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PhD Thesis

The involvement of the central nervous system in chemotherapy-induced peripheral neuropathy, and the symptom burden and health-related quality of life in patients with multiple myeloma

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This thesis is dedicated to my parents and Jason.

Summary

Background: Modern treatments extend life expectancy in patients with multiple myeloma (MM) and a high incidence of chemotherapy-induced peripheral neuropathy (CIPN) has evolved. The impact of disease and treatment on health-related quality of life (HRQoL) in MM is poorly characterised. This work aimed to investigate (i) the neuroanatomical functional correlates of pain processing in CIPN and (ii) HRQoL and symptom burden in advanced, intensively-treated myeloma.

Methods: *First study*: twelve neurophysiologically assessed patients with CIPN and 12 healthy volunteers underwent fMRI to determine the brain's haemodynamic response to noxious heat stimuli applied to the extremities. *Second study*: detailed HRQoL and neuropathy assessments, serum IL-6 and TNF-α levels were measured in 32 patients with MM (median age=61yrs, duration of disease=5.5yrs) who had previously undergone 3 lines of treatment.

Results: *First study*: Neurophysiological testing in MM patients confirmed peripheral neuropathy in the feet. Significant hypo-activation of the right superior frontal gyrus and hyper-activation in the precuneus were present in response to foot stimulation in the CIPN-myeloma compared to healthy control groups. Significant positive correlation existed between neuropathy score and frontal opercula activation, during foot stimulation in CIPN-myeloma patients. *Second study*: Thirty-two MM patients (duration of disease=5.5yrs) were recruited. Physical functioning was significantly compromised (p<0.001) and associated with progressive work disability and concerns regarding loss of independence. Fatigue and pain were the predominant symptoms, impacting negatively on physical functioning (p<0.001). Half of the patients reported neuropathic pain. This sub-group scored lower measures of physical, role and social functioning on EORTC QLQ-C30 and future perspectives from the EORTC MY20 assessments compared to patients without neuropathy. Average pain and pain interference from the BPI-SF were positively correlated (p<0.05 and p<0.005, respectively) with serum IL-6 levels.

Conclusion: Patients with MM suffer from a high peripheral neuropathy symptomatology burden, leading to a worsening functioning and increased pain. FMRI data indicates that heat-pain stimuli evoke differential activation of distinct cortical regions, suggesting different central pain processing mechanisms in CIPN-myeloma patients. Despite disease control and supportive care, intensively-treated myeloma survivors have significantly compromised HRQoL related to symptom burden.

Author's Declaration

The two studies that form the basis of this thesis are partly the result of collaborative work with the Academic Unit of Radiology, Academic Unit of Supportive Care, and the departments of Haematology and Diabetes. My contributions were the following:

- The primary role in the conception and design of the first study. Write up of the protocol, research ethics and NHS approval.

- Obtained the funding from the academic unit of supportive care and academic unit of radiology, University of Sheffield and won the NAPP Research Award by the Association of Palliative Medicine, 2009 for the first study. Myeloma UK funded the second study.

- Recruitment, clinical and neurophysiological assessments of the subjects in the first study and most of the second study.

- MR imaging: Prof I.D Wilkinson designed MR protocols and the scanning was performed by departmental radiographers and clinically assessed by a neuroradiologist. I organised the appointments and consented the subjects for the MR scans. I was present for all the scans and co-ordinated the heat-pain stimulation and processed and analysed all MR data of CIPN-myeloma patients and healthy volunteers under the supervision of Prof I.D Wilkinson and Dr M. Hunter.

- Collation of all data and subsequent statistical analysis for both studies including the questionnaires; worked under the supervision of Prof I.D. Wilkinson and Prof S.H. Ahmedzai for the first study; Dr J. Snowden, Prof C.Eiser and Prof S.H. Ahmedzai for the second study.

- Write up of thesis and papers for publications (first author).

Dr Elaine Cachia

Table of Contents

List of figures

List of tables

Abbreviations

ACC	Anterior cingulate cortex	**MNI**	Montreal neurological institute
AMPA	Amino-3-hydroxy-5-methyl-4-isoxazole propionate	**MPT**	Melphalan, prednisolone, thalidomide
B$_o$	Magnetic field	**MRI**	Magnetic Resonance Imaging
BOLD	Blood oxygen level dependent	**Ms**	Milliseconds
BPI-SF	Brief Pain Inventory short form	**mV**	Millivolt
CDT	Cold detection threshold	**NCS**	Nerve conduction study
CGRP	Calcitonin gene related peptide	**NCV**	Nerve conduction velocity
CHEPS	Contact heat evoked potential device	**NES**	Nerve electrophysiological studies
CI	Confidence interval	**NIS**	Neuropathy impairment score
CIPN	Chemotherapy induced peripheral neuropathy	**NMDA**	N-methyl-D-aspartate
CMAP	Compound muscle action potential	**NPS**	Neuropathic pain scale
CNR	Contrast to noise ratio	**NRS**	Numerical rating scale
CNS	Central nervous system	**NTSS-6**	Neuropathy total symptom score
CPAQ	Chronic pain acceptance questionnaire	**PAG**	Periaqueductal grey
Cronbach's α	Cronbach's alpha	**PASW**	Predictive analytics software
CBF	Cerebral blood flow	**PCS**	Pain catastrophizing scale
CSF	Cerebro-spinal fluid	**PCS SF-12**	Physical component summary

