathic pain include NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3) and members of the GDNF family of neurotrophic factors [245]. NGF levels rise substantially in inflamed or damaged tissue, and enhanced retrograde neuronal transport of NGF occurs simultaneously. Blocking NGF activity blocks the effects of inflammation on sensory nerve function

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[177]. In the treatment of painful neuropathies, while NGF may prevent or reverse the peripheral neuropathy, it produces pain on administration, limiting the doses employed in clinical trials of diabetic[10] and HIV neuropathies [252]. Although the main body of evidence implicates a peripheral mechanism of action for NGF in nociception, increases in the levels of NGF have been found in the CSF of patients with painful syndromes such as fibromyalgia [91] and chronic daily headache, which has correlated with elevations of CSF substance P and calcitonin-gene related peptide (CGRP) [249].

### 2.4.3.2 Neuroimmune mediators: BDNF

Brain-derived neurotrophic factor (BDNF) is another member of the neurotrophin family that appears to act as a central pain modulator in both inflammatory [99] and neuropathic [38, 160] pain states. BDNF is involved in hippocampal long-term potentiation (LTP) [126] and there is growing evidence of a parallel role in the plasticity of spinal nociceptive processes. It is co-localised with substance P and CGRP [224] and may also control the release of GABA in the superficial dorsal horn of the spinal cord [160, 223]. Like IL-6, with which BDNF appears to have reciprocal actions[200], the effect of BDNF on pain facilitation varies in different models studied. While blocking the action of endogenous BDNF reduces pain behaviours in inflammatory [99] and neuropathic [83, 321] pain models, the results of studies utilising exogenous BDNF have, on balance, demonstrated an antinociceptive effect [38, 70, 160]. In addition, intrathecal grafts of BDNF-expressing fibroblasts have shown an antinociceptive effect and even some recovery of neurological function in a spinal cord injury model [259].

### 2.4.3.3 Neuroimmune mediators: GDNF

Glial cell line-derived neurotrophic factor (GDNF) belongs to a family of growth factors within the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily. The GDNF family of ligands (GDNF, artemin, persephin and neurterin) promotes the survival of several distinct neuronal populations, such as motoneurons, nociceptive sensory neurons and dopaminergic neurons [245]. Intrathecal GDNF has prevented and reversed pain behaviours in neuropathic pain models [24][25] and did



not produce any changes in behaviour in sham-operated animals or alter baseline nociceptive thresholds. Given that it promotes the survival of central dopaminergic neurons, it has undergone clinical trials in Parkinson's Disease [208], but has produced undesirable side effects in vivo such as weight loss and allodynia.

### 2.4.4 Neuroimmune mediators: Growth Factors

#### 2.4.4.1 Neuroimmune mediators: VEGF

Vascular endothelial growth factor (VEGF) has primarily been studied as an angiogenic factor and peripherally may have a role as a biomarker of disease severity in peripheral vascular disease [269] and in CRPS [216]. Within the central nervous system, VEGF has roles as a modulator of calcium flux in neurons, in synaptic plasticity, and as a neuronal and glial protective factor [207]. VEGF has been implicated in the pathogenesis of neuropathic pain behaviour in a chronic constriction injury model, with higher levels of VEGF potentiating pain responses via P2X<sub>2/3</sub> receptors in the dorsal root ganglion [162]. Endogenous VEGF has also been suggested to play a role in the nonspecific sprouting of myelinated axons and allodynia following spinal cord injury [202].

### 2.4.5 Neuroimmune mediators: Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases capable of breaking down extracellular matrix proteins. They also are known to interact with molecules including cell surface receptors, apoptotic ligands, and chemokines, and they play an active role in several neurodegenerative diseases such as multiple sclerosis [326] and Guillain-Barré Syndrome [158]. MMPs have recently been shown to have distinct roles at different stages in the pathogenesis of neuropathic pain in an animal model. MMP-9 induces microglial activation and the acute phase of neuropathic pain behaviour while MMP-2 maintains late-phase neuropathic pain and astrocyte activation. Both MMP-9 and MMP-2 exert these effects via cleavage of IL-1 $\beta$  [135]. Patients with migraine, with and without aura, have been found to have higher circulating levels of MMP-2 and MMP-9 than controls [175].

### 2.5 Central Sensitisation 2.0: Role of Toll-like Receptors

Each of the above-named mediators are analogous to the e-mails and text messages that neurons and glia send each other. Cells are continuously talking and listening to each other but they need a modem to transmit and receive local and remote signals; toll-like receptors (TLRs) are specialised transmembrane pattern recognition receptors that form part of the innate immune systems ability to distinguish self from both non-self and damaged self [176]. TLRs respond to both external pathogens and endogenous danger signals of tissue damage by recognising signature ligands called pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs) respectively. TLR activation may be the common link that accounts for gender differences in the prevalence of chronic pain syndromes and the recognition of and response to available analgesics [203]. TLR<sub>4</sub> is the most studied in the context of pain and activation appears essential for the development of allodynia and behavioural changes. The clinical effect of three disparate classes of analgesics, tricyclic antidepressants (TCAs), COX inhibitors and opioids may be dependent on, and mediated by, TLR activation.

#### 2.5.1 Tricyclic antidepressants

Tricyclic antidepressants have been used off-label in low doses in the treatment of a variety of chronic pain conditions. Amitriptyline, the prototypical TCA, has multiple molecular targets that would account for its analgesic effect, among them serotonin, noradrenaline and acetylcholine receptors, and sodium and calcium channel blockade [280]. Other novel mechanisms of action have now been demonstrated, such as agonist activity at TrkA and TrkB, effectively mimicking the effect of NGF and BDNF [125], MMP-dependent increases in GDNF production [109] and potentiation of morphine analgesia via the inhibition of TLR<sub>2</sub> and TLR<sub>4</sub> [116].

### 2.5.2 COX inhibitors

The induction of central cyclooxygenase-2 (COX-2) and subsequent production of prostanoids leads to central sensitisation following peripheral inflammation[247]. Central prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is used as a surrogate marker of central sensitisation in several animal pain models [15, 68, 294, 315]. However there is a ceiling effect to the analgesia conferred by NSAIDs and selective COX-2 inhibitors and concerns about gastrointestinal and thromboembolic adverse effects associated with long-term use [215]. The selective COX-2 inhibitors may also induce tolerance with sustained use [239] with a reduced analgesic effect over time. COX inhibitors have been shown to suppress TLR<sub>4</sub>-mediated central PGE<sub>2</sub> synthesis but not the associated tactile allodynia, whereas minocycline attenuated allodynia but not expression of spinal PGE<sub>2</sub> [246]. This suggests that in the context of TLR activation, COX inhibitors may be of limited clinical utility.

### 2.5.3 Opioid-induced hyperalgesia

Chronic systemic morphine activates spinal glia and upregulates proinflammatory cytokines, which temporally correlated to the development of opioid tolerance [232]. This pattern of spinal neuroimmune activation has also been observed with chronic, but not acute, intrathecal morphine [131]. Disrupting glial activation [233], neuronal-glia signalling [131] and central cytokine production enhances acute intrathecal morphine analgesia and reverses and attenuates morphine-induced tolerance and hyperalgesia [131, 232].

This modulation of opioid analgesia may be mediated directly by actions on opioid receptors and endogenous opioid peptides or indirectly via other endogenous neuromodulators that counteract opioid responses. Glial expression of mu opioid receptors is increased in response to morphine [169], suggesting progressively increasing responses with repeated administration. However, in addition to inducing the release of cytokines, morphine can also stimulate chemokine release, such as CCL1/MCP-1, CCL5/RANTES and CCL12 [309]. Chemokine receptor binding, at CCR1, CCR5 and CXCR4, can desensitise or deactivate opioid receptors on the same cell via intracellular signalling cascades. It is not clear whether

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this heterologous desensitisation is uni- or bi-directional and if binding at opioid receptors modulates chemokine receptor binding [2].

Originally described in relation to peripheral immune cell interactions, this relationship also seems to extend to the central nervous system. Pretreatment with CXCL12 or CCL5 to the periaqueductal grey matter prior to morphine administration suppresses analgesia and CXCL12 receptor antagonism with AMD3100 reverses this effect equally at mu, delta and kappa opioid receptors [2]. The overall clinical effect would appear to be a diminished neuronal response to chronic opioids secondary to chemokines of glial origin.

TLR<sub>4</sub> activation on astrocytes, microglia and neurons may produce each of the above mediators and exposure to opioids themselves not an absolute requirement to produce the phenomenon [310]. The opioid antagonist, naloxone, attenuates opioid-induced hyperalgesia (OIH) by antagonism of TLR<sub>4</sub>, a finding confirmed in TLR<sub>4</sub> knockout mice [117]. The morphine metabolite, morphine-3-glucuronide, has also been demonstrated to activate microglial TLR<sub>4</sub> [161], raising the possibility that it is the body's own attempt to clear a drug that renders it capable of sensitising or activating TLR<sub>4</sub> receptors and producing hyperalgesia.

### 2.6 Neuroimmune activation in human pain

In the absence of a means to easily monitor glial function in real time, we are left with analysis of CSF biomarker content for the study of chronic pain, which is in most cases opportunistic following sample collection for diagnostic lumbar puncture or prior to intrathecal drug administration. Pro-inflammatory cytokines propagate through the CSF by bulk flow [298] and since the total volume of CSF turns over two to three times per day, cytokines are therefore capable of diffusing over very long distances within hours.

There is only one prospective study that has identified a central inflammatory mediator as a predictor of the development of chronic pain. Kotani et al. [147] enrolled 170 patients with herpes zoster and collected CSF at presentation and at full crusting of the herpetic rash. CSF was analysed for assays of IL-1, TNF-, IL-6 and IL-8. These were compared to healthy age-matched controls undergoing spinal anaesthesia. In multivariate analysis, only three variables, advanced age,

## 2.7 Clinical lumbar back pain syndromes

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degree of acute pain and IL-8 concentration at time of crusting of the acute rash, were significant predictors of post herpetic neuralgia at six months and one year. This followed on from previous studies which correlated a reduction in CSF IL-8 following intrathecal methylprednisolone with a corresponding reduction in severity and duration of neuralgia [146].

Alexander et al. have conducted two studies comparing levels of CSF inflammatory markers with those of controls with and without pain. In one study, they demonstrated an elevation in CSF levels of IL-1 and IL-6 in patients with CRPS that had been present for an average of two years[5]. In the second study to elucidate the contribution of chemokines and glial activation to the pathogenesis of CRPS, patients with a mean duration of symptoms of 8.4 years displayed a pattern of elevated IL-6, low levels of IL-4 or IL-10, increased glial fibrillary acidic protein (GFAP) and MCP-1 and increases in at least two of the following markers nitric oxide metabolites, calcium or glutamate[4]. Inada et al.[120] pilot study examining the role of intrathecal betamethasone in intractable cancer pain from vertebral metastases demonstrated that a reduction in pain scores was related to a reduction in CSF levels of IL-8 and PGE2.

In addition to the finding of low anti-inflammatory cytokines in CRPS, an inverse relationship between CSF levels of IL-10 and reported pain has also been found in painful peripheral neuropathy[16], suggesting that an imbalance between pro- and anti-inflammatory cytokines may contribute to the maintenance of chronic pain regardless of primary pathophysiology.

Patients with painful lower limb osteoarthritis necessitating a joint replacement have been found to have higher levels of CSF of GDNF, IL-8 and IL-1 $\beta$  than matched controls [168].

## 2.7 Clinical lumbar back pain syndromes

### 2.7.1 Facet joint arthropathy

Pain arising from the lumbar zygoapophysial joints was first described by Goldwaithe in 1911 and the term “facet syndrome” was coined by Ghormley in 1933 to refer to the onset of lumbosacral pain occurring after a rotational strain that may

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or may not contain a radicular component [293]. Predisposing factors include spondylolisthesis and degenerative disc disease both of which cause increased loading of the facet joints. Occasionally, it can arise from a specific traumatic event although the more common clinical scenario is inflammation arising from cumulative low-grade degenerative changes. Paraspinal tenderness, unilateral pain and a diffuse pattern of radiation that does not extend below the knee are some of indicators of lumbar facets as a potential source of pain [314].



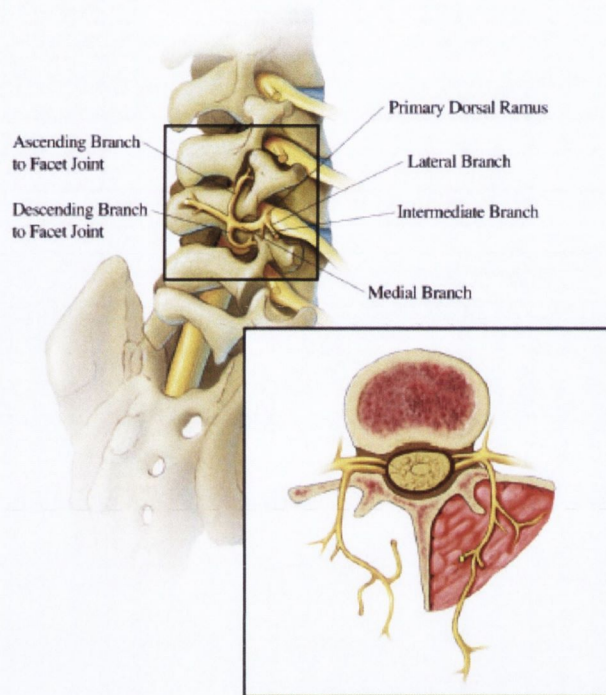
**Figure 2.5:** Pain referral patterns from lumbar facet joints. Reprinted from Pain Practice, Jul 26, 2010, Van Kleef et al., Pain Originating from the Lumbar Facet Joints, ©2010, with permission from John Wiley and Sons

Each joint receives its nerve supply from the medial branches of two segmental nerves, at that level and one level above and is richly innervated with encapsulated, unencapsulated, and free nerve endings [49]. In addition to nociceptors, the joint capsule and surrounding structures are innervated with mechanosensitive neurons and sympathetic efferent fibres [14] suggesting multiple potential neural pathways of pain transmission and help explain some of the pain referral patterns observed in clinical practice. Inflammation can cause radicular pain if the segmental nerve becomes irritated by the release of inflammatory cytokines from the joint capsule [118]. Other changes in response to facet joint inflammation that include an increase within the dorsal root ganglion (DRG) in the

## 2.7 Clinical lumbar back pain syndromes

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numbers of brain-derived neurotrophic factor (BDNF)-immunoreactive neurons and a phenotype shift to larger neurons [212].



**Figure 2.6:** Right lateral oblique view of the lumbar vertebral bodies and the dorsa rami medial branches. reprinted from *Anesthesiology*, Vol 106, Cohen et al., Pathogenesis, Diagnosis, and Treatment of Lumar Zygoapophysial (Facet) Joint Pain, ©2007, with permission from Wolters Kluwer Health

The prevalence, diagnosis and treatment of facet joint arthropathy remains the subject of ongoing debate and controversy. Various clinical criteria have been proposed to aid in differentiating painful facet joints from other anatomical structures [238], however the current gold standard remains controlled diagnostic blocks with local anaesthetic. The prevalence of painful facets has been variously reported as between 15%-52% and does increase with age and in the presence of arthritis [293]. Clinical practice for the interventional treatment of facetogenic pain has moved away from intra-articular injection of steroid towards radiofrequency (RF) neurotomy of the medial branches supplying the joint. When performed correctly, thermal RF produces controlled and reproducible lesion dimensions [260] and can be repeated without any loss in efficacy[254]. There have

## 2. PAIN: FROM MOLECULES TO MIND

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been two studies comparing thermal RF and pulsed radiofrequency (PRF) for lumbar facet pain[148, 283]. In both studies, while there was no difference between RF and PRF in the short term, the duration of effect of RF was sustained for longer in the RF treated patients. Therefore, RF medial branch neurotomy remains the preferred technique for lumbar facet pain.

### 2.7.2 Failed Back Surgery Syndrome

Failed Back Surgery Syndrome (FBSS) refers to persistent or recurrent low back or leg pain and affects approximately 30% of patients who have undergone technically adequate lumbar spinal surgery [32]. It may arise as a consequence of post-operative changes in spinal anatomy or environment or if the surgery did not accomplish its intended goal. FBSS is a descriptive label for a constellation of symptoms that may pre-date the actual operation and that can occur in the absence of surgery, therefore there is no specific pattern of signs or symptoms that is pathognomic. The commonality between patients with FBSS is a neuropathic pain syndrome that can result in greater levels of pain, lower quality of life and a higher rate of unemployment (78%)compared to other chronic pain illness models [284]. With the explosion in rates of lumbar spinal fusion surgery, with a 220% increase in the decade between 1990 and 2000 [67] and an estimated 250,000 new laminectomies each year as of 2002 [261], and given an average reported failure rate of 30%, there are significant numbers of new patients with FBSS each year. There is conflicting evidence on the significance of post-operative processes, such as an inflammatory reaction and epidural fibrosis [7, 23], or a biomechanical change such as new-onset facet joint instability [214]. Studies identifying the aetiology of FBSS have been summarised by Hussain et al.[115] as shown in Table 2.1.