CTD	Cyclophosphamide, thalidomide and dexamethasone	**PET**	Positron Emission Tomography
CTDa	Attenuated Cyclophosphamide, thalidomide and dexamethasone	**P$_{FWE-corr}$**	P family-wise error corrected (statistics for SPM)
CVAD	Cyclophosphamide, vincristine, doxorubicin, dexamethasone	**PFC**	Prefrontal cortex
DPN	Diabetic peripheral neuropathy	**PNS**	Peripheral nervous system
DRG	Dorsal root ganglion	**QoL**	Quality of life
ECG	Electrocardiogram	**QST**	Quantitative sensory testing
EEG	Electroencephalography	**r**	Spearman's rho
EORTC QLQ-C30	European Organisation for Research & Treatment of Cancer core quality of life questionnaire	**RF**	Radiofrequency
EORTC QLQ-MY20	European Organisation for Research & Treatment of Cancer myeloma specific module	**Rt**	Right
EPI	Echo planar imaging	**RVM**	Rostral ventromedial medulla
FID	Free induction decay	**S1, S2**	Somatosensory cortex
FOV	Field of view	**SD**	Standard deviation
fMRI	Functional magnetic resonance imaging	**SF**	Social functioning
GE	Gradient echo	**SE**	Spin echo
GLM	General linear model	**SF-12**	Short Form-12 health survey
g/dl	Grams per decilitre	**SF-36**	Short Form-36 health survey

g/l	Grams per litre	**s-LANSS**	Self-report Leeds Assessment of Neuropathic Symptoms and Signs
HADS	Hospital anxiety and depression score	**SMA**	Supplementary motor area
HRQoL	Health-related quality of life	**SNAP**	Sensory nerve action potential
HRV	Heart rate variability	**SNR**	Signal to noise ratio
HSCT	Haemopoietic stem-cell transplantation	**SPM**	Statistical parametric mapping
HV	Healthy volunteer	**SPMs**	Statistical parametric maps
Ig	Immunoglobulin	**SD**	Standard deviation
IL	Interleukin	**t**	T statistics
IQR	Interquartile range	**T**	Tesla
JND	Just noticeable differences	**TE**	Echo time
k_E	Threshold level in SPM	**TNF-α**	Tumour necrosis factor alpha
LEPs	Laser evoked potentials	**TNSr**	Total Neuropathy Score, reduced
Lt	Left	**TR**	Repetition time
M_o	Net magnetisation	**VAD**	Vincristine, doxorubicin and dexamethasone
MCS SF-12	Mental component summary	**VDT**	Vibration detection threshold
MDT	Mechanical detection threshold	**vs**	Versus
mmol/l	Millimoles/litre	**μmol/l**	Micromole/litre
MM	Multiple myeloma	**μV**	Microvolt

Thesis Outline

The objective of this thesis is to determine whether differences exist in central pain processing as assessed by functional magnetic resonance imaging during noxious thermal stimulation between patients with chemotherapy-induced peripheral neuropathy and healthy volunteers and to characterise the HRQoL and late effects in patients with advanced but stable myeloma, clinically and in terms of HRQoL.

Chapter 1: I will present background knowledge relevant for understanding the research presented in this thesis. In the first section, I will discuss multiple myeloma followed by pain and chemotherapy-induced peripheral neuropathy and will finish on the topic of neuroimaging. I will finish by discussing the objectives of the thesis with specific primary and secondary aims for the two studies that I have incorporated in this thesis.

Chapter 2: Investigation of the central pain processing using functional magnetic resonance imaging of multiple myeloma patients with chemotherapy-induced peripheral neuropathy and compare this to healthy volunteers.

Chapter 3: Investigation of the late effects of pain, peripheral neuropathy and psychosocial issues in intensively treated transplanted multiple myeloma patients.

Chapter 4: An overall conclusion from both studies.

Chapter 5: The final conclusion and future work will be discussed in this chapter.

Chapter 1

1 Background

1.1 Multiple myeloma

Multiple myeloma (MM) accounts for 10-15% of all haematological malignancies and is the second most common haematological cancer (Greenlee et al. 2001). The male to female ratio is 1.5:1 and the annual incidence of myeloma in the UK is approximately 60-70 per million (Cancer Research UK 2010). The prevalence is estimated at around 180-270 per million of population, although likely to increase due to increased awareness and diagnosis, better treatment options and an ageing population (Kumar et al. 2008). Essentially older adults are affected by MM (median age at presentation is 70 years); it is uncommon to be diagnosed in those less than 40 years old and only 15% of patients are aged <60 years (Smith et al. 2006; Bird et al. 2011).

Pathophysiology

Multiple myeloma is a plasma cell disorder originating in the bone marrow with subsequent over-abundance of monoclonal paraprotein (M protein). In normal physiology, B cells are derived from lymphocytes that matured and differentiated in the bone marrow. Once released in the blood, they establish colonies in the peripheral lymphoid tissues. When a B-cell receptor binds to an antigen, the B cell differentiates into active plasma cells in the germinal centre of lymphoid organs whilst others become dormant memory cells. Plasma cells produce antibodies which are secreted in the blood; these are known as immunoglobulins (Ig) and are grouped in 5 sub-classes: about 80% is IgG, 15% is IgA, 5% is IgM, 0.2% is IgD, and a trace is IgE. Antibodies are involved in the humoral arm of the adaptive immune system. The memory cells persist in the circulation for long-term protection against the same antigen. In MM, there is proliferation of malignant plasma cells and a subsequent overabundance of monoclonal paraprotein. The type of MM corresponds to the particular type of immunoglobulin that the myeloma cells produce. The most common type of myeloma is IgG followed by IgA. The rare myelomas include up to 7% of all myelomas and consist of plasma cell leukaemia, IgD, IgM, IgE and non-secretory myeloma (Bird et al. 2011).

Patients with MM usually present clinically with symptoms of bone disease, anaemia, hypercalcaemia impaired renal function, recurrent infections and hyperviscosity. Other patients are diagnosed following the incidental finding of a raised erythrocyte sedimentation rate, serum protein, serum globulin or plasma viscosity. The plasma cell proliferation may interfere with the normal production of blood cells, leading to leucopoenia, anaemia, and thrombocytopenia. The plasma cells may cause soft-tissue masses, termed plasmacytomas or produce skeletal lytic lesions. The monoclonal antibodies produced, lead to impaired humoral immunity making patients more susceptible to infections, and the overproduction of these antibodies may lead to hyperviscosity, amyloidosis and renal failure. The overproduction of monoclonal IgG, IgA, and/or light chains may be identified with densitometry of the monoclonal peak on electrophoresis, immunochemical measurement of total immunoglobulin isotype level, and urinary total protein and light chain excretion.

No single major causative agent has been identified for MM but risk factors include genetic components and race, exposure to radiation and environmental external agents, increasing age, diet, obesity (body mass index ≥ 30kg/m^2) and lifestyle behaviours. An increased risk of MM and obesity has been reported and a high body mass index correlates with a particular genotype that increases interleukin (IL) 6 production (Kyle et al. 2003). The development of MM is likely to be a stepwise process where genetic defects such as translocations accumulate in the malignant plasma cell, changes occur in the bone marrow microenvironment that support the tumour growth leading to failure of the immune system to control the disease (Richardson and Anderson 2004). This interaction between MM cells and bone marrow stromal cells leads to enhanced expression and secretion of cytokines and chemokines that stimulate proliferation and survival of MM cells. This also leads to activation of signal transduction pathways that promote cell proliferation, stimulate neoangiogenesis and protection from apoptosis. The best characterised myeloma growth factor is the cytokine IL-6 which has been reported to have an important role in the pathogenesis and malignant growth of MM (Bommert et al. 2006). Other growth and anti-apoptotic factors including vascular endothelial growth factor, insulin-like growth factor, tumour necrosis factor alpha (TNF-α) and fibroblast growth factors are secreted.

Management

Multiple myeloma is a treatable but incurable disease. Patients with asymptomatic MM do not require interventions but need to be closely monitored by a haematologist as the overall risk of progression is 10% per year for the first five years but declines after this (Kyle et al. 2007). Symptomatic MM is defined by the presence of myeloma-related organ or tissue impairment which includes hypercalcaemia, renal insufficiency, anaemia or bone lesions (International Myeloma Working Group 2003). Disease-directed treatment is only needed for symptomatic MM. By delaying treatment, side effects associated with chemotherapy can be minimised. Even though treatment directed at MM is postponed in asymptomatic patients, supportive care is still given to prevent and manage symptoms and complications.

1.1.1 Disease specific treatment

The choice of treatment modalities in MM has increased over the last several decades, and has been associated with significant increases in life expectancy (Child et al. 2003; Klepin and Hurd 2006; Smith et al. 2006; Bird et al. 2011). Induction therapy followed by high-dose chemotherapy with haemopoietic stem-cell transplantation (HSCT) is the standard of care for MM patients who can tolerate this treatment (Morgan et al. 2012). However certain patients will be unable to receive HSCT because of poor performance status, advanced age, or co-morbidities and therefore treatment decisions take into account individual patient factors and patient choice. These patients may be offered single-agent or combination chemotherapy, which is less intensive. For patients where high-dose chemotherapy is planned, the aim of induction treatment is to stimulate high remission rates quickly with minimal toxicity and to preserve haemopoietic stem cell function to ensure successful mobilsation of peripheral blood stem cells (Bird et al. 2011). Studies have shown that stem cell transplantation can result in significantly more disease-free individuals and better overall survival than conventional chemotherapy at five-years (overall survival 52% versus 12%) (Attal et al. 1996; Barlogie et al. 1999). Delivery of more intensive treatment including haematopoietic stem cell transplantation in younger patients and introduction of novel agents, has resulted in more profound improvements in overall survival, with over half of patients under 50 years old predicted to survive beyond a decade following diagnosis of the disease (Pulte et al. 2011).

Conventionally, the standard care for patients who were planning to receive high dose chemotherapy and HSCT was the induction therapy based on high dose dexamethasone, such as vincristine, doxorubicin and dexamethasone (VAD). This treatment led to substantial haematological toxicity and the need for central venous access thus increasing the incidence of venous catheter-related infections and thrombosis. There have been several studies on the use of thalidomide for induction and the latest Medical Research Council Myeloma IX trial compared cyclophosphamide, vincristine, doxorubicin and dexamethasone (CVAD) with cyclophosphamide, thalidomide and dexamethasone (CTD) and the results showed higher response rates in the CTD arm (Morgan et al. 2012). Also thalidomide is an oral administration and has been shown to have a reduced incidence of infection and cytopenia. Thalidomide-containing combination regimens are now widely used in the UK following the demonstration that it can be safely and effectively given and that stem cell mobilisation and harvesting are not adversely affected by its use (Bird et al. 2011). If the patient is unable to tolerate or has contraindications to thalidomide then bortezomib in combination with an alkylating agent (melphalan or cyclophosphamide) and dexamethasone is recommended as an option for the first-line treatment of MM if high-dose chemotherapy with stem cell transplantation is considered inappropriate (Doss et al. 2011).

Maximum response to induction therapy usually occurs after four to six cycles. Currently, it is recommended to treat to at least partial response, which usually occurs within four to six cycles and to substitute to another regimen if there is evidence of disease progression after two cycles or partial response after four cycles. Peripheral blood stem cell harvesting is usually carried out within four to six cycles for all induction regimens following stimulation with growth factors (Bird et al. 2011). The ideal regimen for mobilising stem cells is not clear but cyclophosphamide with granulocyte colony-stimulating factor is commonly used. High dose melphalan is the standard conditioning prior to HSCT. Previously, total body irradiation was given, which resulted in increased toxicity with no improvement in response rate or progression free survival. As well as autologous transplantation as discussed above, another form of transplantation is allogeneic stem cell transplantation, which involves a healthy donor that has a tissue type that matches the recipient. This can result in long-term disease-free survival, however its' use has been controversial because of the significant transplant related morbidity and mortality (Kumar 2009).