The pain in FBSS is a combination of nociceptive and neuropathic and must be compared to the pre-operative presentation that led to surgery. Leg pain may be due to failure to surgically correct or recurrence of an intervertebral disc herniation, which may be detected on radiological investigation. Back pain as the sole or dominant feature is more difficult to treat and may be the result of incorrect diagnosis (and therefore inappropriate or inadequate surgery), spinal stenosis, epidural fibrosis or nerve root injury or scarring [32]. Neuropathic pain



## 2.7 Clinical lumbar back pain syndromes

**Table 2.1:** Studies of FBSS Aetiologies

Burton et al. [31]	Waguespack et al. [300]	Slipman et al. [261]
Lateral spinal stenosis 58%	Foraminal stenosis 29%	Stenosis (all types) 21.5%
Adhesive arachnoiditis 16%	Painful discs 17%	IDD 21.5%
Recurrent HNP 12%	Pseudarthrosis 14%	Fibrosis 14.5%
Epidural fibrosis 8%	Neuropathic pain 9 %	Recurrent HNP 12.4%
Central stenosis 7%	Recurrent HNP 6%	DDD 9.1%
Others <5% each	Others <5%	Others <6%

HNP, herniated nucleus pulposus; IDD, internal disc disruption; DDD, degenerative disc disease

is associated with sensory abnormalities and typically is initially confined to the same distribution as the sensory abnormality but then extends over time due to secondary changes in central processing neurons [165].

The treatment options for FBSS are conservative management, minimally invasive interventional procedures and revision surgery, determined by the dominance of back or leg pain and the presence or absence of other neurological Rehabilitation has better results if intensive and multidisciplinary [104]. Minimally invasive procedures that may be indicated by clinical and radiological findings may include epidural injections, epidural adhesiolysis, facet joint procedures, disc interventions, and spinal cord stimulation [115]. Revision surgery is associated with a high failure rate and in the study by North, 62% of patients chose to cross over to spinal cord stimulation [206]. Spinal cord stimulation has been shown to be efficacious and cost-effective in this population in several studies and reviewed by Hussain et al. [115] and Chan et al. [40](Table 2.2).

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**Table 2.2:** Studies of spinal cord stimulation in FBSS

Study	Control Group	SCS: Con- trols	Results and outcome measures
Kumar (2002) [151]	CMM	60: 40	QoL in SCS vs CMM improved by 27% vs 12%. SCS cost neutral by 2.5 years
Kumar (2007) [149]	CMM	52: 40	Pain relief >50% in 48% of SCS vs 9% of CMM
Kumar (2008) [150]	CMM	41: 41	Pain relief >50% in 37% SCS vs 2% CMM in “intention-to-treat” analysis
North (2005) [204]	Reoperation	19: 26	Major pain relief in 39% SCS vs 12% reoperation; reduced opioids in 87% SCS vs 58% of reoperation group
North (2007) [205]	Reoperation	19: 21	Cost reduction: \$48,357 SCS vs \$105,929 reoperation

SCS, spinal cord stimulation; QoL, quality of life; CMM, Conventional Medical Management

### 2.8 Interventional Technologies

When definitive treatment that addresses the primary process that is not possible then interventional procedures may form part of the armamentarium of therapeutic modalities available to reduce the burden of chronic pain. They may be broadly divided into ablative or augmentative. The earliest recorded ablative procedure for pain relief is probably trephination, which existed in several cultures across diverse geographical locations [236]. Similarly, the earliest recordings of the use of neurostimulation to relieve pain can be dated back to the Fifth Dynasty of ancient Egypt, when natural sources of electricity such as the Nile catfish and torpedo fish [22] were employed. Interestingly, the torpedo fish and the Nile

catfish, both sources of bioelectricity, produce a current similar in voltage and frequency to modern stimulators.

### 2.8.1 Radiofrequency Neurotomy

Radiofrequency (RF) is an alternating current with a frequency of 500,000 Hz. The RF generator produces current that may be directed through body tissue through an insulated electrode. The high current density at the interface of the electrode and organic matter, which acts as a resistor, produces heat and a mean of ablating biological tissue in a targeted manner [262]. The biological effects of RF may be due to heat, the high intensity electric fields, or a combination of the two [47]. Thermal destruction of neural tissue is thought to be reversible below 45°C although when used in clinical practice, temperatures up to 80°C may be generated at the electrode tip. It is the tissue that heats up the electrode and once the desired temperature is reached, the output of the generator compensates for the rate of heat washout which in turn depends on the vascularity of the tissue. Other applications of RF include ablation of tumour metastases and accessory conduction pathways that cause cardiac arrhythmias. As a technology for pain relief, RF was originally applied more centrally to the spinal cord [241] and trigeminal ganglion [276] before it found use for the treatment of spinal pain.

The longstanding explanation of the mechanism of action of an RF procedure was of a heat lesion that interrupted afferent nociceptive pathways. Postoperative discomfort is attributed to a reactive swelling and ultimate recurrence of pain is attributed to nerve regeneration. There is a temperature gradient within an RF lesion and some techniques account for this and aim to include the target structure in a moderate temperature zone. The rationale for this is to effect disruption of small unmyelinated fibres while sparing large fibres and reducing deafferentation sequelae [159]. This concept of tissue destruction has been revised in view of findings that are inconsistent with this theory. There is a discrepancy between pain relief and other sensory findings and the effectiveness of RF when applied to the DRG for certain procedures where the electrode is distal to the nociceptive input. The alternative proposed mechanism is an effect due to the electrical field produced, which has led to to the modification of pulsing the current.

## 2. PAIN: FROM MOLECULES TO MIND

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Pulsed RF (PRF) is not associated with a histological nerve lesion in laboratory studies. The electrode tip delivers a large current density to the nerve, but the pauses between pulses allows dissipation of any heat produced, thereby preventing any thermal lesion. Application of PRF has been associated with changes in myelin configuration in the axons of pain afferents [74] and with an increase in expression of c-Fos, a marker of neuronal activity, in the segmental dorsal horn which does not occur with continuous RF [107]. Spinal mechanisms may include enhancement of noradrenergic and serotonergic descending pain inhibitory pathways [101] and attenuation of microglial activation in the dorsal horn [43].

Thermal or continuous RF remains the more efficacious for certain pain conditions, such as trigeminal neuralgia and lumbar medial branch neurotomies [290]. However, PRF may ultimately be applicable to a broader number of conditions.

### 2.8.2 Spinal Cord Stimulation

Spinal cord stimulation (SCS) arose as a direct clinical spin-off from the “gate control” theory of pain [180]. Since its first use in 1967 [257], SCS has achieved a proven record in the management of certain chronic pain conditions. The clinical use has steadily increased and it has been estimated that more than 14,000 new systems for DCS are implanted every year worldwide. However, some patients who are initially good responders may subsequently experience a diminished response over time [138]. Other recognized phenomena include post-stimulation analgesia and a significant improvement in the quality of sleep.

A recent review of DCS for Failed Back Surgery Syndrome (FBSS) included 1 randomized-controlled trial (RCT), 1 cohort study and 72 case studies [281]. The RCT showed significant benefit ( $p = 0.047$ ) in patients with FBSS reporting 50% or more pain relief with SCS (37.5%) compared with back re-operation (11.5%) [204]. Moreover, a review of 14 studies of cost-effectiveness demonstrated that DCS is cost-beneficial, and although more expensive initially, proves to be less expensive than traditional treatment after an average of 2.5-3 years [281].

The current literature suggests a significant benefit in the use of DCS to treat pain due to CRPS type I (Level A evidence) and CRPS type II (Level D evidence)[282], with a low incidence of serious complications [21]. A formal

## 2.8 Interventional Technologies

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cost effectiveness analysis based on the RCT showed that treatment with SCS in CRPS resulted in a lifetime cost saving of \$58,471 per patient compared to the standard treatment alone [138].

SCS is not without limitations even in those conditions where it has demonstrated effectiveness:

- There is at least a 30% non-response rate.
- There is a diminution in effect over time.

Most of the work on the proposed mechanism of action of SCS has focused on GABA-ergic effects. This was based on observations that the antinociceptive and anti-allodynic effects of SCS were enhanced by the administration of the *GABA<sub>B</sub>* agonist, baclofen, and abolished by GABA antagonists [272][56] [184]. The effect of augmenting SCS is less pronounced with the use of *GABA<sub>A</sub>* agonists. This may be because *GABA<sub>A</sub>* receptor-mediated inhibition is strongly determined by  $K^+ Cl^-$  cotransporter 2 (KCC2) functionality [127], which in turn is downregulated by BDNF [328]. Other proposed mechanisms suggest a potential role of adenosine [183] and the release of serotonin and substance P at the level of the periaqueductal grey [164]. Most recently,  $^1H$  MR spectroscopy showed an increase in GABA and decrease in glucose in the ipsilateral thalamus after nine minutes of SCS in 20 patients with FBSS [195].

## 2. PAIN: FROM MOLECULES TO MIND

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# 3

## Methodology

### **3.1 Patient Recruitment**

#### **3.1.1 Ethical approval**

Ethical approval was granted by the Clinical Research Ethics Committee of the Cork Teaching Hospitals for the study protocol. Patients were recruited via the Department of Pain Medicine, St. James' Hospital, Dublin and samples handled and stored at the Department of Physiology, Trinity College Dublin and the Trinity College Institute of Neuroscience.

#### **3.1.2 Inclusion and exclusion criteria**

##### Inclusion Criteria:

- Group 1: Patients with lumbar facet joint arthropathy (FJA) scheduled for radiofrequency medial branch neurotomy.
- Group 2: Patients with a functioning spinal cord stimulator implanted for Failed Back Surgery Syndrome (FBSS).

### 3. METHODOLOGY

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- Patients who have agreed to participate in the study following informed consent.

#### Exclusion criteria:

- 1. Patient refusal to participate in study
- 2. Conditions known to alter CSF cytokine and neurotrophin levels:
  - Multiple Sclerosis
  - Post-Traumatic Stress Disorder
  - Parkinsons Disease
  - Recent major surgery or trauma
  - Stroke
  - Cognitive impairment
  - Depression
  - Recent surgery or trauma
  - Recent use of corticosteroids or anticoagulant medications.

#### **3.1.3 Patient recruitment: RF Group**

We received ethical approval to obtain samples of cerebrospinal fluid (CSF) and serum from patients undergoing RF neurotomy for the treatment of lumbar facet joint arthropathy diagnosed by a positive response to medial branch nerve blocks. Fifty-five consecutive patients were approached for samples at baseline and two hours post-procedure. Seventeen patients consented to CSF sampling and twenty to serial serum sampling. All patients had the the Brief Pain Inventory (BPI), Pittsburgh Sleep Quality Index (PSQI) and Short Form Health Survey-36 (SF-36) administered prior to their procedure. CSF samples were obtained by atraumatic lumbar puncture with a 25 gauge Whitacre needle. Patients underwent unilateral RF at three lumbar levels as determined by previous diagnostic facet joint injections. All procedures were performed without sedation in the prone position under fluoroscopic guidance. 1% lignocaine was applied to the subcutaneous tissue prior



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## 3.2 Measurement tools and procedures

to RF needle insertion. Once needle position was verified by fluoroscopy stimulation was carried out. A positive test for sensory stimulation was achieved when the patient declared stimulation of the painful area at  $<0.3V$ . Motor testing was performed to  $1.0V$ . Radiofrequency was delivered to  $80^{\circ}C$  for 120 seconds. 0.5mls of 1% lignocaine was administered to the medial branch nerve immediately prior to RF treatment.

### 3.1.4 Patient recruitment: SCS group

The SCS group were patients who had a spinal cord stimulator implanted for FBSS and were asked to volunteer following informed consent. Nine patients consented to CSF sampling. Patients were instructed to turn off their spinal cord stimulators at midnight and attend between 8 and 10AM the following morning for CSF and serum sampling. They had a BPI, PSQI and SF-36 administered prior to any interventions. Their stimulators were then switched on at the settings that they had been using for adequate paraesthesia and pain relief. CSF and serum samples were taken at baseline and then the stimulators were turned on. Under radiological guidance to avoid damage to the stimulator cables, CSF samples were taken again after five minutes of stimulation. Eleven patients consented to serum sampling at baseline and after four hours of stimulation.

## 3.2 Measurement tools and procedures

### 3.2.1 Brief Pain Inventory

The Brief Pain Inventory (BPI) has become one of the most widely used tools for assessing clinical pain. Originally developed to assess pain related to cancer, it has now been shown to be an appropriate tool for measuring pain in a variety of clinical conditions. It is a short, self-administered questionnaire designed to assess the intensity of pain and the degree to which pain interferes with daily activities and quality of life [136], giving it both a sensory and a reactive component.

Chronic pain can be variable, so the BPI asks the patient to rate their pain over the previous 24 hours, at the time of completing the questionnaire, as well as rating it at its worst, least, and average, on a numerical rating scale from zero

### 3. METHODOLOGY

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to ten. The worst pain rating has the greatest test-retest reliability and the other ratings providing information on variability[48]. The BPI also assesses the degree to which pain interferes with patient function and employs a numeric rating scale from zero (“no interference”) to ten (“interferes completely”) to assess the impact of pain on several dimensions of daily living:

- Physical activity
- Work
- Mood
- Ability to walk
- Sleep
- Relationships with other people

The BPI has a wide range of applications, from epidemiological studies to routine clinical assessment of pain and in examining the effectiveness of pain treatments and interventions. It has been validated in a wide range of clinical and cultural contexts.

#### 3.2.2 Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a retrospective 19-item self-report questionnaire that assesses sleep quality and quantity over a one-month period. These items yield seven component scores across different domains associated with sleep quality;

- Subjective sleep quality
- Sleep Latency
- Sleep duration
- Habitual sleep efficiency
- Sleep disturbances

- Use of sleeping medication
- Daytime dysfunction

All component scores range from 0 to 3 and sum to a Global Score ranging from 0 to 21. A higher score indicates poorer quality sleep and an empirically derived Global Score of  $>5$  distinguishes poor sleepers from good sleepers. The mean Global Score in a heterogenous outpatient chronic pain population has been reported as  $11.57 \pm 4.36$  (mean  $\pm$  SD,  $n=51$ ) [263].

### 3.2.3 Short form-36

The short form-36 (SF-36) is a multi-purpose, short-form health survey with 36 questions. It was developed as a generic measure as opposed to targeting a specific age, disease or treatment group. The experience with the SF-36 has been documented in almost 4,000 publications and has been judged to be the most widely evaluated generic patient-assessed health outcome measure [86]. It is useful in estimating the burden of disease relative to general population norms for eight scales as illustrated in Figure 3.1.

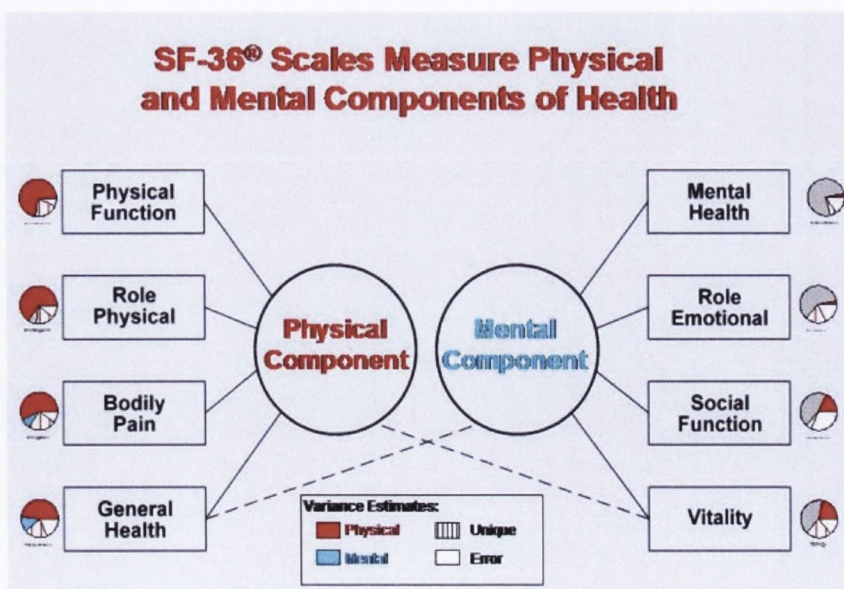


Figure 3.1: SF-36 scales measure physical and mental components of health

### 3. METHODOLOGY

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The SF-36 consists of eight scales which are each the weighted sums of 2-10 questions in their section. There also two summary measures, mental and physical, that in turn aggregate the scales, reducing the number of statistical comparisons when assessing different diseases, patient groups or within-group response to treatment. This represents a combination of brevity and comprehensiveness and the eight scales and two summary measures rarely fail to detect significant differences in physical or mental health status in group level comparisons [305].