Patients who do not respond to the induction therapy (no change in M-protein and no evidence of clinical progression or progressive disease) are reviewed on a case-by-case basis to decide whether the patient is still suitable for high dose therapy. Patients who are clinically stable (have refractory but non-progressive disease), may still benefit from consolidation with high-dose therapy and they might receive different chemotherapy regimens or might enter clinical trials.

When high dose chemotherapy is not planned, the main aim of treatment is to stabilise the disease (termed the plateau phase) for as long as possible with minimal treatment related toxicity, thereby prolonging survival and maximising quality of life (QoL). Previously, the combination of oral melphalan and prednisolone regimens were the mainstay of treatment in this group. Nowadays, alternative regimens comprising MPT (melphalan, prednisolone and thalidomide) or CTD are used. MPT, as first line treatment in elderly patients has shown superior response rates and prolongation of progression free survival and overall survival (Dimopoulos and Terpos 2010). Attenuated doses of CTDa are given in elderly less fit patients. In the non-intensive arm of the Myeloma IX trial, early results demonstrate superior response rates for CTDa over melphalan and prednisolone (Morgan et al. 2011). Bortezomib and lenalidomide are also effective when used in combination with steroids with or without an alkylating agent. However, the National Institute for Health and Clinical Excellence (NICE) recommends that lenalidomide in combination with dexamethasone is only given to patients who have received two or more previous therapies (NICE 2009).

There is no standard recommended treatment for relapsed patients because of disease heterogeneity and variability in patient specific factors including co-morbidities, age, bone marrow function and the persistence of toxicities related to previous therapy (Bird et al. 2011). Data shows that thalidomide, bortezomib and lenalidomide based regimens used in treating relapsed patients are usually given in combination with corticosteroids and sometimes with an alkylating agent, most commonly cyclophosphamide. In patients who had a good response to the initial transplant procedure (>18 months to disease progression), a second HSCT may be considered (Bird et al. 2011).

1.1.2 Neuronal damage and symptoms arising from anti-myeloma therapies

Thalidomide, bortezomib, and vincristine have been associated with the development of peripheral neuropathy in MM and their mechanisms of action are looked at in more detail below.

Thalidomide

This is a TNF-1α antagonist, which is widely used as an induction treatment, as a maintenance treatment and for the management of relapsed-refractory disease, usually in combination with steroids and other chemotherapy. Thalidomide is used in MM because of its immunomodulatory and anti-angiogenic activity. Other mechanisms of action in MM include stimulation of the secretion of alpha-interferon and IL-2, induction of apoptosis, and regulation of adhesion molecule expression (Hideshima et al. 2000). The most common side effects of thalidomide are deep vein thrombosis, peripheral neuropathy, constipation and fatigue, which often restrict dose and treatment duration rather than drug effectiveness. The reported incidence of thalidomide neuropathy varies in the literature from 25% to 75% (Cavaletti et al. 2004; Mileshkin et al. 2006; Palumbo et al. 2008). The relationship between the development of neuropathy, cumulative dose, dose-intensity of thalidomide and the duration of exposure is controversial. In a study by Plasmati et al, 31 newly diagnosed MM patients were treated with thalidomide and at the end of treatment, 83% of the patients had clinical and electrophysiological evidence of a sensory axonal, length-dependent polyneuropathy (Plasmati et al. 2007).

Bortezomib

Bortezomib is a proteasome inhibitor; its mechanism of action targets the proteasome and causes the cell cycle to arrest. It has anti-angiogenic properties, causes induction of the stress response, apoptosis of MM cells and also targets the interaction between the tumour cell and the bone marrow microenvironment (Raab et al. 2009). The anti-proliferative, pro-apoptotic, and anti-tumour activities of bortezomib result from proteasome inhibition and depend on the altered degradation of a host of regulatory proteins. This is the standard treatment for first relapse in the UK (based on NICE guidance) and has been studied extensively in large clinical trials. The most common side effects reported are peripheral neuropathy, transient thrombocytopenia, fatigue, and gastrointestinal disorders (nausea and constipation

or diarrhoea). Phase II studies (SUMMIT, CREST) showed that treatment-emergent neuropathy was reported in 35% of patients (Richardson et al. 2006) whilst the frequency of bortezomib-associated peripheral neuropathy was evaluated in the phase III trial (APEX) with 37% of patients having treatment-emergent peripheral neuropathy, including 9% ≥ grade 3 (Richardson et al. 2009).

Vincristine

Vincristine is a vinca alkaloid; it is a mitotic inhibitor and exerts cytotoxicity via inhibition of tubulin polymerisation into microtubules, destabilising the mitotic spindle. Vincristine is a chemotherapeutic agent, which is part of the conventional chemotherapy, VAD. Vincristine is frequently associated with peripheral neuropathy (Haim et al. 1994; Verstappen et al. 2005). In rats, there was a high incidence of abnormal spontaneous discharge in A-fibre and C-fibres with confirmed vincristine evoked neuropathic pain (Xiao and Bennett 2008).

Neuronal consequences of myeloma chemotherpay

Apart from the sensory disturbances mentioned above, another less common complication of chemotherapy is autonomic dysfunction. The autonomic nervous system has two subsystems: sympathetic and parasympathetic. The afferent nerves from both systems transmit impulses from sensory organs, muscles, the circulatory system and all the organs of the body to the controlling centres in the medulla, pons and hypothalamus and often these impulses do not reach our consciousness, but elicit largely automatic or reflex responses via the parasympathetic and sympathetic nerves (Streeten 2012). Autonomic neuropathy is caused by damage to unmyelinated nerve fibres, which lead to clinical manifestations including cardiac conduction abnormalities, hypotension, impotence, and bowel and bladder dysfunction. The autonomic neurons are vulnerable as they are contained in ganglia, which lie outside the blood-brain barrier and are supplied by fenestrated capillaries that allow free passage of molecules between the extracellular fluid in the ganglia and the circulation (Windebank and Grisold 2008).

A recent immunofluorescence study using punch biopsies taken from the distal leg and thigh of three patients with relapsed MM treated with bortezomib alone or bortezomib and thalidomide showed marked reduction of both adrenergic and cholinergic autonomic fibres, innervating epidermal annexes predominantly at the

distal leg site (Giannoccaro et al. 2011). Interestingly, the cell bodies of both the autonomic and sensory systems lie outside the blood-brain barrier. It could be speculated as the autonomic system functions unconsciously therefore there must be sufficient neuronal loss to cause noticeable symptoms such as constipation or dizziness (Windebank and Grisold 2008). Nowadays, new anti-myeloma agents have increased the options available for patients in the relapse setting. However, the side effects of treatment limit the choices available for a number of patients.

1.1.3 Symptom burden in multiple myeloma

Patients with MM have the highest level of symptoms and the lowest level of QoL among patients with haematological cancers (Johnsen et al. 2009). This could be because these patients endure more bone pain and pathological fractures, increased fatigue and recurrent infections. Chemotherapy-induced peripheral neuropathy causes numerous debilitating symptoms, impairs functional capacity, and results in dose reductions or possible cessation of chemotherapy (Bhagra and Rao 2007). Unfortunately, the neuropathy is usually only partly reversible and in the worst cases it is completely irreversible. In addition to physical problems that accumulate in patients with MM, psychological and social factors impact on overall QoL (Larsen et al. 2003; Gulbrandsen et al. 2004; Sherman et al. 2004; Straus et al. 2006). A recent qualitative study demonstrated the impact of MM on patients' and caregivers' emotional, role, social and work-related areas of life in 20 `survivors` of MM (Molassiotis et al. 2011). Psychological problems included those associated with cognition and learning, social relationships, compromised education and work opportunities, and sexual and family relations. Although QoL has been explored previously in relation to specific treatments, including transplantation (Larsen et al. 2003; Gulbrandsen et al. 2004; Sherman et al. 2004; Straus et al. 2006), there is little information relating to the overall impact of modern clinical management strategies in patients with MM, particularly in the context of co-morbidities of an ageing population (Klepin and Hurd 2006). Furthermore, compared with some other cancers with prolonged survival, relatively little is known in MM about psychosocial and broader holistic needs, particularly with modern clinical management strategies (Mols et al. 2005).

1.2 Pain

'Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' which is always subjective (Merskey 2002). Pain is an essential early warning sign that helps protect us from the dangerous environment and that is why the sensation of pain is unpleasant so that we cannot ignore it. Acute pain is usually transitory and lasts until the noxious stimulus is removed or the principal damage or pathology has healed; it usually responds to with analgesic medications and treatment of the precipitating cause. However, some painful conditions persist beyond healing time and this leads to chronic pain. There is a range of chronic clinical pain conditions and usually they are categorised by their site and type of injury. Plastic changes can occur place in the periphery, spinal cord and brain following injury and this may increase the degree of the perceived pain and may play a role in the development of chronic pain (Petersen-Felix and Curatolo 2002). This pain is mediated by high-threshold peripheral and central neurons, which respond to noxious stimuli. The transmission and modulation of painful stimuli are mediated through the nociceptor neurons (Aβ, Aδ and C fibres), the spinal cord and the brain processes.

There are four elements to the pain process. Initially, *transduction* occurs when pain fibres are activated due to a noxious stimulus, which leads to depolarisation of nociceptors. This conversion of membrane potentials to action potentials is called transformation. The stimulus is then transmitted via sensory nerve fibres to the spinal cord through the dorsal horn and this is known as *transmission*. This leads to direct activation of motor neurons, restricting movement, which is protective. After transmission to the second order neuron, the pain stimulus is transmitted via the spinothalamic tract to the thalamus. Descending pathways control transmission by either inhibiting or enhancing it and this is known as *modulation*. Pain *perception* is processed in the higher brain cortex and this leads to the knowledge of and reaction to pain.

The somatosensory system consists of the modalities of nociception, mechanoreception, proprioception, thermoreception and visceroception providing awareness of sensory information from the skin, the viscera and the musculoskeletal system (Cruccu et al. 2008).