#### 3.2.4 Lumbar puncture and CSF collection

Potential risks of lumbar puncture include haematoma, infective sequelae, post-dural puncture headache (PDPH) and neuronal injury. Our unit has extensive experience in neuraxial blockade and the insertion of intrathecal catheters in the treatment of chronic pain conditions, both for diagnostic purposes and the administration of drugs to the CSF. The incidence of PDPH after a single shot spinal is dependent on needle size and design. It is the practice in our institution to use a 25G Whitacre needle, which is associated with an incidence of PDPH of 0-14.5% [288] The co-investigators in this study have a minimum of seven years experience in Anaesthesia and Pain Medicine. We have analysed a series of 132 patients in our institution that underwent placement of a 22G intrathecal catheter over a 27G stylet yielding a PDPH rate of 1.5%. As these patients are undergoing interventional treatments, they are automatically screened for any risk factors for developing haemorrhagic and infective sequelae prior to attendance and all of these procedures are performed in theatre under sterile conditions. The safety and acceptability of the research lumbar puncture has recently been reiterated in a series of three hundred and forty two patients with a low incidence of adverse events (PDPH 0.093%) and was well tolerated [221].

All lumbar punctures were performed with a 25G Whitacre needle under strict asepsis at the L<sub>3</sub>L<sub>4</sub> or L<sub>4</sub>L<sub>5</sub> interspaces. For patients with an implanted spinal cord stimulator, radiological guidance was added to prevent damage to stimulator leads. The initial 0.5mL was discarded and then 3mL of CSF was collected in a sterile 5mL container on ice, centrifuged at 2000g for ten minutes, divided into 0.5mL aliquots in propylene Eppendorf safe-lock tubes (Sigma-Aldrich, Dorset,

## 3.2 Measurement tools and procedures

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UK) and frozen on site for later transport in a Cryo Porter DP-80 portable -80°C freezer (Cryo Porter DP-80 (Dairei, Japan). The samples were maintained at -80°C for transport to the freezer room at the Department of Physiology, Trinity College Dublin.

### 3.2.5 Lumbar radiofrequency neurotomy

Patients underwent unilateral radiofrequency (RF) treatment of the medial branches at three lumbar levels as determined by previous diagnostic facet joint injections. All procedures were performed without sedation in the prone position under fluoroscopic guidance. 1% lignocaine was applied to the subcutaneous tissue prior to RF needle insertion. PRF was performed using 10cm electrodes through straight 20 gauge cannulae with a 2mm active tip (CSK-TC10, Cosman Medical, Mass., USA) and a Radionics RFG-3C generator (Cosman Medical, Mass., USA). Once needle position was verified by fluoroscopy, sensory stimulation was carried out at 50Hz and needle tip repositioned as necessary to optimise stimulation. A positive test for sensory stimulation was achieved when the patient declared stimulation of the painful area at <0.3V. Motor testing was performed to 1.0V. PRF was commenced up to 80°C for 120 seconds. 0.5mls of 1% lignocaine was administered to the medial branch nerve immediately prior to lesioning.

### 3.2.6 Spinal cord stimulation

Patients with a spinal cord stimulator implanted for the treatment of pain arising from FBSS were invited to participate. Patients with co-existing neurological disorders, concurrent use of corticosteroids or anticoagulants were excluded. Following informed consent, nine patients volunteered to participate. Patients were instructed to turn off their stimulator at midnight and attended for CSF and serum sampling between 8 and 10AM the following day. On arrival, the Brief Pain Inventory (BPI) and the Short Form-36 Health Survey (SF-36) were administered to each patient and a baseline serum sample was obtained. A lumbar puncture was performed aseptically with a 25 gauge Whitacre needle under radiological guidance to minimise the risk of damaging the stimulator leads. 1.5mL of CSF was collected and the stylet replaced in the needle. The stimulators

### 3. METHODOLOGY

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(Synergy<sup>®</sup> or Restore<sup>®</sup>, Medtronic, Minneapolis, USA) were switched on at the stimulation parameters that the patient had been using to achieve satisfactory pain relief. After five minutes of stimulation, a second sample of CSF was collected. The stimulator remained on and a second serum sample was obtained after four hours of stimulation. Stimulation parameters ranged from pulse widths of 210-360  $\mu$  s (mean  $282 \pm 46 \mu$  s), frequencies of 40 – 100 Hz (mean  $73 \pm 20$  Hz), and amplitudes of 3 – 7.4V (mean  $4 \pm 1.4$  V).

#### 3.2.7 Sample analysis

Levels of cytokines, chemokines or other biomarkers in serum, plasma, cell supernatant samples were measured using SearchLight<sup>®</sup> protein arrays (Aushon BioSystems, Billerica, MA). Briefly, samples were incubated for one hour on the array plates that were pre-spotted with capture antibodies specific for each protein biomarker. Plates were decanted and washed three times before adding a cocktail of biotinylated detection antibodies to each well. After incubating with detection antibodies for 30 minutes, plates were washed three times and incubated for 30 minutes with streptavidin-horseradish peroxidase. All incubations were done at room temperature with shaking at 200 rpm. Plates were again washed before adding a chemiluminescent substrate. The plates were immediately imaged using the SearchLight<sup>®</sup> imaging system, and data was analyzed using SearchLight Array Analyst software.

Reported limits of detection for each of the analytes under investigation are summarised in Table 3.1

#### 3.2.8 Human Cytokine Serum and Plasma Assay Protocol

1. Addition of Diluent 2: 50  $\mu$ L of Diluent 2 were dispensed into each well. The plates were sealed with an adhesive plate seal and incubated for 30 minutes with vigorous shaking (300-1000 rpm) at room temperature.
2. Addition of Sample or Calibrator: 50  $\mu$ L of Calibrator or Sample Solution was added into separate wells of the plate. The plates were sealed and incubated for 30 minutes with vigorous shaking (300-1000 rpm) at room temperature.

### 3.2 Measurement tools and procedures

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**Table 3.1:** Assay ranges

Human Analyte	Curve Range (pg/mL)
BDNF	3.1 - 3,200
GDNF	1.5 - 1,500
IL-10	0.4 - 400
<i>IL - 1<math>\beta</math></i>	0.2 - 200
IL-2	0.4 - 400
IL-4	0.8 - 800
IL-6	0.2 - 200
IL-8	0.4 - 400
MCP-1	0.8 - 800
MMP-2	31.1 - 32,000
MMP-9	48.8 - 50,000
<i>TNF<math>\alpha</math></i>	2.3 - 2,400
TNFR1	2.3 - 2,400
VEGF	4.9 - 5,000

3. Wash and Addition of Detection Antibody Solution: The plates were washed three times with PBS + 0.05% Tween-20. 50  $\mu$ L of the 1X Detection Antibody Solution was added into each well of the plate. The plates were sealed and incubated for 30 minutes with vigorous shaking (300-1000 rpm) at room temperature.
4. Wash and Read: The plates were washed three times with PBS + 0.05% Tween-20. 150  $\mu$ L of 2X Read Buffer T was added to each well of plate. The plates were read on the the Searchlight<sup>®</sup> imaging system.

The CSF samples were run both undiluted and diluted 1:10. For the majority of analytes the data was reported from the undiluted samples. However, all samples for MCP-1 were reported at 1:10 because the undiluted samples were too high on the standard curve. One sample for MMP-2 (#2) was reported at 1:10 because the undiluted sample was too high on the standard curve. The limit of detection for each assay is defined by the low standard (for example the low standard for IL-2 is 0.4 pg/ml).

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#### 3.2.9 Statistical analysis

All data was coded and entered into GraphPad Prism 5.0 (GraphPad Software, California, USA). All univariate datasets were tested for normality using the D'Agostino-Pearson normality test. When testing paired data or assessing correlations between datasets, the appropriate nonparametric test was used unless both datasets followed a normal distribution. Data are reported as the mean  $\pm$  standard deviation unless otherwise indicated and all datasets were tested for a normal distribution using the D'Agostino-Pearson test. Correlations between clinical variables and CSF protein levels were performed by Spearman's ranked correlation co-efficient. Differences between FJA and FBSS groups were analysed using Wilcoxon ranked sum test with a Holm-Bonferroni correction for multiple comparisons. Changes in serum protein concentrations within the RF and SCS groups before and after treatment were analysed with a paired Student's t-test or Wilcoxon ranked sum test. Changes in serum protein concentrations two hours after PRF treatment were analysed with either a paired Student's t-test or Wilcoxon matched pair test as appropriate. A  $p$  value of  $<0.05$  was considered to be statistically significant for correlations.



# 4

## CSF mediators in chronic low back pain

### 4.1 Abstract

Chronic low back pain is on the increase. The ideal management is multidisciplinary and follows biopsychosocial principles but treatment outcomes may be disappointing. Spinal microglial activation is associated with chronic pain. We examined the relationship between neuroimmune mediators in cerebrospinal fluid in patients with arthritic and neuropathic low back pain. This may be used in biomarker development to refine existing treatment modalities. We enrolled patients with painful lumbar facet joints (n=17) and Failed Back Surgery Syndrome (n=8). We assayed CSF levels of BDNF, GDNF, IL-1, IL-4, IL-6, IL-8, IL-10, MCP-1, MMP-2, TNF and VEGF. Pain intensity and reported pain relief were assessed by the Brief Pain Inventory. Sleep quality was assessed using the Pittsburgh Sleep Quality Inventory and healthy-related quality of life by the SF-36 short form. CSF IL-8 inversely correlated with pain ( $p < 0.001$ ) and positively correlated with pain relief ( $p < 0.001$ ). CSF levels of GDNF, IL-6, IL-8, MCP-1 & MMP-2 were significantly higher in patients with FBSS than patients with facet joint arthropathy ( $p < 0.01$ ). FBSS appears to be associated with a greater spinal neuroimmune response. There is a close relationship between different neu-

## 4. CSF MEDIATORS IN CHRONIC LOW BACK PAIN

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roimmune mediators which suggests they cannot be studied in isolation. There is a complex non-linear relationship between spinal neuroimmune activation, pain and reported pain relief.

### 4.2 Introduction

Chronic disabling back pain is on the increase across all demographic groups [80], making its effective prevention and treatment a public health concern. It is generally accepted that the biopsychosocial model represents the ideal approach to managing chronic low back pain. The biopsychosocial model is also a means of understanding how pain and disease may be modulated at multiple levels of organisation, from molecular to societal. The reported treatment benefits for various modalities, including interdisciplinary rehabilitation, is moderate at best [46]. One explanation for this is that pain in any group of patients will contain a heterogeneous mix of nociceptive, inflammatory and neuropathic pain mechanisms, which may respond to varying degrees to available pharmacological, physical, interventional and psychological strategies.

Spinal microglial activation has been implicated in the onset and maintenance of several persistent pain states, from inflammatory [278] to neuropathic [51] pain models. Release of central neuroimmune mediators may have a role in cytokine-induced sickness behaviour [137], which may also account for the disturbances of mood and sleep observed in patients with chronic low back pain. The resilience of an individual in the face of physical, psychological and social stress is mediated by genetic and environmental influences [77]. This resilience may in turn determine response to treatment and be influenced by the neuroimmune phenotypes of inflammatory and neuropathic pain to varying degrees.

Facet joint arthropathy (FJA) typically arises from cumulative low-grade degenerative changes. The prevalence of painful facets has been variously reported as between 15%-52% and does increase with age and in the presence of arthritis [293]. Inflammation can cause radicular pain if the segmental nerve becomes irritated by the release of inflammatory cytokines from the joint capsule [118]. Other changes in response to facet joint inflammation that include an increase within the dorsal root ganglion (DRG) in the numbers of brain-derived neurotrophic

factor (BDNF)-immunoreactive neurons and a phenotype shift to larger neurons [211].

Failed Back Surgery Syndrome (FBSS) refers to persistent or recurrent low back or leg pain and affects approximately 30% of patients who have undergone technically adequate lumbar spinal surgery [32]. It may arise as a consequence of post-operative changes in spinal anatomy or environment or if the surgery did not accomplish its intended goal. The commonality between patients with FBSS is a neuropathic pain syndrome that can result in greater levels of pain, lower quality of life and a higher rate of unemployment (78%) compared to other chronic pain illness models [284]. There is conflicting evidence on the significance of post-operative processes, such as an inflammatory reaction and epidural fibrosis [7][23], or a biomechanical change such as new-onset facet joint instability [214].

We hypothesized a difference in neuroimmune expression in two different chronic low back pain syndromes, a low-grade inflammatory process in FJA versus the postsurgical neuropathic aetiology of FBSS.

## 4.3 Materials and methods

### 4.3.1 Patients and procedures

Following ethical approval and informed consent, we recruited seventeen patients with FJA that had been diagnosed with controlled local anaesthetic blocks and that were scheduled for radiofrequency (RF) lumbar medial branch neurotomy. Patients with a history of co-existing neurological disorder or mood disturbance, recent trauma or surgery, or recent or current chemotherapy or corticosteroids were excluded. Forty-five consecutive patients were approached and seventeen consented to participate. Immediately prior to radiofrequency neurotomy, cerebrospinal fluid (CSF) samples were obtained by atraumatic lumbar puncture with a 25 gauge Whitacre needle. Patients underwent unilateral radiofrequency neurotomy at three lumbar levels as determined by previous diagnostic facet joint injections. The FBSS subjects were patients in whom leg pain was the predominant complaint versus back pain after surgery who had achieved a 50% or more analgesic response to SCS. Following informed consent, eight patients volunteered

## 4. CSF MEDIATORS IN CHRONIC LOW BACK PAIN

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to participate. Patients were instructed to turn off their stimulator at midnight and to attend for CSF sampling between 8 and 10 AM the following day. A lumbar puncture was performed aseptically with a 25-gauge Whitacre needle under radiological guidance to minimize the risk of damaging the stimulator leads.

### 4.3.2 Evaluation of pain severity, physical function and sleep quality

The Brief Pain Inventory (BPI), Short Form (36) Health Survey (SF-36) and Pittsburgh Sleep Quality Index (PSQI) were administered to each patient to assess pain severity, overall health quality and sleep quality respectively.

### 4.3.3 Measurement of CSF protein levels

CSF samples were centrifuged at 2000g for 10 min and frozen at  $-80^{\circ}\text{C}$  until analysed. CSF protein levels of  $\text{TNF}\alpha$ ,  $\text{IL-1}\beta$ , IL-4, IL-6, IL-8, IL-10, MCP-1, MMP-2, BDNF and VEGF, were quantified by multiplex chemiluminescent immunoassay kits (Aushon Biosystems, USA). The detection limits, as reported by the manufacturer, were 2.3 pg/ml for  $\text{TNF}\alpha$  0.2 pg/ml for IL- $\beta$ , IL-6 and IL-10, 0.8 pg/ml for IL-4 and MCP-1, 0.4 pg/ml for IL-8, 3.1 pg/ml for BDNF, 4.9 pg/ml for VEGF and 31.3 pg/ml for MMP-2. Samples were assayed in duplicate.

### 4.3.4 Data Analysis

Data are expressed as the mean  $\pm$  SD, unless stated otherwise. Correlations between age, baseline pain scores and CSF protein levels were performed by Spearman's ranked correlation co-efficient of the pooled data ( $n=25$ ). After applying a Bonferroni correction for multiple comparisons, a  $p$  value of less than 0.001 was considered statistically significant. The difference in median CSF concentrations of proteins between FJS ( $n=17$ ) and FBSS ( $n=8$ ) patients was tested with a Mann Whitney U Test. We used the Holm-Bonferroni method [112] to correct for multiple comparisons which set a level of significance of less than 0.007.

## 4.4 Results

### 4.4.1 Subject demographics

We recruited seventeen patients with FJA and eight patients with FBSS. Baseline demographics such as age, pain scores, sleep quality and quality of life did not differ significantly between the two groups (Table 4.1).

**Table 4.1:** Clinical variables of FJA versus FBSS patients

Clinical variable	FJA (n=17)	FBSS (n=8)	<i>p</i> value
BPI Q3 (worst pain)	8 (4-10)	7 (1-9)	0.053
BPI Q4 (least pain)	4 (1-9)	3 (0-6)	0.16
BPI Q6 (pain right now)	5.5 (1-10)	6 (0-8)	0.40
Age (years)	45 (31-78)	47 (26-68)	0.79
PSQI Global	13 (2-20)	15 (10-19)	0.26
SF-36 PCS	44.5(11-71)	37 (12-51)	0.11
SF-36 MCS	57.5 (19-89)	42 (3-79)	0.42

FJA, Facet Joint Arthropathy; FBSS, Failed Back Surgery Syndrome; BPI, Brief Pain Inventory; PSQI, Pittsburgh Sleep Quality Inventory; SF-36, Short Form (36) Health Survey; PCS, Physical Component Score; MCS, Mental Component Score. *p* value of Mann Whitney U Test. Data are median and range.