1.2.1 Nociception

Nociception is described as the neural processes of translating and processing noxious stimuli (Loeser and Treede 2008). Nociception protects us to prevent injuries by producing a reflex withdrawal from the noxious stimulus and an unpleasant sensation resulting in behavioural approaches to evade future interaction with such stimuli (Latremoliere and Woolf 2009). Around 90% of all afferent fibres in cutaneous nerves are nociceptive. These fibres are termed Aβ, Aδ and C fibres. C fibres form the largest group; around 90% of these are found in cutaneous nerves. The Aβ fibres are specialised mechanoreceptors for discriminative touch, vibration and proprioception. The Aδ and C fibres respond to noxious stimuli which could be mechanical, chemical or thermal (usually above approximately 45°C). The diameter of axons of sensory neurons is directly correlated to the speed of transmission of pain and also whether the neurons are myelinated; C fibres have small diameter unmyelinated axons bundled in fascicles surrounded by Schwann cells whist initial fast-onset pain is mediated by myelinated A-fibre nociceptors (Djouhri and Lawson 2004). Peripheral nerves also contain efferent fibres to the sympathetic nervous system. The signals from the nociceptors are then transmitted to the spinal cord to contribute to motor and sympathetic reflexes. These signals are also processed in the brain where the pain related signals are relayed to the cerebral cortex which is responsible for the recognition and localisation of pain and also determines the motivational-affective components of the pain perception (Lautenbacher and Fillingim 2004). Nociceptors have their cell bodies in dorsal root ganglia (DRG) and the cell body gives off a single process, which, divide into a peripheral and a central branch. The distribution of a peripheral nerve is followed by the peripheral branch of each nociceptive DRG cell. The terminals of nociceptors innervate a target organ. The central branch enters into the spinal cord and transmits sensory information to the central nervous system (CNS) (Bingham et al. 2009) (Figure 1.1). The grey matter of the spinal cord is divided into lamina from posterior to anterior (lamina I-IX). Afferent fibres transmitting cutaneous sensations end principally in laminae I to VI. Proprioceptive impulses reach laminae V and VI and these laminae also receive fibres from the cerebral cortex (Khurana and Arushi 2009). More specifically, most nociceptive Aδ and C fibres terminate superficially in laminae I–II, and a smaller number reaches deeper laminae, whereas Aβ fibres predominantly terminate in laminae III–VI (D'Mello and Dickenson 2008).
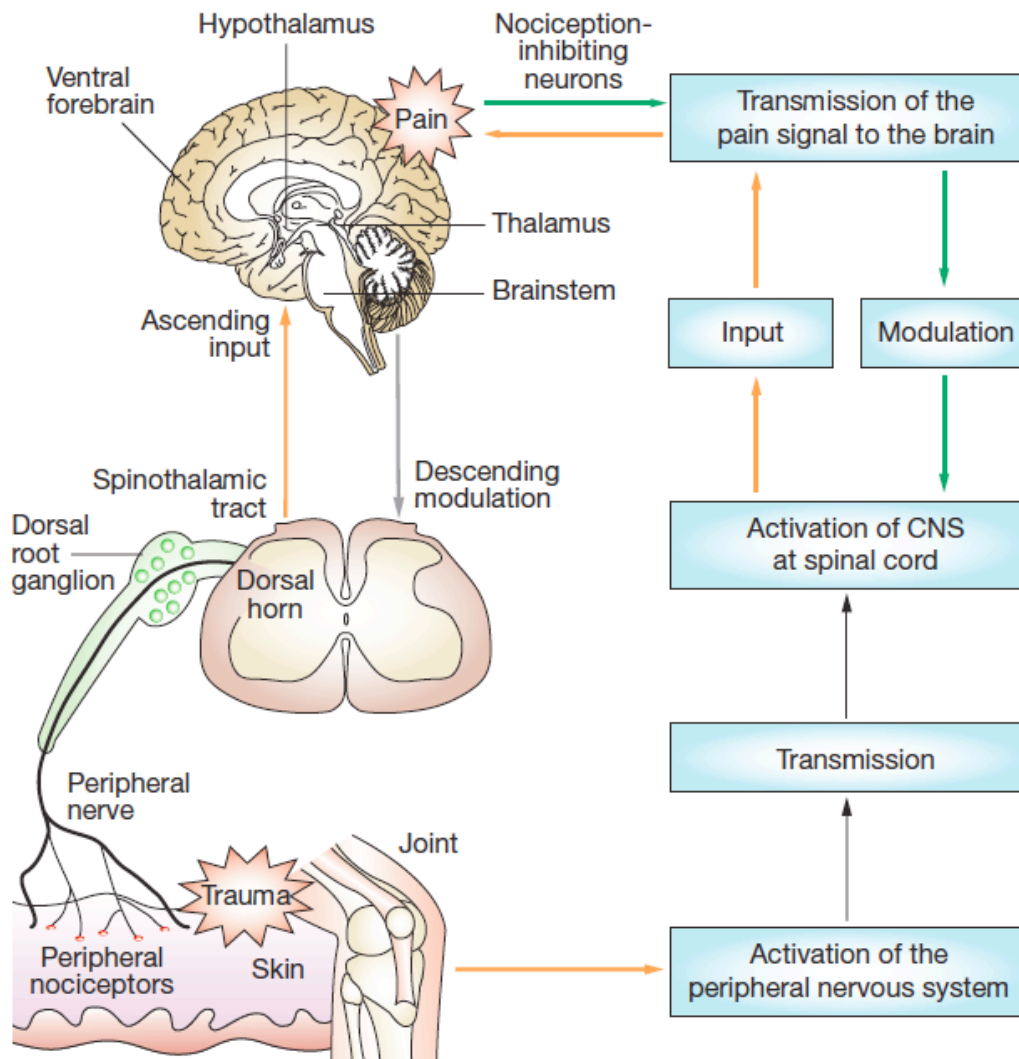
Figure 1.1: The pain pathway.

Nociceptor activation by noxious stimuli produces impulses that travel to the dorsal horn and then are transmitted via the ascending pathway to the thalamus and cortex.

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1.2.2 Ascending pathways

Second-order neurons send their sensory inputs to the brain via the ascending pathways (Table 1.1). The dorsal column (medial-lemniscal system) includes the fasciculus gracilis, fasciculus cuneatus, and medial lemniscus, which relay fine discriminative tactile sense, position sense, and vibratory sense. The anterolateral system includes the spinotectal, spinothalamic, spinohypothalamic, spinoreticular and spinomesencephalic tracts. These tracts convey chiefly pain and temperature sensation, together with pressure, non-discriminative touch, and proprioceptive

sensation, which is involved in pain transmission. The somatosensory pathways to the cerebellum, which include the anterior, posterior, and rostral spinocerebellar, and the cuneocerebellar tracts, transmit mainly proprioceptive (but also some pain and pressure) information (Patestas and Gartner 2009) (Figure 1.2) The body's interaction with the environment and the body's condition are sensed by the ascending sensory pathways which relay to the brain.

Table 1.1: Ascending sensory tracts
Ascending sensory tracts, their anatomical tracts and which modalities of sensation they transmit.