### 4.4.2 Correlations of clinical variables and CSF proteins

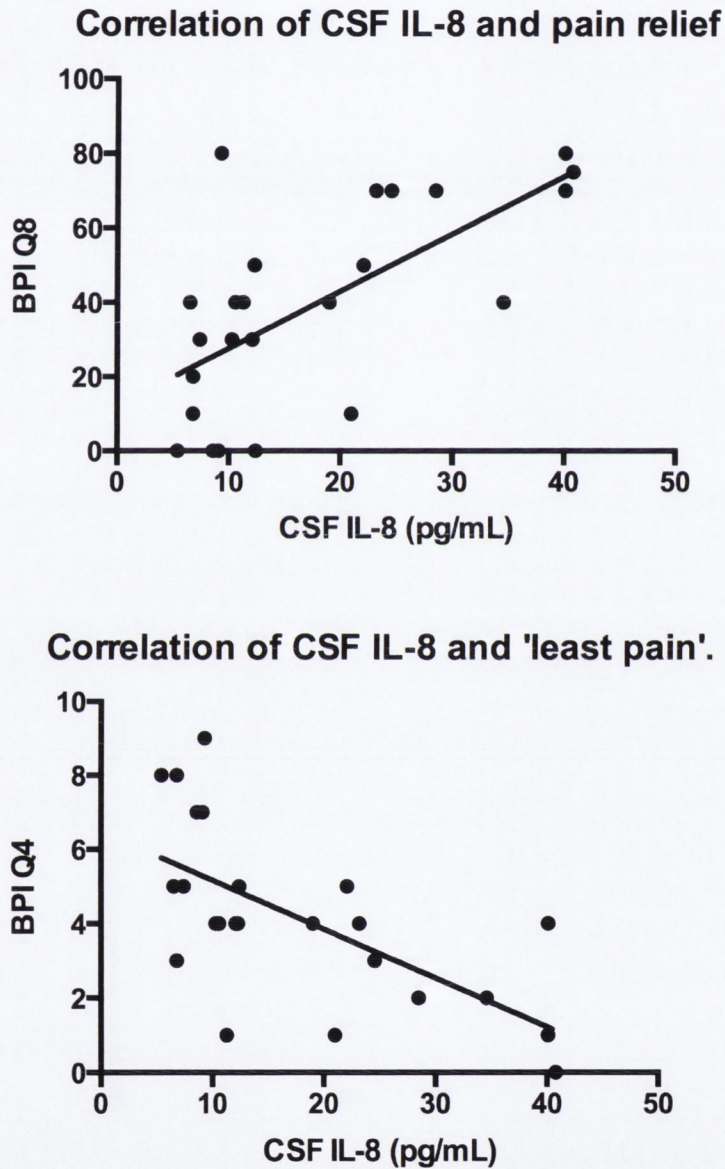
Following a Bonferroni correction for multiple comparisons, CSF concentrations of IL-8 retained an inverse relationship with several pain indices, most notably least pain ( $p = 0.0007$ ) and a positive relationship with reported pain relief ( $p = 0.0005$ ).

### 4.4.3 Correlations between CSF proteins

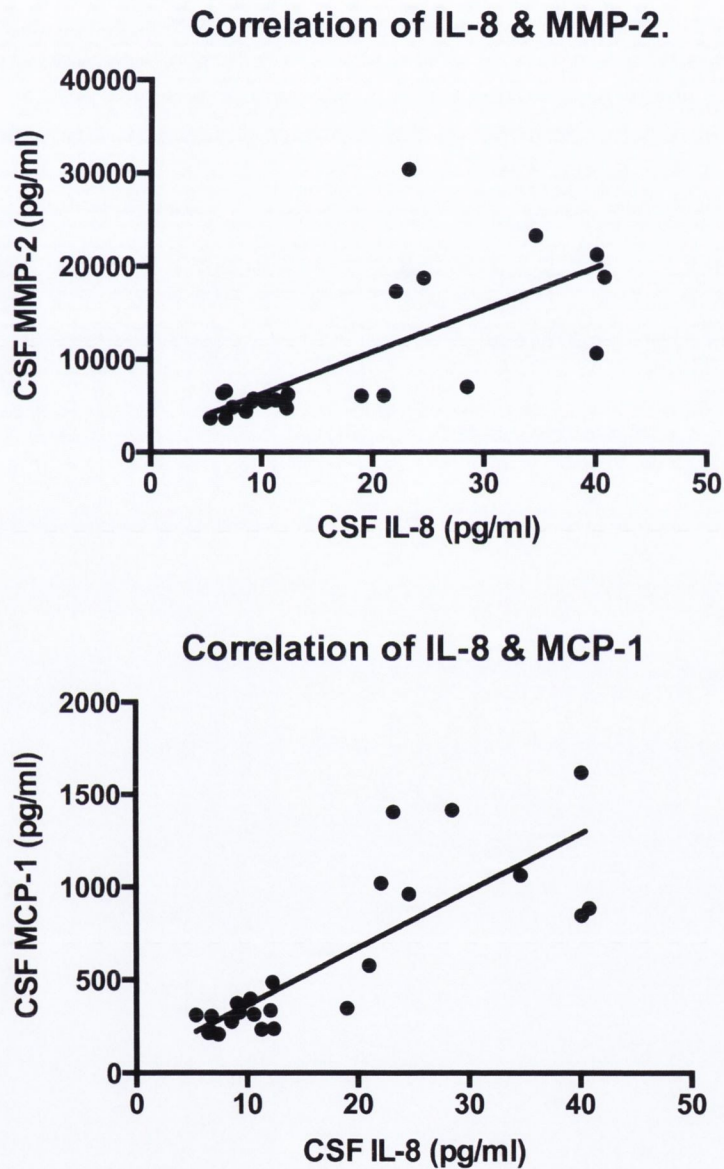
With the exception of IL-6 and VEGF, each of the proteins under study exhibited significant positive correlations with each other. MMP-2 (Figure 4.2) had the greatest number of very significant correlations ( $p < 0.001$ ) with MCP-1, IL-8, BDNF and GDNF in descending order of number of significant associations (Table 4.2).

#### 4. CSF MEDIATORS IN CHRONIC LOW BACK PAIN

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**Figure 4.1:** Correlation between cerebrospinal fluid levels of interleukin-8 (IL-8) and reported pain relief in Q8 (Pearson  $r = -0.658, p = 0.0005$ ) and reported pain in Q4 (Spearman  $r = -0.653, p = 0.0007$ ) of the Brief Pain Inventory (BPI).



**Figure 4.2:** Correlation of cerebrospinal fluid levels of interleukin-8 (IL-8) with levels of matrix metalloproteinase-2 (MMP-2), Pearson  $r = -0.707, p < 0.001$ , and with levels of monocyte chemotactic protein (MCP-1), Pearson  $r = 0.810, p < 0.001$ .

#### 4. CSF MEDIATORS IN CHRONIC LOW BACK PAIN

**Table 4.2:** Correlations between CSF protein levels (n=25)

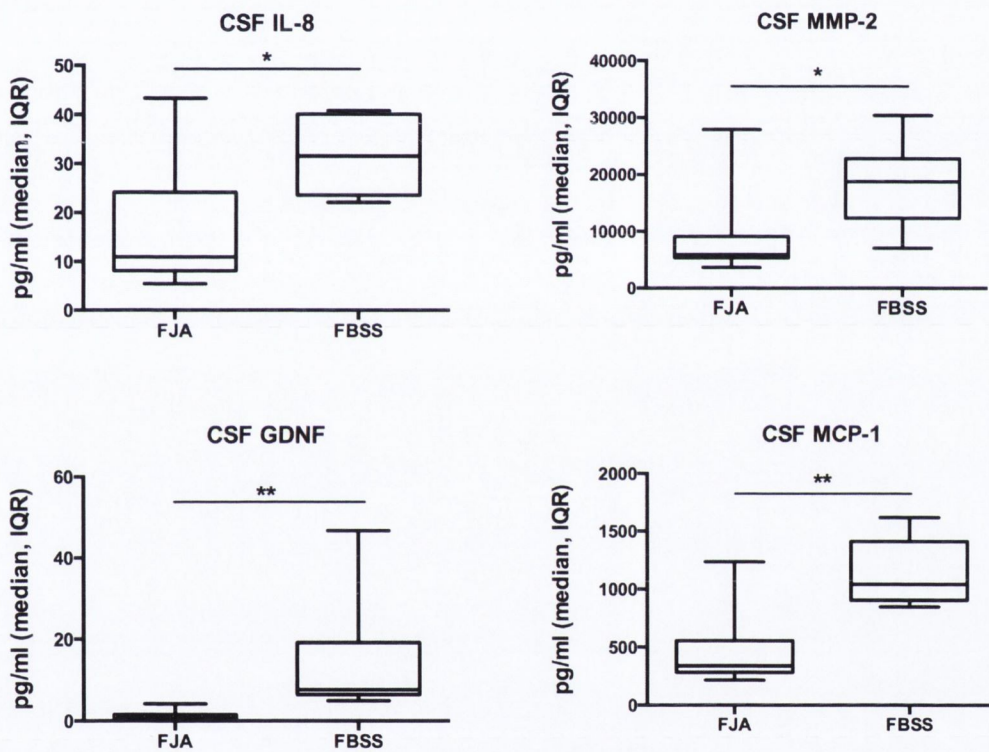
CSF Variable	IL-6	IL-8	MCP-1	MMP-2	GDNF	BDNF	VEGF
IL-6		0.596	0.575	0.422	0.168	0.327	-0.193
IL-8	0.596		0.820*	0.707*	0.531	0.590	0.019
MCP-1	0.575	0.820*		0.810*	0.496	0.655*	-0.082
MMP-2	0.422	0.707*	0.810*		0.643*	0.832*	0.207
GDNF	0.168	0.531	0.496	0.643*		0.459	0.325
BDNF	0.327	0.590	0.655*	0.832*	0.459		0.405
VEGF	-0.19	0.019	-0.082	0.207	0.325	0.405	

IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemotactic protein-1; BDNF, brain-derived neurotrophic factor; GDNF, glial cell-derived neurotrophic factor; MMP-2, matrix metalloproteinase-2; VEGF, vascular endothelial growth factor. Pearson product-moment correlation co-efficient with Bonferroni correction for multiple comparisons, \* $p < 0.001$ .

#### 4.4.4 Differences in median protein levels between FJA & FBSS

Median levels of IL-6 ( $p = 0.0099$ ), IL-8 ( $p = 0.003$ ), GDNF ( $p < 0.0001$ ), MCP-1 ( $p = 0.0001$ ) and MMP-2 ( $p = 0.0014$ ) were higher in patients with FBSS than patients with FJA. After applying the Holm-Bonferroni correction for multiple comparisons, the differences in IL-6, IL-8, GDNF, MCP-1 and MMP-2 remained significant (Figure 4).





**Figure 4.3:** Differences in cerebrospinal fluid levels of GDNF, IL-8, MCP-1 and MMP-2 in patients with facet joint arthropathy (FJA), n=17, compared to patients with Failed Back Surgery Syndrome (FBSS), n=8. Mann Whitney U Test,  $*p < 0.05$ ,  $**p < 0.01$

### 4.5 Discussion

To the best of our knowledge this is the first study to report the relationships between CSF neuroimmune mediators, pain and quality of life in two groups of chronic low back pain patients. The subjects were not matched for age, gender, SF-36 and sleep quality score although there were no overall differences in median values between the two groups. The FBSS group were patients whose pain had responded positively to spinal cord stimulation and had turned off their stimulator for at least eight hours. We cannot definitively exclude a longer-term effect of spinal cord stimulation or say if these differences would also be observed in axial or non-radicular pain, which do not typically respond to spinal cord stimulation [292]. Previously published work has identified elevations in CSF levels of IL-6 and MCP-1 in patients with Complex Regional Pain Syndrome (CRPS) relative to controls [6]. Both CRPS and FBSS respond well to spinal cord stimulation and it is possible that the analgesic effect involves a modulation of glial activity in these two aetiologically distinct pain syndromes. The pro-inflammatory cytokines tumour necrosis factor- $\alpha$ (TNF- $\alpha$ ) and interleukin-1 $\beta$ (IL-1 $\beta$ ) and the anti-inflammatory cytokines interleukin-4 (IL-4) and interleukin-10 (IL-10) were undetectable in the CSF of our patients. Whether this represents a limitation of the assay or is an indicator these cytokines do not play an ongoing role in chronic back pain is unclear. Reduced expression of the anti-inflammatory cytokines IL-4 and IL-10 has been demonstrated in CRPS [6][289], therefore it is possible that failure or exhaustion of the anti-inflammatory response may also play a role in chronic low back pain.

Our study does indicate a difference in concentrations of mediators involved in neuroglial signalling in patients with FBSS compared to those with painful lumbar facet joints. Low back pain, regardless of aetiology, is a puzzling entity [66], where organic pathology is poorly predictive of pain intensity. Dichotomous thinking about neuropathic versus inflammatory mechanisms may be counterproductive as it is likely that both co-exist to varying degrees. Radicular pain, which is usually considered to be caused by mechanical compression and neuropathic in nature, may be produced by periganglionic inflammation via the release of pro-inflammatory cytokines and prostaglandins [8]. Some inflammatory and immune

processes are beneficial to nerve regeneration after injury [167]. However, this attempt at neuroprotection may have the unintended consequence of potentiating and amplifying pain [52] and producing some of the observed disturbances of mood and sleep in chronic low back pain [69]. This is relevant to the effective and appropriate treatment of back pain. There are various pharmacological, interventional, physical and psychological therapies that may be effective in managing low back pain. The challenge lies in aligning the specific therapy to the individual who will benefit most.

The correlations between IL-8 and BDNF, GDNF, MCP-1 and MMP-2 may be suggestive of co-expression in response to a common trigger. In acute inflammation, gene expression of IL-8, IL-6 and MCP-1 is upregulated, correlates with pain intensity, and is not reduced by ketorolac [304]. Higher CSF levels of GDNF have been reported in patients with osteoarthritis [168] (Lundborg, 2010). While GDNF is felt to attenuate neuropathic pain [24], it induces muscle pain through the activation of cyclooxygenase-2 [198], therefore having several mechanistic roles in the chronic low back pain of multiple aetiologies. Amitriptyline, a tricyclic antidepressant frequently prescribed for chronic pain with a neuropathic component, increases the MMP-dependent production of GDNF [109].

#### 4. CSF MEDIATORS IN CHRONIC LOW BACK PAIN

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# 5

## FJA: serum mediators after lumbar RF

### 5.1 Abstract

Our aim was to characterise the relationship between serum levels of neuroimmune markers and pain, sleep quality and level of function in patients undergoing radiofrequency (RF) denervation for the treatment of painful lumbar facet arthropathy and evaluate any change in response to the RF neurotomy. We conducted a prospective, observational study of 20 patients with painful lumbar facet arthropathy undergoing RF neurotomy. Pain severity, sleep quality and quality of life were assessed by administering the Brief Pain Inventory (BPI), Pittsburgh Sleep Quality Index (PSQI) and SF-36 Short Form health Survey. Serum samples were taken prior to procedure and again at two hours post-procedure. Samples were analysed for IL-6, IL-8, GDNF, BDNF, MCP-1, MMP-2 and VEGF. A Numeric Rating Scale (NRS) was also administered at 2 hours to assess change in pain. RF neurotomy resulted in an acute reduction in pain ( $p = 0.007$ ), BDNF ( $p = 0.0076$ ) and MCP-1 ( $p = 0.0001$ ) at two hours post-procedure. Pain correlated with poor sleep ( $p = 0.001$ ) and poorer quality of life ( $p = 0.009$ ). IL-8 and IL-6 correlated with baseline pain ( $p < 0.05$ ) and age ( $p < 0.01$ ). Post-procedure pain was inversely correlated with serum IL-8 ( $p = 0.047$ ) and VEGF ( $p = 0.017$ ).

## 5. FJA: SERUM MEDIATORS AFTER LUMBAR RF

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Our findings suggests a role for serum neuroimmune mediators in the pain arising from lumbar facet joints, the physiological response to a neuroablative procedure and the resulting analgesia. This may open up new lines of investigation to aid refinement of radiofrequency technology and in biomarker development for patient selection.

### 5.2 Introduction

Persistent low back pain remains a pressing healthcare problem because of its prevalence [255], chronicity, and association with disturbances of mood and sleep [210]. In approximately 15 to 40% of patients, this pain has been identified as originating in the facet joints and facet interventions are among the most commonly performed interventional procedures in pain management centres. Despite the frequency of diagnostic and therapeutic interventions for facetogenic pain, there is significant geographic variation in what is considered to be best practice in terms of the sequence and timing of interventions and it remains the subject of ongoing debate [49][293]. Current evidence supports controlled facet joint nerve blocks with local anaesthetic as the diagnostic intervention and in therapeutic studies, either lumbar facet joint medial branch blocks (MBB) or lumbar radiofrequency neurotomy (RFN) [60]. The response to MBB is not necessarily predictive of pain relief from RF denervation [50]. Therefore, there may be a distinct biological effect from the thermal lesioning of a nerve in the context of the chronic inflammation of osteoarthritis of the facet joints that does not occur in response to a nerve block with local anaesthetic.

Facet joint procedures have the potential to result in meaningful and sustained improvements in function [173]. Facet joint syndrome most frequently arises from repetitive strain and capsular stretch due to mechanical loading of the joint. There is evidence that capsular stretch results in hyperalgesia of greater magnitude and longer duration than occurs after complete joint disruption [156]. Upstream of the chronic inflammation within the joint, there is upregulation of cytokine messenger RNA within the dorsal root ganglion [156] and neuronal hyperexcitability can occur immediately within the dorsal horn after capsular stretch

[228]. This hyperexcitability may act as a substrate for a neuropathic component to the pain [100] in addition to the inflammatory pain of an intra-articular osteoarthritic process and can occur as early as six hours after mechanical facet joint injury [55]. Neuronal excitability and inflammation may be propagated by the release of pro-inflammatory cytokines and prostaglandins [264]. The role of glial cells and neuroimmune activation in the pain of chronic facet arthropathy has yet to be characterised. Central neuroimmune activation may account for some of the psychological and behavioural sequelae of chronic low back pain such the disturbances of mood and sleep [97]. Recently, circulating interleukin-6 (IL-6) has been found to correlate with pain and sleep disturbance in patients with chronic low back pain [105]. Patients with painful osteoarthritis undergoing joint replacement have higher cerebrospinal fluid levels of glial cell-derived neurotrophic factor (GDNF and interleukin-8 (IL-8) than pain-free controls [168]. Whether this is also true of osteoarthritis of lumbar facet joints is unclear. It may be that the prolonged analgesic effect of facet joint procedures may be related to a modulation of an immune-mediated process, not simply an ablation of sensory afferents.

The biological effect by which a high-frequency, low-energy radiofrequency current can produce sustained analgesia for up to two years is still not completely understood. Conventional RF induces distance-dependent tissue destruction [33]. However, while sensory disruption may be transient, the pain relief may be of much longer duration [229]. A more recent refinement of RF, pulsed radiofrequency (PRF) is based on the principle that the desired clinical effect is not due to heat lesion. Results for PRF treatment appear to be equivalent to conventional RF for cervical but not lumbar facet interventions [290]. The current hypothesis is that by the application of RF to the first order neuron, there is increased transcription of c-Fos and other early genes within the dorsal horn [262] and this mediates the duration of effect. Other evidence of a biological effect comes from work with pulsed RF. In addition to effects on c-fos at the level of the dorsal horn, there is evidence of enhancement of noradrenergic and serotonergic descending pain inhibitory pathways [101] and of a transient modulation of hippocampal excitatory transmission [34]. Therefore radiofrequency may exert

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a biological effect remote from the site of application and by means other than simple disruption of afferent input.

Despite the evidence for neuroimmune activation and the role of mediators released by glial cells in the maintenance of persistent pain states, there have been no studies to date looking at the effect of RF neurotomy on levels of immune mediators. We tested the hypothesis that serum levels of neuroimmune mediators are associated with reported pain, sleep quality and level of function and are altered by lumbar RF neurotomy.

### **5.3 Materials and methods**

#### **5.3.1 Patients and procedures**

We received ethical approval to recruit twenty consecutive patients undergoing lumbar radiofrequency neurotomy who had a prior diagnosis of lumbar facet joint arthropathy. Painful lumbar made on a positive response to diagnostic blocks of a 50% reduction in pain. Patients with a history of co-existing neurological disorder or mood disturbance, recent trauma or surgery, or recent or current chemotherapy or corticosteroids were excluded. Forty-five consecutive patients were approached and twenty consented to participate. Serum samples were taken pre-procedure at the time of intravenous cannula insertion and two hours post-procedure. Patients underwent unilateral radiofrequency neurotomy at three lumbar levels as determined by previous diagnostic facet joint local anaesthetic blocks. All procedures were performed without sedation in the prone position under fluoroscopic guidance, as is the standard practice in our institution. Skin and subcutaneous tissue were infiltrated with 1% lignocaine prior to RF needle insertion. Once needle position was verified by fluoroscopy stimulation was carried out. A positive test for sensory stimulation was achieved when the patient declared stimulation of the painful area at  $<0.3V$ . Motor testing was performed to  $1.0V$ . Radiofrequency thermal lesioning was carried out at  $80C$  for 120 seconds. 0.5mls of 1% lignocaine was administered to the medial branch nerve immediately prior to lesioning.



### 5.3.2 Evaluation of pain severity, physical function and sleep quality

Prior to RF neurotomy, the Brief Pain Inventory (BPI), Short Form (36) Health Survey (SF-36) and Pittsburgh Sleep Quality Index (PSQI) were administered to each patient to assess pain severity, overall health quality and sleep quality respectively. A numeric rating scale (NRS) was administered to assess pain two hours post-procedure at the time of collecting the second serum sample.

### 5.3.3 Measurement of serum protein levels

Serum samples were centrifuged at 2000g for 10 min and frozen at -80°C until analysed. Serum protein levels of interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1), matrix metalloproteinase-2 (MMP-2), brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF), were quantified by multiplex chemiluminescent immunoassay kits (Aushon Biosystems, USA). The detection limits, as reported by the manufacturer, were 0.2 pg/ml for IL-6 and IL-1, 0.8 pg/ml for MCP-1, 0.4 pg/ml for IL-8, 1.5 pg/ml for GDNF, 3.1 pg/ml for BDNF, 4.9 pg/ml for VEGF and 31.3 pg/ml for MMP-2. Samples were assayed in duplicate.

### 5.3.4 Data Analysis

Data were expressed as the mean  $\pm$  SD and tested for a normal distribution. Correlations between age, gender, BPI, SF-36 and PSQI and serum protein levels were performed by Spearman's ranked correlation coefficient. A  $p$  value of  $<0.05$  was considered statistically significant. The difference in mean pain scores before and after treatment was assessed by using a Student's  $t$ -test of replies to BPI Q.6 and the NRS administered two hours post-procedure. The difference in mean serum protein levels before and after treatment was tested using a paired Student's  $t$ -test. The difference in median serum protein levels before and after treatment was tested using a paired Wilcoxon Ranked Sum test. We used the Holm-Bonferroni method [112] to correct for multiple comparisons which set a

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level of significance that ranged from a value of  $<0.007$  for the most significant result to a  $p$  value of  $<0.05$  for the least significant result.

### **5.4 Results**

#### **5.4.1 Subjects**

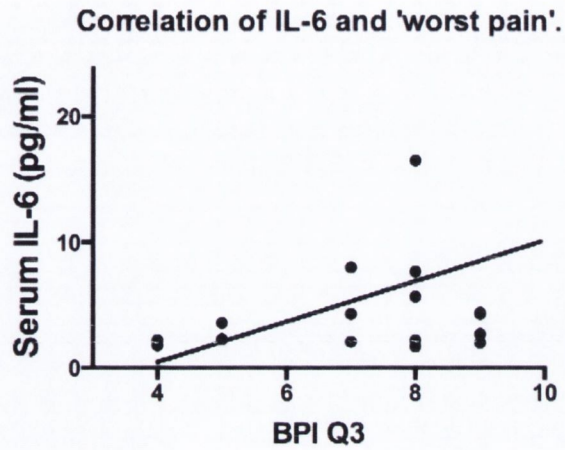
We recruited twenty consecutive patients undergoing radiofrequency lumbar medial branch rhizotomy who consented to participate. The eleven men and nine women ranged in age from 31 to 78 years (mean  $48 \pm 13$  years). The average pain score as assessed by Q.5 of the BPI, on a numerical rating scale of zero to ten, for the preceding 24 hours was  $6.4 \pm 1.5$ . The SF-36 scores ranged from 16 to 69 ( $47.3 \pm 18$ ). 18 out of 20 patients had a PSQI score of greater than 5, indicating poor sleep quality, with an overall mean of  $12.4 \pm 4.4$ .

#### **5.4.2 Relationships between clinical variables**

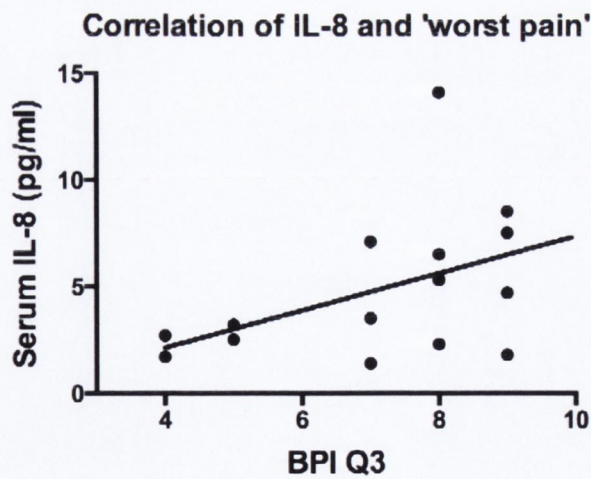
Pain right now (BPI Q.6) correlated with pain ratings for the previous 24 hours, poorer sleep quality ( $p=0.001$ ) and lower physical and mental quality of life ( $p=0.009$ ). Worst ( $p=0.04$ ) and average ( $p=0.03$ ) pain scores for the previous 24 hours (BPI Q.3 and BPI Q.5) both correlated with lower mental, but not physical, health scores on the SF-36. Poor sleep quality correlated with lower physical health scores ( $p=0.004$ ). There was no significant correlation between age and pain scores, sleep quality, and physical or mental health scores.

#### **5.4.3 Relationships between clinical variables and serum protein levels**

Serum concentrations of IL-6 ( $p=0.014$ ) and IL-8 ( $p = 0.007$ ) (Figure 5.1 & 5.2) positively correlated with worst pain in the previous 24 hours (BPI Q.3). Age also correlated positively with serum concentrations of IL-8 ( $p=0.003$ ) and IL-6 ( $p=0.002$ ). The male patients ( $n=11$ ) had a higher median serum VEGF at baseline than the female patients ( $n=9$ ) ( $p=0.03$ ).



**Figure 5.1:** Correlation between serum interleukin-6 (IL-6) and Q3 of the Brief Pain Inventory (BPI), Spearman  $r = 0.583$ ,  $p = 0.014$ ,  $n=20$ .



**Figure 5.2:** Correlation between serum interleukin-8 (IL-8) and Q3 of the Brief Pain Inventory (BPI), Spearman  $r = 0.585$ ,  $p = 0.007$ ,  $n=20$ .

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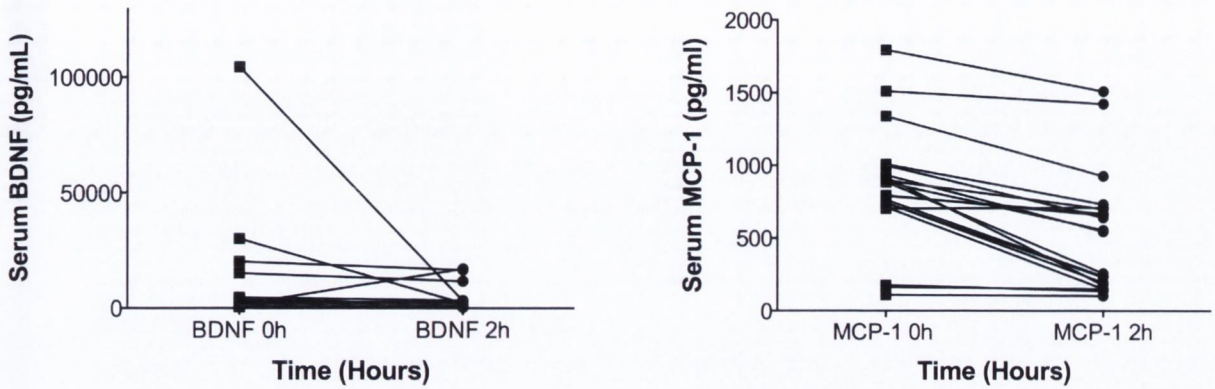
### 5.4.4 Changes in pain and serum protein levels after lumbar RF neurotomy

Two hours after RF neurotomy, mean pain scores were significantly reduced from 5.6 to 2.4 to 3.2 to 2.9 ( $p=0.007$ ) and inversely correlated with serum IL-8 ( $p=0.047$ ) and VEGF ( $p=0.0017$ ) at that time point. Two hours after lumbar RF neurotomy, there was a reduction in serum concentrations of MCP-1 ( $p=0.0001$ ), BDNF ( $p=0.0076$ ), GDNF ( $p=0.034$ ) and MMP-2 ( $p=0.011$ ), (Table 5.1). After applying the Holm-Bonferroni correction for multiple comparisons, only the changes in MCP-1 ( $p=0.0001$ ) and BDNF ( $p=0.0076$ ) remained significant (Figure 2).

**Table 5.1:** Serum protein levels before and after RF neurotomy

Serum protein levels	Pre-RF	RF + 2 hours	<i>p</i> Value
IL-6	6.5 ± 6.0	6.0 ± 5.5	0.20
IL-8	5.4 ± 3.2	5.2 ± 4.9	0.40
BDNF	10060 ± 23563	3498 ± 5190	0.0076
GDNF	2185 ± 5959	2050 ± 5621	0.035
MCP-1	844 ± 409	536 ± 409	0.0001
MMP-2	143525 ± 21652	132919 ± 20313	0.011
VEGF	392 ± 302	362 ± 350	0.05

BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; GDNF, glial cell-derived neurotrophic factor; IL, interleukin; MCP-1, monocyte chemotactic protein-1; MMP-2, matrix metalloproteinase-2; SD, standard deviation; VEGF, vascular endothelial growth factor. *p*-value of paired Students *t*-test or Wilcoxon Ranked Sum test.



**Figure 5.3:** Serum concentrations of brain-derived neurotrophic factor (BDNF) and monocyte chemotactic protein-1 (MCP-1) two hours after lumbar radiofrequency neurotomy in patients with painful facet arthropathy,  $n=20$ . Wilcoxon Ranked Sum test for BDNF ( $p=0.0076$ ), paired t-test for MCP-1 ( $p=0.0001$ ).

## 5.5 Discussion

The management of chronic pain has evolved from targeting a unidimensional reduction of pain to a consideration of changes in mood, sleep quality, and level of function. The use of interventional procedures in the management of chronic low back pain is subject to regional variation, depending on national resources and institutional practices. In our study, there was an acute reduction in pain scores two hours post-procedure. Unfortunately it is not possible to forecast long-term outcome and functional improvement from our sample size and assessment period. This timeframe was chosen for pragmatic purposes to minimally inconvenience potential participants, as it did not result in delayed discharge after an ambulatory procedure. It is generally accepted that analgesic effect of lignocaine lasts for 90 minutes and may contribute the observed response. However, an acute and sustained reduction in the amplitude of excitatory postsynaptic potentials has been demonstrated within ten minutes of the application of continuous radiofrequency energy [33].

As expected, reported pain severity correlated with poor sleep and lower quality of life scores. It is not possible to determine causality or directionality, although it is interesting to note that sleep quality correlated with scores of physical,

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but not mental, function. The therapeutic implications of this association, and its potential influence on clinical outcome, are worthy of further study. The relationship between worst pain and lower score on the mental component of the SF-36 is also in keeping with the previous report that depression predicted a shorter duration of success of lumbar RF denervation [273].

IL-8 and IL-6 both correlated with worst pain in the preceding 24 hours and with age. These molecules have been implicated in several pain models, such as acute post-surgical inflammatory pain [304], fibromyalgia [303] to radicular pain from intervertebral disc herniation [30]. The chemokine MCP-1 has been found to be co-expressed with IL-8 and IL-6 as part of an inflammatory cascade and has also been implicated as a neuronal-microglial messenger in facilitating neuronal excitability and neuropathic pain conditions [174]. Although MCP-1 did not correlate with any clinical variables in this patient sample, serum levels were significantly reduced two hours post-procedure. MCP-1 has also recently been implicated as one of five biomarkers of analgesic response to morphine in patients with cancer [106], therefore the observed reduction in levels may not be unique to the mechanism of action of radiofrequency neurotomy as an analgesic modality.

The other significant change in response to RF neurotomy was a reduction in serum BDNF concentrations. BDNF appears to act as a central pain modulator in both inflammatory and neuropathic pain states. BDNF modulates both fast excitatory and inhibitory signals, as well as slow peptidergic neurotransmission in spinal cord [181]. Facet joint inflammation has been associated with a phenotype switch to larger BDNF-expressing neurons within the DRG [212]. Serum BDNF reflects the amount of BDNF stored and transported by platelets [251], therefore the acute reduction in serum levels seen may represent a central offloading of BDNF from the periphery in response to the neurotomy.

It is possible that each of these mediates a different aspect of the multidimensional chronic pain experience, including sleep quality and level of function. One curious aspect of the relationship between these inflammatory chemotactic mediators is the opposite role exhibited with pain and analgesia. In fibromyalgia, IL-8 has been prospectively associated with pain severity [9] and after multidisciplinary treatment, inversely associated with pain intensity [303]. We found

a similar apparent contradiction, the baseline concentrations of IL-8 positively correlated with worst pain in the preceding 24 hours and the post-procedure concentrations inversely correlated with the pain score two hours post-procedure. This may represent either a down-modulation of IL-8 receptors, as is seen in other examples of IL-8-mediated inflammation [225]. IL-8 has been shown to evoke a sympathetically-mediated hyperalgesia that does not respond to inhibition of prostaglandin production [59]. It is possible that the inversion of the relationship between IL-8 and reported pain might represent an attenuation of that sympathetic hyperalgesia.

The other mediator that also inversely correlated with post-procedure pain scores was serum VEGF. Frequently co-expressed with IL-8 as an angiogenic factor in response to hypoxia and in tumorigenesis [193], VEGF has also now been implicated in neuropathic pain [163] and treatment-resistant depression [296]. The finding that median serum VEGF levels were over twice as high in men than women is interesting and worthy of further investigation, given the implication of VEGF in the action of antidepressants and electroconvulsive therapy in the context of depression [306], which might overlap with the pharmacological and interventional treatment of chronic pain, respectively.