Ascending sensory pathways	Anatomical tracts	Functional component
Dorsal column – medial lemniscal	Fasciculus gracilis Fasciculus cuneatus	Discriminative fine touch, vibratory sense, position sense
Anterolateral	Spinothalamic Spinoreticular Spinotectal Spinohypothalamic Spinomesencephalic	Pain, temperature, nondiscriminative touch, pressure, proprioceptive sensation
To cerebellum	Anterior, Posterior, and Rostral spinocerebellar Cuneocerebellar	Proprioception, some pain and pressure

At the level of the spinal cord, sensory incoming nociceptive signals can sometimes be prevented from reaching the CNS at the synaptic level of the spinal cord dorsal horn. Pain is filtered in the substantia gelatinosa of the dorsal horn grey matter. The gate-like mechanism, which inhibits or facilitates afferent impulses into the spinal cord before it causes pain awareness and response, is known as the gate control theory of pain (Melzack and Wall 1965). Nowadays, the pain experience is known to be much more complex and does not only involve noxious stimuli. C fibres and Aδ fibres transmit impulses in the substantia gelatinosa thus inhibiting the inhibitory interneuron and activating the second order spinothalamic tract neuron that projects to the thalamus to keep the gate open. Pain relief is obtained when the inhibitory interneuron is activated, e.g by rubbing, therefore stimulating the Aδ/Aβ fibres which could lead to inhibition of some nociceptive transmission to the CNS (Wallace 2005).

1.2.3 Descending pathways

Once the pain impulses reaches the brain, descending pathways projecting from cerebral structures such as rostral ventromedial medulla (RVM), periaqueductal grey (PAG), hypothalamus, dorsal reticular nucleus, in the medulla, to the dorsal horn are sent which could either suppress (descending inhibition) or potentiate (descending facilitation) passage of nociceptive messages to the brain (Millan 2002) (Figure 1.2). Although this is not completely understood, modulation results in descending inhibition of nociception by the release of neurotransmitters such as norepinephrine and endogenous opioids. Modulatory processes can also increase descending facilitation of nociception through multiple chemical mediators including glutamate and aspartate and this consequently leads to pain. This results in increased synaptic strength and facilitatory output for pain expression and behavioural hyperalgesia. Serotonin plays both an inhibitory and facilitatory action. Serotonin is important in descending facilitation pathways to the spinal cord, however in certain areas of the dorsal horn, it can act via excitatory receptors to facilitate neurotransmitter release (Stahl 2008).
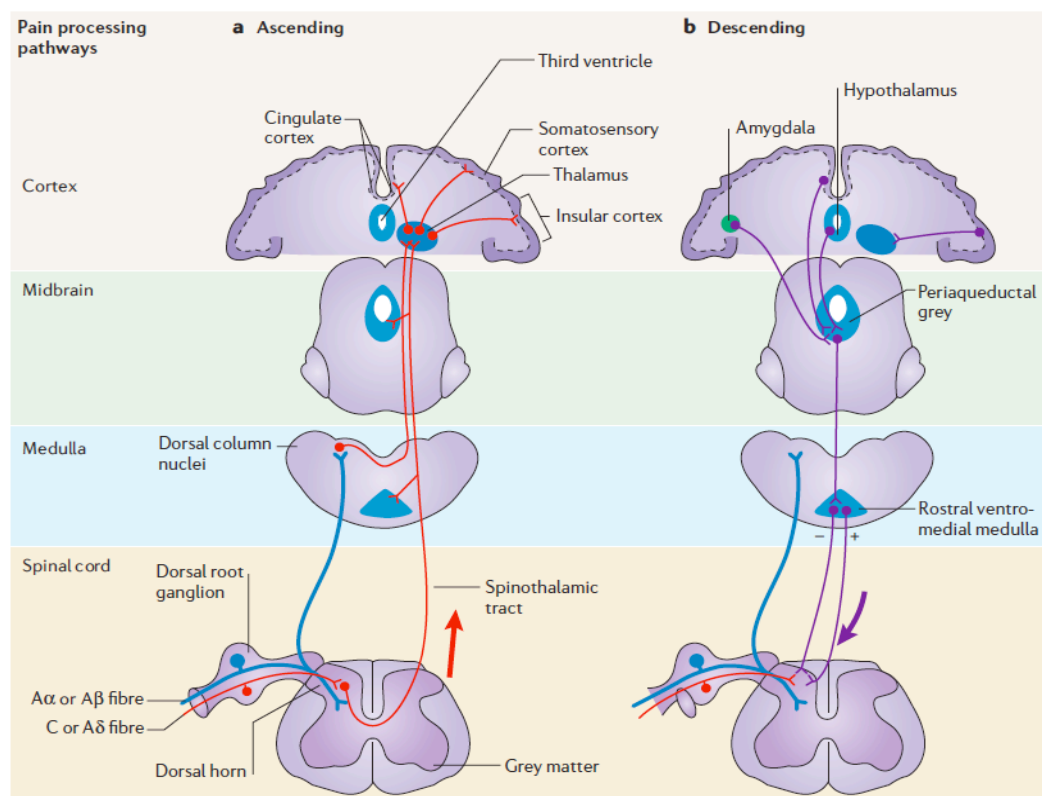
Figure 1.2: Transmission and modulation of somatosensory information
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There is increasing evidence that glial cells, mainly astrocytes and microglia, play an important role in chronic pain. Astrocytes are the most abundant glial cells, whilst microglia are resident macrophage-like cells in the CNS. In pain free states, these glial cells are at rest, however during injury/disease, these cells become active and contribute in the pathogenesis of chronic pain by astrocytes forming networks and leading to activation of intracellular signalling pathways promoting neuronal-glial interactions, which can enhance pain states (Gao and Ji 2010).