There was also a reduction in MMP-2 and GDNF two hours after RF neurotomy, although this was no longer significant after applying the Holm-Bonferroni correction. MMP-2 is a matrix metalloproteinase, a family of zinc-dependent endopeptidases that are tightly regulated cleaving components of the extracellular matrix, cytokines and chemokines. In the context of nociception and chronic pain, MMP-2 has been implicated in the aetiology of neuropathic pain [135] and migraine [154]. More directly, matrix metalloproteinases cleave the precursor of BDNF, pro-BDNF, to its active form, a process that can switch the effect of BDNF on synapses from depressing to potentiating [323]. There was also a reduction in serum GDNF after RF denervation although this was even less significant than the change in MMP-2. GDNF is produced by chondrocytes [61] and is generally felt to be pro-algesic and may represent part of the inflammatory state arising from chronic osteoarthritis [168]. In our patient group, serum GDNF had a negative, but non-significant, correlation with all indices of pain. A larger number of patients and multiple sampling time points might better delineate the

## **5. FJA: SERUM MEDIATORS AFTER LUMBAR RF**

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timeline of the changes in MMP-2 and GDNF and their relationship with other inflammatory mediators, offering an insight into the dynamic interaction between them that tilts the balance towards persistent pain.



# 6

## FBSS: CSF mediators & response to SCS

### 6.1 Abstract

Spinal cord stimulation (SCS) is an efficacious therapy for chronic neuropathic pain whose precise mechanism of action is unclear. Mediators produced by glial and immune cells are now believed to modulate neuronal transmission and promote chronic neuropathic pain. We postulated a relationship between cerebrospinal fluid (CSF) concentrations of neuroimmune mediators and SCS. We measured CSF concentrations of the chemokine, monocyte chemoattractant protein-1 (MCP-1), and the neurotrophins, brain-derived neurotrophic factor (BDNF) and glial-cell derived neurotrophic factor (GDNF), and the growth factor, vascular endothelial growth factor (VEGF), and tested for relationships with stimulation parameters and clinical response in nine patients with failed back surgery syndrome (FBSS). Patients with FBSS had higher CSF concentrations of BDNF ( $p = 0.01$ ), GDNF ( $p = 0.01$ ) and MCP-1 ( $p = 0.0001$ ) than matched controls. CSF concentrations of BDNF and VEGF correlated with reported pain ( $p = 0.04$ ). Five minutes of SCS resulted in a reduction in median VEGF concentrations ( $p = 0.01$ ). Patients with FBSS have altered CSF levels of BDNF, GDNF and MCP-1. CSF VEGF correlates with pain and is reduced by SCS. CSF

## 6. FBSS: CSF MEDIATORS & RESPONSE TO SCS

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GDNF correlates with SCS frequency. This may offer novel insights into both the mechanism of action of SCS in FBSS and the variation in clinical response that may be encountered.

### 6.2 Introduction

Spinal cord stimulation (SCS) has been in clinical use in the treatment of chronic neuropathic pain for over 40 years. It has become the mainstay of treatment for patients with failed back surgery syndrome (FBSS) and is associated with sustained pain relief and significant improvements in functional capacity and health-related quality of life when compared with conventional medical management [149]. Alterations in extracellular  $\gamma$ -amino butyric acid (GABA) have been associated with SCS and the administration of the GABA<sub>B</sub> receptor agonist baclofen has been shown to convert non-responders into responders [57]. GABA<sub>A</sub> receptor-mediated postsynaptic inhibition may be impaired or even inverted due to alterations of K<sup>+</sup> Cl<sup>-</sup> cotransporter 2 (KCC2), leading to altered Cl<sup>-</sup> conductance and altered anion gradients. The most recent study of the role of KCC<sub>2</sub> found reduced intracellular GABA during the time frame of SCS in responders [127]. Published evidence over the last two decades strongly implicates glial and immune activation in the neuroplastic changes underpinning chronic neuropathic pain [320]. Several mediators involved in signalling between microglia and neurons, including neurotrophins, growth factors, and chemokines, have been shown to alter GABAergic synapses and may explain the spectrum of clinical response to SCS. For instance, brain-derived neurotrophic factor (BDNF) released from microglia reverses the anion potential of lamina I neurons causing GABA receptor activation to become depolarizing [52]. Vascular endothelial growth factor (VEGF) has been implicated recently in the pathogenesis of neuropathic pain with higher levels of VEGF potentiating pain responses [162] and VEGF also may influence glutamatergic and GABAergic transmission through its receptor VEGFR-2 [37]. The chemokine, monocyte chemoattractant protein-1 (MCP-1), has been shown to closely mirror spinal glial activation in the spinal cord following nerve injury [327] and potently inhibits GABA-induced currents in spinal cord neurons [96]. The influence of spinal neuroimmune activation and SCS on each

other and on analgesic response is unknown. We set out to explore possible relationships between SCS parameters, analgesic response, and cerebrospinal fluid (CSF) concentrations of BDNF, MCP-1, and VEGF, each of which have been implicated in the pathophysiology of neuropathic pain. GDNF sensitises nociceptors in several pain models and shows a differential temporal expression in skin and muscle after a nociceptive skin incision [268]. In a model of intervertebral disc degeneration, there was a gradual and persistent increase in GDNF in both the dorsal root ganglion (DRG) and thalamus [133], suggesting a role in the pathophysiology of pain in FBSS.

## 6.3 Materials and methods

### 6.3.1 Patients and procedures

We received ethical approval to recruit patients with a spinal cord stimulator implanted for the treatment of pain arising from FBSS. Patients with coexisting neurologic disorders, concurrent use of corticosteroids, or anticoagulants were excluded. Following informed consent, nine patients volunteered to participate. Patients were instructed to turn off their stimulator at midnight and to attend for CSF sampling between 8 and 10 AM the following day. Prior to CSF sampling, the Brief Pain Inventory (BPI) and the Short Form (36) Health Survey (SF-36) were administered to each patient. The SCS parameters that each patient had been using to achieve satisfactory analgesia were recorded. A lumbar puncture was performed aseptically with a 25-gauge Whitacre needle under radiological guidance to minimize the risk of damaging the stimulator leads. About 1.5 mL of CSF was collected and the stylet replaced in the needle. At this point, the stimulator was turned on at each patient's individual parameters. After five minutes of stimulation at these settings, a second sample of CSF was collected. Controls were patients with chronic lumbar back pain without either FBSS or a SCS and who were matched for age, gender, and SF-36 score.

## **6. FBSS: CSF MEDIATORS & RESPONSE TO SCS**

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### **6.3.2 Measurement of CSF Protein Concentrations**

CSF samples were centrifuged at 2000 g for ten minutes and frozen at -80C until analyzed. CSF concentrations of MCP-1, BDNF, and VEGF were measured by multiplex chemiluminescent immunoassay kits (Aushon Biosystems, Billerica, Mass USA). The detection limits, as reported by the manufacturer, were 1.5 pg/ml for GDNF, 3.1 pg/mL for BDNF, 4.9 pg/mL for VEGF, and 0.8 pg/mL for MCP-1. Samples were assayed in duplicate.

### **6.3.3 Data Analysis**

Data are reported as the mean  $\pm$  standard deviation unless otherwise indicated and all datasets were tested for a normal distribution. Data were assumed to be independent of each other and correlations between stimulation parameters, CSF protein concentrations, reported pain (BPI Q.5), reported analgesia (BPI Q.8), and SF-36 score were sought. Changes in CSF protein concentrations after stimulation were analyzed with a Wilcoxon ranked sum test. A p value of  $< 0.05$  was considered to be statistically significant. Analysis was performed using GraphPad Prism 5.0.

## **6.4 Results**

### **6.4.1 Subjects and Stimulation Parameters**

Nine patients with a SCS implanted for FBSS consented to participate. The six men and three women ranged in age from 26 to 68 years (mean  $47 \pm 13.7$  years). Pain on average in the preceding 24 hours, as assessed by Q.5 of the BPI, ranged from 10 to 80mm (mean  $39 \pm 22.6$  mm). Reported pain relief from SCS in the preceding 24 hours as measured by Q.8 of the BPI ranged from 40% to 80% (mean  $65.6\% \pm 13.4\%$ ). SF-36 scores ranged from 28 to 75 (mean  $50.4 \pm 17.2$ ). SCS parameters ranged from pulse widths of 210 – 360 ms (mean  $282 \pm 46$  ms), frequencies of 40 – 100 Hz (mean  $73 \pm 20$  Hz), and amplitudes of 37.4 V (mean  $4 \pm 1.4$  V).

**Table 6.1:** Characteristics of SCS patients

Age	Gender	Amplitude (V)	Frequency (Hz)	Pulse width (ms)	Pain VAS (mm)	Pain relief; BPI Q8 (%)	SF-36 score
49	F	4.0	60	300	10	80	34
61	M	3.0	40	360	20	70	62
26	M	4.0	80	210	50	50	70
41	M	3.0	80	300	30	40	28
49	F	3.0	60	310	20	75	56
68	M	4.5	60	310	60	70	33
33	M	7.4	80	240	80	70	56
57	F	3.2	100	240	30	70	75
38	M	4.5	100	270	50	65	40

F, female; M, male; SCS, spinal cord stimulation; SF-36, Short Form (36) Health Survey; VAS, visual analog scale.

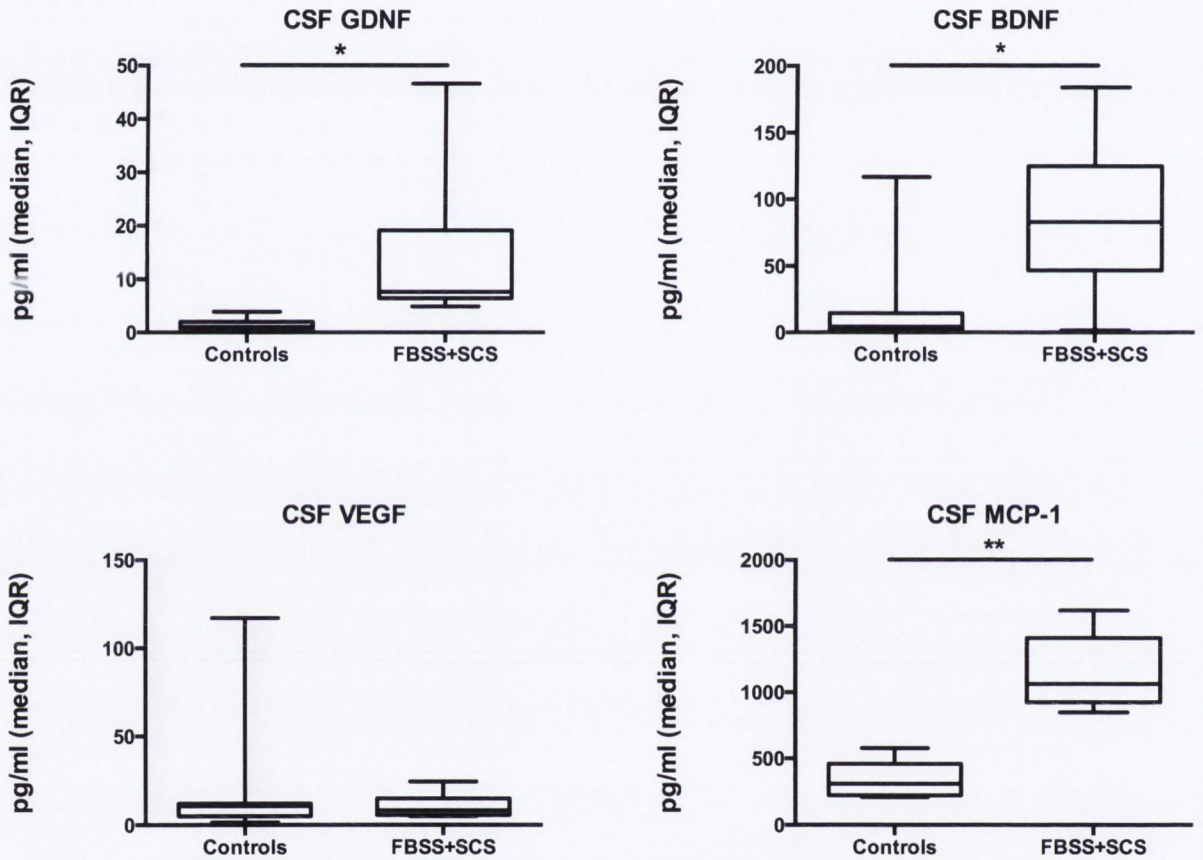
### 6.4.2 CSF Proteins, pain and SCS

In patients with FBSS and a SCS, CSF concentrations of BDNF ( $p = 0.011$ ), GDNF ( $p = 0.010$ ) and MCP-1 ( $p < 0.001$ ) were significantly higher than in controls. CSF concentrations of BDNF and VEGF exhibited positive correlations with reported pain ( $p = 0.04$ ). After five minutes of stimulation at each patients own stimulation parameters, median CSF concentrations of VEGF were significantly decreased ( $p = 0.01$ ).

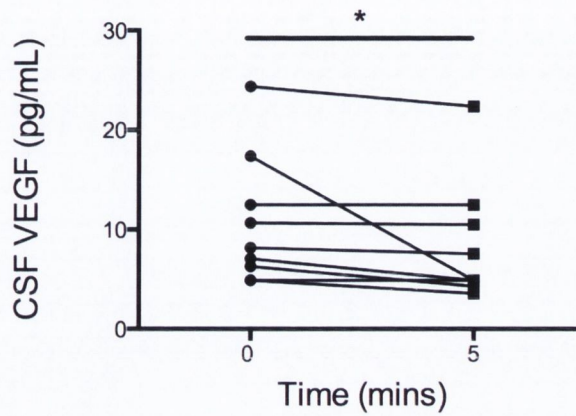
### 6.4.3 CSF proteins and SCS parameters

There was a significant positive correlations between log-transformed CSF levels of GDNF and stimulation frequency ( $p = 0.048$ ).

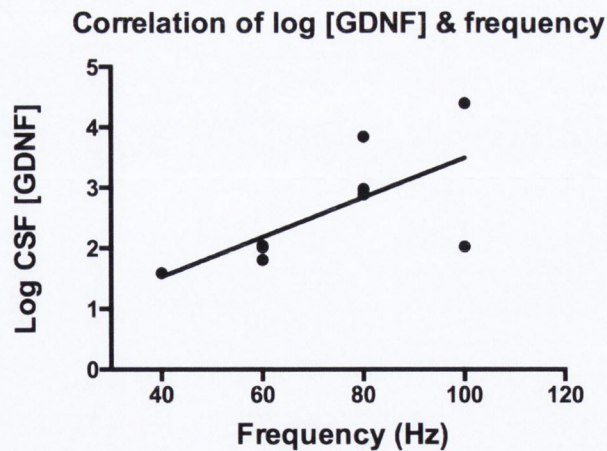
## 6. FBSS: CSF MEDIATORS & RESPONSE TO SCS



**Figure 6.1:** Difference in cerebrospinal fluid (CSF) concentrations of brain-derived neurotrophic factor (BDNF), monocyte chemoattractant protein-1 (MCP-1), glial cell-derived neurotrophic factor (GDNF) and vascular endothelial growth factor (VEGF) in patients with failed back surgery syndrome (FBSS) and a spinal cord stimulator (SCS) vs. matched controls, N = 9. Mann Whitney U Test,  $*p < 0.05$ ,  $**p < 0.01$ .



**Figure 6.2:** Cerebrospinal fluid (CSF) concentration of vascular endothelial growth factor (VEGF) (median, interquartile range) after five minutes of spinal cord stimulation in patients with failed back surgery syndrome,  $N = 9$ . Wilcoxon ranked sum test,  $p = 0.01$ .



**Figure 6.3:** Correlation between log cerebrospinal fluid (CSF) concentration (pg/ml) of glial cell-derived neurotrophic factor (GDNF) and SCS frequency in Hertz (Hz), Pearson's  $r = 0.671$ ,  $p = 0.048$ .

## 6. FBSS: CSF MEDIATORS & RESPONSE TO SCS

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**Table 6.2:** CSF Protein Levels in SCS Patients vs. Matched Controls

CSF levels (pg/ml)	SCS (mean $\pm$ SD)	Controls (mean $\pm$ SD)	<i>p</i> Value
MCP-1	1176 $\pm$ 278	339 $\pm$ 133	0.0001
BDNF	87 $\pm$ 55	19.5 $\pm$ 39.6	0.011
VEGF	10.7 $\pm$ 6.5	40.5 $\pm$ 91.7	0.374
GDNF	14.75 $\pm$ 14	52.3 $\pm$ 143	0.010

BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; MCP-1, monocyte chemoattractant protein-1; SCS, spinal cord stimulation; SD, standard deviation; VEGF, vascular endothelial growth factor; GDNF, glial cell-derived neurotrophic factor. *p*-value of Mann Whitney U test.

### 6.5 Discussion

Data from humans with chronic pain is frequently observational in nature. We invited patients with a positive response to SCS to volunteer and minimized the potential risks associated with a lumbar puncture by excluding patients on any anticoagulant therapy and performing a single lumbar puncture under radiological guidance in each patient. The small size of our study group is mitigated somewhat by the wide range of ages and SF-36 scores in the patients who volunteered. Our controls were patients with chronic lumbar back pain who were matched for age, gender, and SF-36 scores. However, it is not possible from our study protocol to delineate whether differences in baseline concentrations of CSF proteins are due to the pathophysiology of FBSS or the result of SCS over time. Also, it is not possible to exclude the lumbar puncture itself as a confounding effect on any observed changes.

BDNF is increased in response to nerve injury, colocalized with substance P and calcitonin gene-related peptide within the dorsal horn of the spinal cord, and modulates the release of GABA and glycine in lamina II of the dorsal horn [18]. BDNF is involved in hippocampal long-term potentiation and there is growing evidence of a parallel role in the plasticity of spinal nociceptive processes and in nerve regeneration after spinal cord injury [259], therefore a potential relationship with SCS is worthy of further study. The role of MCP-1 in neuropathic pain may include inhibition of GABAergic transmission and by enhancing excitatory



synaptic transmission and NMDA receptor activity in dorsal horn cells in response to nerve injury

VEGF has primarily been studied as an angiogenic factor and peripherally may have a role as a biomarker of disease severity in peripheral vascular disease [269] and in the pathophysiology of Complex Regional Pain Syndrome [216]. Within the central nervous system, VEGF has roles as a signal modulator of calcium flux in neurons, in synaptic plasticity, and as a neuronal and glial protective factor [207]. Release of VEGF may occur in response to NMDA receptor activation [244] and may be associated with the shedding of large extracellular vesicles from astrocytes and neurons containing VEGF and other angiogenic factors [227]. The observed reduction in CSF concentration of VEGF after five minutes of stimulation may represent more avid binding of VEGF to cell surface receptors, leaving less of the soluble form free within CSF.

The positive correlation between GDNF and stimulation frequency is interesting given the recent development of high-frequency modes of SCS to treat patients with refractory back pain [291]. Stimulation parameters have changed relatively little over the last 40 years, although different trends in stimulation parameters depending the aetiology of the pain have been reported [93]. A potential relationship between GDNF and stimulation frequency may offer promising developments in the use of SCS for spasticity and dystonia, an area only recently receiving attention and noteworthy for involvement of GDNF in dopaminergic neuronal development.

## 6. FBSS: CSF MEDIATORS & RESPONSE TO SCS

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# 7

## Conclusions and outlook

Most acute low back pain can be easily managed in non-specialist clinics. As it progresses to chronic pain, it becomes difficult to treat due to overlapping inflammatory and neuropathic processes. The well-documented role of immune mediators in sickness behaviour becomes more central to deciphering the progression of an acute pain to a syndrome of low mood, poor sleep and limited function. We have medications that are limited by side effects; psychological and physical therapies that are a “hard sell” as they send mixed messages about the veracity of the patient’s symptoms and do not have the support of a mechanistic explanation of their effect; and interventional technologies that work phenomenally well, just not for everybody. The aim of this thesis was to answer some of the ‘known unknowns’ surrounding the epiphenomena experienced by patients and witnessed by clinicians.

### 7.1 Limitations

1. Small sample size, particularly in the FBSS group. Our study was not powered to address questions relating to long-term clinical outcome or gender

## 7. CONCLUSIONS AND OUTLOOK

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differences in response. A larger sample size would be also be reassuring validation of our observed results.

2. Sampling at single time point or a short interval between serial samples. This was for pragmatic reasons to maximise recruitment and to satisfy ethics board conditions that patients were minimally inconvenienced. Serial sampling over a longer timeframe would answer questions about directionality and hierarchy of responses.
3. The FBSS patients all had functioning stimulators implanted. Therefore we cannot say with absolute certainty that any observed differences are pathognomic of FBSS or if they are due a longer-term effect of SCS. Given the short half-lives of many of these mediators, leaving the stimulators switched off for up to ten hours seemed a reasonable compromise.

### 7.2 Concluding remarks

FBSS appears to be associated with a greater spinal neuroimmune response. There is a close relationship between different neuroimmune mediators which suggests they cannot be studied in isolation. There is a complex non-linear relationship between spinal neuroimmune activation, pain and reported pain relief. The multiple sites of action of mediators such as IL-8 and GDNF would suggest that they play roles in both inflammatory and neuropathic pain syndromes to varying degrees.

It is not possible to draw definitive conclusions with regard to the mechanism of action of radiofrequency procedures from our study, which was observational in nature. We report several findings that are in keeping with current lines of investigation in pre-clinical studies and this is the first report of a potential role of MCP-1 and BDNF in the analgesic action of radiofrequency treatment. This, and the association of IL-6, IL-8 and VEGF with pain, may be useful in biomarker development to refine patient selection and radiofrequency technology, and to improve prediction of clinical outcomes.

Spinal cord stimulation may influence spinal levels of VEGF and there may be a relationship between stimulation parameters such as frequency and levels

of mediators such as GDNF. The effect of SCS on these mediators may explain both its mechanism of action and account for the variation in observed clinical response. Different patterns of expression in FBSS versus CRPS may explain the previously reported trend towards different stimulation parameters in both conditions.

### 7.3 Future outlook

We believe the observed findings in this thesis further bolster existing lines of investigation of the interaction of the immune system and nociceptive pathways in the context of chronic pain. Just as we are more than the sum of our parts, chronic pain is likely both always inflammatory and always neuropathic simultaneously; the distinctions that we observe clinically are merely points on a spectrum.

The reality of immune involvement in chronic pain and response to available therapies is perhaps more complex and intricate than previously appreciated. It is not that the “gate control” and other theories of pain are now obsolete, rather that, with greater scrutiny, what we are seeing is a fractal dimension and what has the appearance of ‘gates within gates within gates’.

To borrow from language of immunisation, herd immunity against chronic pain and disability may be a long way off. However this thesis suggests there may be ways of priming favourable immune responses that may extend the magnitude and duration of response to existing therapies.

## **7. CONCLUSIONS AND OUTLOOK**

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# Neuroimmunomodulation by pulsed RF.

## A.1 Summary

Pulsed radiofrequency (PRF) is a relatively recent modification of existing interventional technology in the treatment of chronic pain. An adaptation of continuous radiofrequency technology, it appears to be safe, effective and well-tolerated. The biological effects and precise mechanism of analgesia of PRF are still unclear. Brain-derived neurotrophic factor (BDNF) is a neurotrophin which supports neuronal survival and synaptic plasticity. BDNF has been implicated in both central and peripheral processes associated with inflammatory and neuropathic pain and has recently been found to be expressed in joints and intervertebral discs. We hypothesized that PRF applied to the lumbar medial branches exerted an influence on BDNF and that this may explain some of the analgesic and biological effect of PRF. We measured BDNF concentrations in the cerebrospinal fluid (CSF) (n=5) of patients undergoing lumbar PRF for the treatment of painful lumbar facet arthropathy. BDNF protein concentrations were measured at baseline and two hours post-procedure by ELISA. The concentrations of BDNF were significantly higher in CSF ( $p < 0.05$ ) after lumbar PRF at two hours. These findings provide evidence of discrete alterations in central BDNF concentrations in response to

## A. NEUROIMMUNOMODULATION BY PULSED RF.

PRF treatment which may underlie its analgesic effect.

### **A.2 Introduction**

Pulsed radiofrequency (PRF) has gained widespread popularity as a treatment modality for a variety of chronic pain conditions since its initial use in 1998. Conceptually, it is an attractive technique, principally because it is minimally invasive, localized and seemingly does not result in permanent nerve destruction. The clinical efficacy of PRF in a variety of painful syndromes is the subject of ongoing evaluation. However, while it would seem that while it is not equivocal to thermal RF for some conditions, it may have novel applications where a thermal neurotomy is contraindicated [290].

PRF is not associated with a histological nerve lesion in laboratory studies. The electrode tip delivers a large current density to the nerve, but the pauses between pulses allows dissipation of any heat produced, thereby preventing any thermal lesion. Application of PRF has been associated with changes in myelin configuration in the axons of pain afferents [74] and with an increase in expression of c-Fos, a marker of neuronal activity, in the segmental dorsal horn which does not occur with continuous RF [107]. Spinal mechanisms may include enhancement of noradrenergic and serotonergic descending pain inhibitory pathways and attenuation of microglial activation in the dorsal horn.

These reported findings are associated with the application of PRF adjacent to a nerve or dorsal root ganglion (DRG). However, the use of PRF has also been successfully reported for discogenic pain where the electrode is placed in the center of the nucleus pulposus, where no nerves are present [130]. This raises the possibility that the mechanism of action of PRF does not completely rely on close approximation to a nerve. Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family that appears to act as a central pain modulator in both inflammatory and neuropathic pain states. BDNF is involved in hippocampal long-term potentiation (LTP) and there is growing evidence of a parallel role in the plasticity of spinal nociceptive processes. It is co-localised with substance P and calcitonin gene-related peptide (CGRP) and may also control the release of GABA in the superficial dorsal horn of the spinal cord. The effect



of BDNF on pain facilitation varies in different models studied. While blocking the action of endogenous BDNF reduces pain behaviours in inflammatory and neuropathic pain models, the results of studies utilising exogenous BDNF have, on balance, demonstrated an antinociceptive effect. We sought to examine the effect of PRF on cerebrospinal fluid (CSF) and serum levels of BDNF in patients with lumbar facet arthropathy.

### A.3 Materials and Methods

#### A.3.1 Patients and Procedures

We received ethical approval to obtain samples of CSF and serum from patients undergoing pulsed RF for the treatment of lumbar facet joint arthropathy diagnosed by a positive response to medial branch nerve blocks. Patients with a history of co-existing neurological disorder or mood disturbance, recent trauma or surgery, or recent or current chemotherapy or corticosteroids were excluded. Five patients consented to CSF sampling. CSF samples were obtained by atraumatic lumbar puncture with a 25 gauge Whitacre needle. Patients underwent unilateral PRF at three lumbar levels as determined by previous diagnostic facet joint injections. All procedures were performed without sedation in the prone position under fluoroscopic guidance. 0.5 ml of 1% lignocaine was applied to the subcutaneous tissue prior to RF needle insertion. Once needle position was verified by fluoroscopy stimulation was carried out. A positive test for sensory stimulation was achieved when the patient declared stimulation of the painful area at  $<0.3V$ . Motor testing was performed to 1.0v. Pulsed RF was carried up to  $42^{\circ}C$  for 120 seconds. 0.5mls of 1% lignocaine was administered to the medial branch nerve immediately prior to PRF treatment. A second lumbar puncture was performed two hours post-procedure.

#### A.3.2 Measurement of CSF BDNF levels

CSF and serum samples were centrifuged at 2000g for ten minutes and frozen at  $-80^{\circ}C$  until analysed. BDNF concentrations were measured using SearchLight<sup>®</sup> Protein Arrays (Aushon BioSystems, Billerica, MA) with a range of sensitivity

## **A. NEUROIMMUNOMODULATION BY PULSED RF.**

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of 3.1 - 3,200 pg/mL. The SearchLight Protein Array is a quantitative multiplexed sandwich enzyme-linked immunoassay (ELISA) containing capture antibodies spotted on the bottom of a 96-well polystyrene microtiter plate. Each antibody captures a specific protein present in the standards and samples added to the plate. The bound proteins are then detected with a biotinylated detection antibody, followed by the addition of streptavidin-horseradish peroxidase (HRP) and lastly, a chemiluminescent substrate. The luminescent signal produced from the HRP-catalyzed oxidation of the substrate is measured by imaging the plate using the SearchLight Imaging System which is a cooled charge-coupled device (CCD) camera. The data is then analyzed using SearchLight Array Analyst software. The amount of luminescent signal produced is proportional to the amount of each protein present in the original standard or sample. Concentrations are extrapolated off a standard curve.

### **A.3.3 Data analysis**

Data are reported as the mean  $\pm$  standard deviation unless otherwise indicated and all datasets were tested for a normal distribution using the Kolmogorov-Smirnov test. Changes in CSF protein concentrations two hours after PRF treatment were analysed with either a paired Student's t-test and a  $p$  value of  $<0.05$  was considered to be statistically significant. Analysis was performed with Graph-Pad Prism 5.0.

## **A.4 Results**

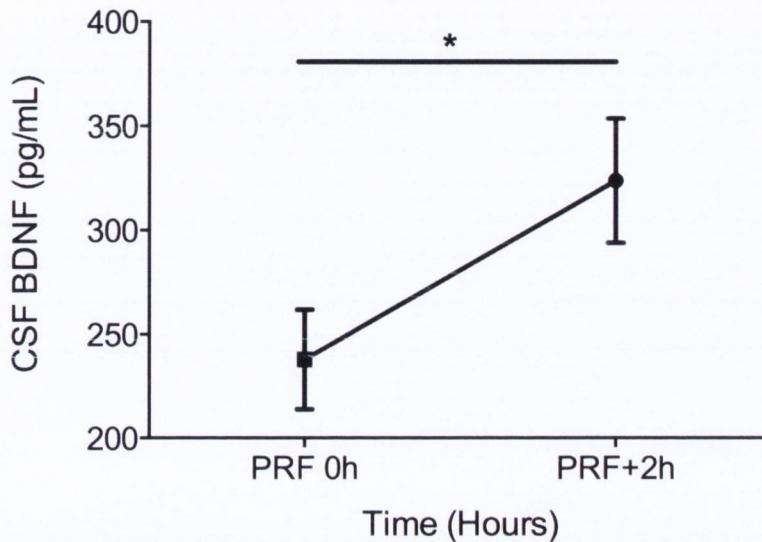
### **A.4.1 Subject Demographics**

Five patients attending for PRF treatment of painful facet arthropathy consented to serial CSF sampling. The three women and two men ranged in age from 24 to 50 years (mean  $37.2 \pm 9.4$  years).

### **A.4.2 Change in BDNF concentrations in response to PRF**

Mean CSF concentration of BDNF ( $n=5$ ) significantly increased from  $237.8 \pm 53.46$  pg/mL at baseline to  $323.8 \pm 62.34$  pg/mL two hours post-procedure ( $p =$

0.011).



**Figure A.1:** Changes in cerebrospinal fluid (CSF) levels of brain-derived neurotrophic factor (BDNF) two hours after lumbar PRF treatment,  $n=5$ . Paired Student's  $t$ -test,  $p = 0.011$ .

## A.5 Discussion

PRF is a safe and effective modification of an older existing treatment but one that appears to have a different mechanism of action and may yet progress to having clinical indications distinct from those for RF neurotomy. The potential utility of PRF in neuropathic pain has been confirmed when applied to the DRG in the treatment of postherpetic neuralgia [141] and in cervical radicular pain that was refractory to transforaminal epidural steroid injections [45]. However, PRF has been found to be less effective than thermal RF for treating trigeminal neuralgia [75] and did not provide any efficacy over sham treatment for post-traumatic neuropathic pain of peripheral nerves [3]. This raises the possibility that PRF has a specific mechanism of action which renders it more effective in certain neuropathic pain aetiologies.

The other novel scenario whereby PRF seemingly diverges from conventional RF is in its efficacy when applied to non-neural tissues, such as the intervertebral

## **A. NEUROIMMUNOMODULATION BY PULSED RF.**

disc [82] and knee joint [134]. In the clinical reports of the successful use of PRF in this manner, pain relief is not dependent on the electrode tip lying in close proximity to neural tissue. The electrical fields produced by PRF are negligible beyond 0.5mm of the electrode tip, therefore the mechanism of analgesia must rely on modulation of a substrate which has a role in both inflammatory and neuropathic pain.

BDNF is expressed in the non-neural tissues where PRF has been demonstrated to produce a pain-relieving effect [201] [98]. Since BDNF is associated with a phenotype shift in neuronal populations, it means by which RF procedures produce analgesia of a much longer duration than can currently be explained.

We only managed to recruit five patients for this study so it is not possible to explain how exactly how this fits in with other observed effects of pulsed RF. Changes in pain behaviour and a downregulation of microglial activity within the dorsal horn have been reported after PRF treatment in models of both lumbar disc herniation [44] and neuropathic pain [218], which would be expected to be associated with a reduction in BDNF. It may be that this is ultimately what happens, after an initial increase. This increase in BDNF may partly explain one of the epiphenomena observed in interventional pain practice.

# B

## Neuroimmunomodulation by SCS

### B.1 Summary

Chronic low back pain can result in significant distress and disability. Pain does not correlate with mechanical or anatomical findings and current evidence suggests a neuroimmune aetiology. Inflammatory mediators may induce glial cells to activate nociceptors and maintain chronic pain. Interventional therapies for low back pain include radiofrequency (RF) treatment and spinal cord stimulation (SCS) for facet joint pain and failed back surgery syndrome (FBSS), respectively. We hypothesized a change in serum concentrations of neuroimmune mediators in response to RF and SCS. We measured serum levels of brain-derived neurotrophic factor (BDNF), monocyte chemoattractant protein-1 (MCP-1), matrix metalloproteinase-2 (MMP-2) and vascular endothelial growth factor (VEGF) in two groups of patients: one undergoing lumbar RF for treatment of painful facet arthropathy and the other of patients with a functioning spinal cord stimulator implanted for FBSS. In the RF group ( $n=20$ ), serum was collected at baseline and two hours post-RF. In the SCS group ( $n=11$ ), patients left their stimulator switched off overnight and serum was collected at baseline and four hours after switching the stimulator on. In the RF group, BDNF ( $p = 0.007$ ), MCP-

## B. NEUROIMMUNOMODULATION BY SCS

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1( $p < 0.0001$ ) and MMP-2 ( $p = 0.011$ ) were significantly reduced two hours post-RF. In the SCS group, only serum MMP-2 was significantly reduced after four hours of stimulation ( $p = 0.01$ ).

### B.2 Introduction

Failed Back Surgery Syndrome (FBSS) refers to persistent or recurrent low back or leg pain and affects approximately 30% of patients who have undergone technically adequate lumbar spinal surgery [32]. It may arise as a consequence of post-operative changes in spinal anatomy or environment or if the surgery did not accomplish its intended goal. The commonality between patients with FBSS is a neuropathic pain syndrome that can result in greater levels of pain, lower quality of life and a higher rate of unemployment (78%) compared to other chronic pain illness models, such as rheumatoid arthritis [284]. There is conflicting evidence on the significance of post-operative processes, such as an inflammatory reaction and epidural fibrosis [7][23], or a biomechanical change such as new-onset facet joint instability [214]. As the number of spinal surgeries has grown exponentially, with consistent recurrence rate of approximately 30%, the number of people affected by FBSS has also increased accordingly.

‘Facet joint arthropathy’ (FJA) most frequently arises from repetitive strain and capsular stretch due to mechanical loading of the joint. There is evidence that capsular stretch results in hyperalgesia of greater magnitude and longer duration than occurs after complete joint disruption [156]. Upstream of the chronic inflammation within the joint, there is upregulation of cytokine messenger RNA within the dorsal root ganglion [156] and neuronal hyperexcitability can occur immediately within the dorsal horn after capsular stretch [228]. This hyperexcitability may act as a substrate for a neuropathic component to the pain [100] in addition to the inflammatory pain of an intra-articular osteoarthritic process and can occur as early as six hours after mechanical facet joint injury [55]. The facet joints have been identified as the source of pain in 16% of patients with recurrent pain after spinal surgery [172].

Spinal cord stimulation (SCS) has been in clinical use in the treatment of chronic neuropathic pain for over 40 years. It has become the mainstay of treat-

ment for patients with failed back surgery syndrome (FBSS) and is associated with sustained pain relief and significant improvements in functional capacity and health-related quality of life when compared with conventional medical management [149]. A reduction in extracellular glutamate and increase in extracellular  $\gamma$ -amino butyric acid (GABA) have been associated with SCS [128] and the administration of the GABA<sub>B</sub> receptor agonist baclofen has been shown to convert non-responders into responders [57]. SCS has demonstrated efficacy for FBSS, Complex Regional pain Syndrome (CRPS), and chronic coronary and peripheral ischaemic pain [128].

There is something specific to FBSS that means it is associated with a greater healthcare burden than comparable arthritic pain and yet is responsive to SCS as a therapy. FBSS would seem to have little in common with Complex Regional Pain Syndrome (CRPS) or pain of vascular origin in terms

We hypothesized a difference in neuroimmune expression in FBSS which would be attenuated by SCS.

## B.3 Materials and Methods

### B.3.1 Patients and Procedures

The RF group consisted of patients attending for RF treatment who had a previous positive response to diagnostic medial branch blocks. Twenty patients consented to serum sampling at baseline and two hours post-procedure. All procedures were performed without sedation in the prone position under fluoroscopic guidance. 1% lignocaine was applied to the subcutaneous tissue prior to RF needle insertion. Once needle position was verified by fluoroscopy stimulation was carried out. A positive test for sensory stimulation was achieved when the patient declared stimulation of the painful area at  $<0.3V$ . Motor testing was performed to 1.0V. Pulsed was carried up to 80°C for 120 seconds. 0.5mls of 1% lignocaine was administered to the medial branch nerve immediately prior to RF treatment.

The SCS group were patients who had been implanted with a spinal cord stimulator for the relief of pain arising from FBSS. Eleven patients consented to serum sampling. Patients were instructed to turn off their spinal cord stimulators

## **B. NEUROIMMUNOMODULATION BY SCS**

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at midnight and attend between 8 and 10AM the following morning for serum sampling. Their stimulators were then switched on and blood samples were taken after four hours of stimulation.

### **B.3.2 Measurement of serum protein concentrations**

Serum samples were centrifuged at 2000g for ten minutes and frozen at -80°C until analysed. BDNF concentrations were measured using SearchLight<sup>®</sup> Protein Arrays (Aushon BioSystems, Billerica, MA). The range of sensitivity for BDNF is 3.1- 3,200 pg/mL, for MCP-1 is 0.8 - 800 pg/mL and for MMP-2 is 31.1 - 32,000 pg/mL. For MCP-1 measurements, the serum samples were diluted 1:10. The SearchLight Protein Array is a quantitative multiplexed sandwich enzyme-linked immunoassay (ELISA) containing capture antibodies spotted on the bottom of a 96-well polystyrene microtiter plate. Each antibody captures a specific protein present in the standards and samples added to the plate. The bound proteins are then detected with a biotinylated detection antibody, followed by the addition of streptavidin-horseradish peroxidase (HRP) and lastly, a chemiluminescent substrate. The luminescent signal produced from the HRP-catalyzed oxidation of the substrate is measured by imaging the plate using the SearchLight Imaging System which is a cooled charge-coupled device (CCD) camera. The data is then analyzed using SearchLight Array Analyst software. The amount of luminescent signal produced is proportional to the amount of each protein present in the original standard or sample. Concentrations are extrapolated off a standard curve.

### **B.3.3 Data analysis**

Data are reported as the mean  $\pm$  standard deviation unless otherwise indicated and all datasets were tested for a normal distribution using the Kolmogorov-Smirnov test. Differences between RF and SCS groups were analysed with a one-way ANOVA and when significant, post hoc comparison were made using Bonferroni's Multiple Comparisons Test where data followed a normal distribution. Changes in serum protein concentrations within the RF and SCS groups before and after treatment were analysed with a paired Student's t-test. A *p* value



of  $<0.05$  was considered to be statistically significant. Analysis was performed with GraphPad Prism 5.0.

## B.4 Results

### B.4.1 Subject Demographics

Twenty patients consented to serum sampling prior to RF treatment and two hours post-procedure. The nine women and eleven men ranged in age from 31 to 78 years (mean  $48.3 \pm 13.16$  years). Eleven patients with spinal cord stimulators consented to serum sampling. The seven men and four women ranged in age from 26 to 68 years (mean  $47 \pm 13.7$  years).

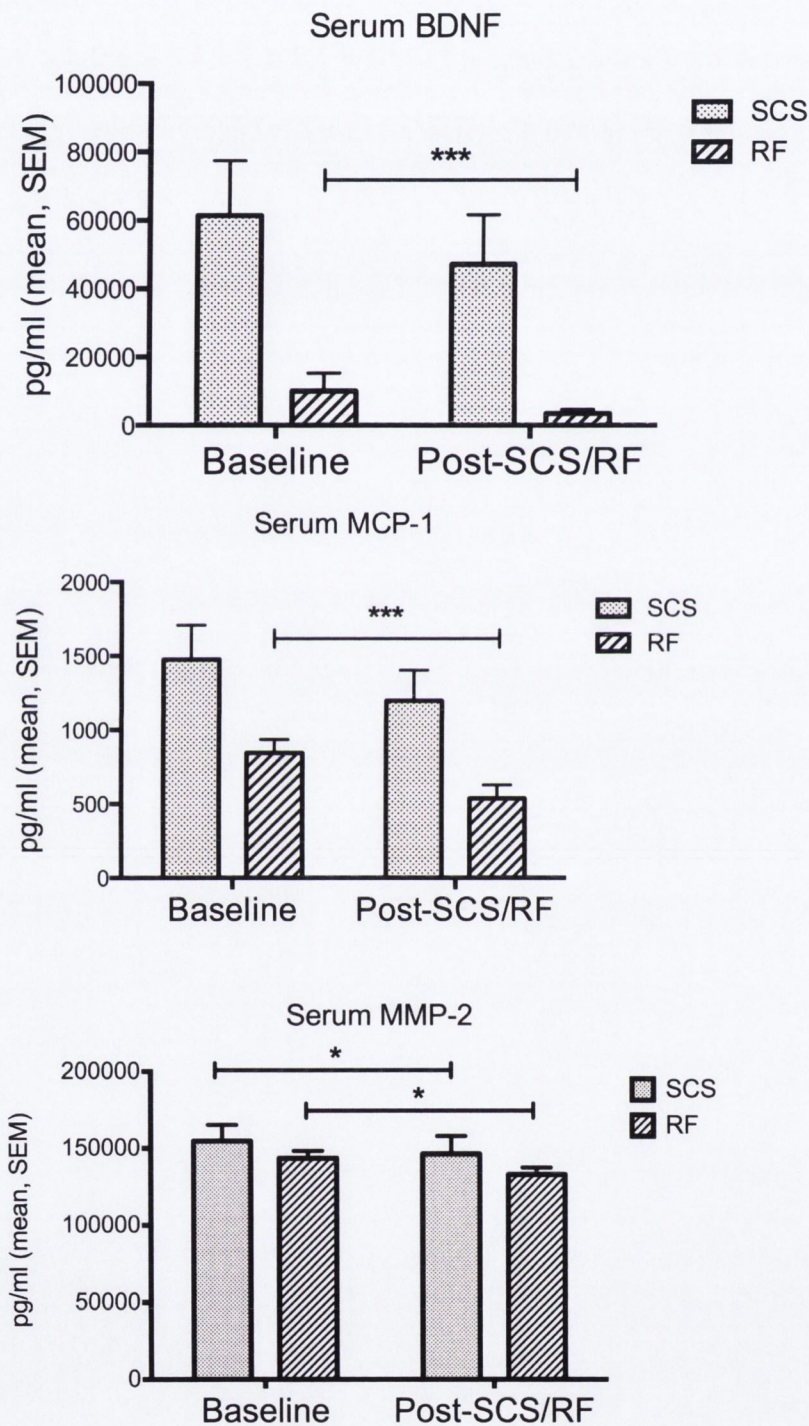
### B.4.2 Serum protein concentrations after RF

Median serum concentration of BDNF ( $n=20$ ) was significantly decreased from 1720 pg/mL to 1262 pg/mL ( $p = 0.007$ ). Mean MCP-1 concentrations were significantly reduced from  $844 \pm 409$  pg/mL at baseline to  $536 \pm 409$  at two hours post-procedure ( $p < 0.0001$ ). Mean MMP-2 concentrations were significantly reduced from  $143,525 \pm 21,652$  pg/mL to  $132,919 \pm 20,313$  pg/mL ( $p = 0.011$ ). Mean serum concentrations of VEGF were not significantly different ( $392 \pm 302$  vs.  $362 \pm 350$  pg/mL,  $p = 0.17$ ).

### B.4.3 Serum protein concentrations after SCS

In the SCS group, there were no significant differences in the mean serum levels of BDNF ( $61,372 \pm 53,147$  vs.  $47,102 \pm 48,065$  pg/mL,  $p = 0.46$ ), MCP-1 ( $1476 \pm 773.6$  vs.  $1195 \pm 694.6$  pg/mL,  $p = 0.056$ ) or VEGF ( $640 \pm 321$  vs.  $583 \pm 329$  pg/mL,  $p = 0.41$ ) after four hours of spinal cord stimulation, while mean serum MMP-2 concentrations were significantly reduced from  $154,644 \pm 35,169$  pg/mL to  $146,266 \pm 38,651$  pg/mL ( $p = 0.011$ ).

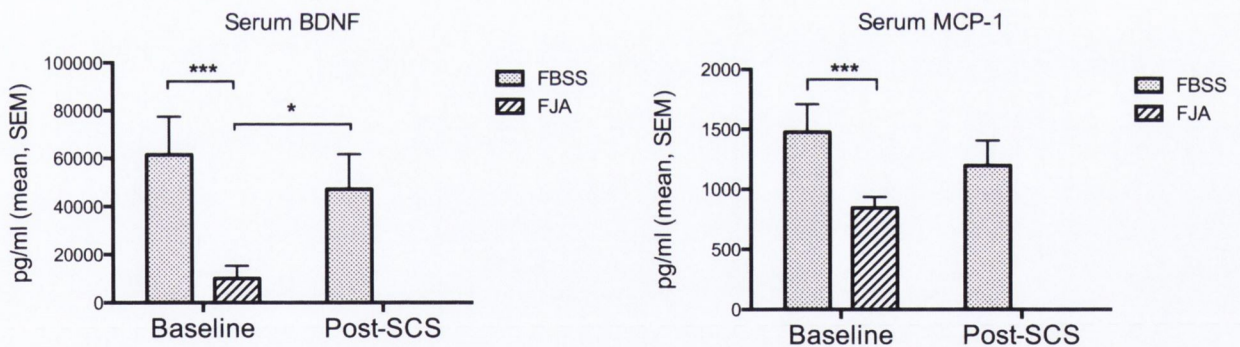
## B. NEUROIMMUNOMODULATION BY SCS



**Figure B.1:** Serum concentrations (mean, SEM) of BDNF, MCP-1, MMP-2 and VEGF after spinal cord stimulation (SCS),  $N = 11$ , and radiofrequency (RF) treatment,  $N = 20$ . Paired Student's  $t$ -test, \* $p < 0.05$ , \*\*\* $p < 0.001$ .

### B.4.4 Comparison of protein concentrations between groups

A one-way ANOVA was used to examine if serum protein concentrations at baseline in patients with a SCS implanted for FBSS were different from serum levels the FJA group and to compare the effect of SCS on any difference. There was significant difference in BDNF concentrations between groups [ $F(3,58)=10.38$ ,  $p = 0.0001$ ]. A post hoc test using Bonferroni's Multiple Comparison Test showed this difference persisted after four hours of SCS ( $47,102 \pm 48,065$  vs.  $10060 \pm 23563$ ). There was a significant difference in mean MCP-1 concentrations between groups [ $F(3,58)=8.22$ ,  $p = 0.0001$ ]. Post hoc comparisons using Bonferroni's Multiple Comparison Test indicated that after four hours of SCS, serum MCP-1 was no longer significantly different from the FJA group. Serum MMP-2 concentrations were not significantly different between SCS and RF groups [ $F(3,58)=1.59$ ,  $p = 0.20$ ]. Serum VEGF concentrations were not significantly different between groups [ $F(3,58)=2.52$ ,  $p = 0.06$ ].



**Figure B.2:** Differences in serum MCP-1 and BDNF between Failed Back Surgery Syndrome (FBSS),  $N = 11$ , and Facet Joint Arthropathy (FJA),  $N = 20$  an effect of four hours of spinal cord stimulation (SCS). 1-way ANOVA with Bonferroni post hoc, \* $p < 0.05$ , \*\*\* $p < 0.001$ .

### B.5 Discussion

Strict diagnostic criteria are felt to improve clinical and research outcomes and aid in developing therapies that are disease-specific. In the context of chronic low back pain, this may not always be feasible as pathologies can co-exist and progress over time. Facet joint pain may be a feature of FBSS and yet, in isolation, is not an indication for SCS. None of the conditions that respond well to FBSS appear to have much in common with each other at first glance; FBSS is a post-surgical, predominantly mechanical entity with superimpose inflammation and fibrosis [115]; CRPS may rise after trauma or spontaneously and is most likely an immune-driven neuroinflammatory process [171]; ; in refractory angina and peripheral vascular disease, pain may be a combination of ischaemic nociceptive pain and [63].

FBSS would seem to be more than just changes in biomechanics and post-surgical fibrosis. Or rather, these events result in a process of neuroinflammation that is more than the inciting events as evidenced by the significant differences in serum levels of BDNF and MCP-1 compared to painful FJA, where there is presumably a low-grade inflammation. Four hours of SCS would appear to ‘normalise’ levels of MCP-1 but not BDNF. Both BDNF and MCP-1 were significantly higher at baseline despite these patients having a spinal cord stimulator implanted for at least six months. While lumbar RF resulted in significant changes in BDNF and MCP-1 in the context of FJA, the magnitude of these reductions would not be significant in the context of the levels observed in the patients with FBSS. While RF and SCS may attenuate levels of neuroimmune mediators, there may be limits to their ability to influence the underlying neuroinflammation and glial activation, although work on pulsed RF does suggest an effect on microglial activation in both mechanical [44] and neuropathic [219] pain models.

Serum MMP-2 was reduced after both RF and SCS, but overall there were no significant differences between groups. MMP-9 and MMP-2 have been implicated in the early and late phases of neuropathic pain, respectively [135]. The role of MMPs in cleaving cytokines, components of the extracellular matrix and the myelin sheath, MMP activity may be a commonality between inflammatory and neuropathic processes [154]. A study of the analgesic effect of electroacupuncture

in a model of neuropathic pain demonstrated a decrease in MMP-2 and MMP-9, pro-inflammatory cytokines, and markers of glial activation [90]. Although each of these technologies may seem to have distinctive mechanisms, they each seem to influence levels of MMP-2, suggesting a common pathway worthy of further investigation.

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