1.2.4 Pain processing in higher levels of the CNS

Once a noxious stimulus reaches the brain, there is activation of a specific network of brain regions which is associated with the perception of pain (Tracey et al. 2000). The PAG located around the cerebral aqueduct within the tegmentum of the midbrain, receives input from ascending spinal pathways and sends projections to thalamic nuclei that process nociception. The anatomical and physiological organisation of the PAG and its descending projections to the RVM and dorsal horn of the spinal cord have been well described in a range of species (Loyd and Murphy 2009). Some of the functions of the midbrain PAG include pain, analgesia, fear, anxiety and vocalisation. With regards to pain, the PAG receives afferents from nociceptive neurons in the spinal cord and transmits impulses to thalamic nuclei that process nociception (Behbehani 1995). The PAG may be an important area that controls peripheral nociceptive perception by descending activity from higher cortical regions (Tracey et al. 2002). In a functional magnetic resonance imaging (fMRI) study on nine healthy volunteers, heat stimulus was applied to their hand and subjects were asked to either focus on or distract themselves from the painful stimuli. When the subjects distracted themselves from the pain, the pain intensity was significantly lower with a corresponding increased activation in the PAG during the distraction condition, suggesting that the PAG is a site for higher cortical control of pain modulation in humans (Tracey et al. 2002).

The thalamus, the largest component of the diencephalon, receives and processes all nociceptive information that is relayed to the cortex. Anatomical studies have demonstrated that ascending sensory spinal pathways terminate within the thalamus before higher order sensory projections are sent to the somatosensory cortex (Wilson et al. 1999). Thalamic nuclei contain many inhibitory interneurons that can modulate the transmission of signals through the thalamus, making filtering an important thalamic function (Swenson 2006). The thalamus is not only a sensory

relay station but also regulates and processes the information that is presented to the cortex (McCormick and Bal 1994). The brain stem, the cortex, and the thalamic reticular nucleus influence the modulation of the thalamus, allowing it to modulate transmission to the cortex in accord with current attentional needs (Sherman and Guillery 2002). The representation of body surface in the form of somatotopic maps is a fundamental feature of somatosensory organisation. Such maps have been demonstrated at all levels of the somatosensory system including the thalamus in many mammalian species.

The basal ganglia consist of the striatum (putamen, caudate nucleus and nucleus accumbens), the globus pallidus, the subthalamic nucleus, and the substantia nigra. They are involved in neuronal pathways involving motivational, associative, emotional and cognitive functions and are a major site for adaptive plasticity in the brain, affecting a broad range of normal behaviours, neurological and psychiatric conditions (Borsook et al. 2010). Basal ganglia lesions have helped us to understand the possible role of basal ganglia in pain and analgesia. In patients who develop infarction of the putamen and globus pallidus, this could result in sensory deficits including pain (Russmann et al. 2003). Efferent pathways from the basal ganglia project to frontal lobe areas including prefrontal, premotor and supplementary motor areas. The basal ganglia have influences on cortical regions involved in motor responses, in performances relating to predicting events, and also play a part in learning and attention (Herrero et al. 2002).

1.2.5 Sensitisation: Peripheral & Central

Following an injury, whether due to inflammation or a nerve injury, hyperalgesia can occur due to an increase in the sensitivity of primary afferent nociceptors in the vicinity of the injury (peripheral sensitisation), and from an increase in the excitability of neurons in the spinal cord (central sensitisation) (Woolf and Thompson 1991). Once an injury has occurred, the Aδ and C fibres become sensitised and have a lower threshold for being activated. The injury also causes a release of inflammatory mediators like prostaglandin, bradykinin, serotonin which lowers the threshold for nociceptive stimuli. The depolarized nociceptive sensory endings release substance P and calcitonin gene related peptide (CGRP), thus contributing to the spread of oedema by producing vasodilatation, an increase in vascular permeability, and the spread of hyperalgesia by leading to the release of histamine from mast cells. The reaction produced by the noxious stimulus may outlast the stimulus (as in chronic

pain) and this can lead to alterations in cytokine and neurotrophin production, and receptor channels. Changes also occur in the dorsal horn due to sustained neuronal activation producing wind-up, early gene induction, neurotrophin production, and the induction of neurotransmitter and receptor synthesis (Hill 2001).

In central sensitisation, there is increased neuronal activation caused by increased membrane excitability and reduced inhibition. This is a demonstration of the plasticity of the somatosensory nervous system in response to inflammation and neural injury (Latremoliere and Woolf 2009). During central sensitisation, neurons in the dorsal horn display changes such as spontaneous activity, a decrease in the threshold for activation by peripheral stimuli, and an increase in their receptive fields (Latremoliere and Woolf 2009). At the synaptic level, glutamate, an excitatory neurotransmitter of primary afferent neurons, binds to several receptors on postsynaptic neurons in the dorsal horn, including ionotropic amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), N-methyl-D-aspartate (NMDA), and kainate receptors and metabotropic (G-protein coupled) glutamate receptor subtypes (Latremoliere and Woolf 2009). Normally, the NMDA receptor is blocked by a magnesium ion in its pore. When activated nociceptors release glutamate and the neuropeptides Substance P and CGRP that, leads to membrane depolarisation that forces magnesium to leave the NMDA receptor pore, allowing glutamate to bind to the receptor. This generates an inward current and allows entry of calcium in the neuron, leading to activation of intracellular pathways, which contributes to the maintenance of central sensitisation (Figure 1.3). Substance P, which is co-released with glutamate at the nociceptor pre-synaptic terminus, also plays a part in the generation of central sensitisation. It binds to the neurokinin-1 G-protein–coupled receptor and causes a prolonged membrane depolarisation, thus contributing to the temporal summation of C fibre–evoked synaptic potentials as well as to intracellular signalling. CGRP potentiates the effects of substance P and participates in central sensitisation through postsynaptic CGRP1 receptors, which activate protein kinase A and C. Calcium influx leads to activation of intracellular kinases which phosphorylate ionotropic NMDA and AMPA glutamate receptors increasing their activity and producing the changes that manifest as central sensitisation. This effect is further potentiated by nitric oxide synthesised by either neuronal or inducible nitric oxide synthases in the dorsal horn (Latremoliere and Woolf 2009).

Normally, inhibitory interneurons continuously release GABA and glycine to decrease the excitability of the output neurons and modulate pain transmission.

However, in central sensitisation, this inhibition can be lost, further increasing the potentiation of the nociceptor signal.
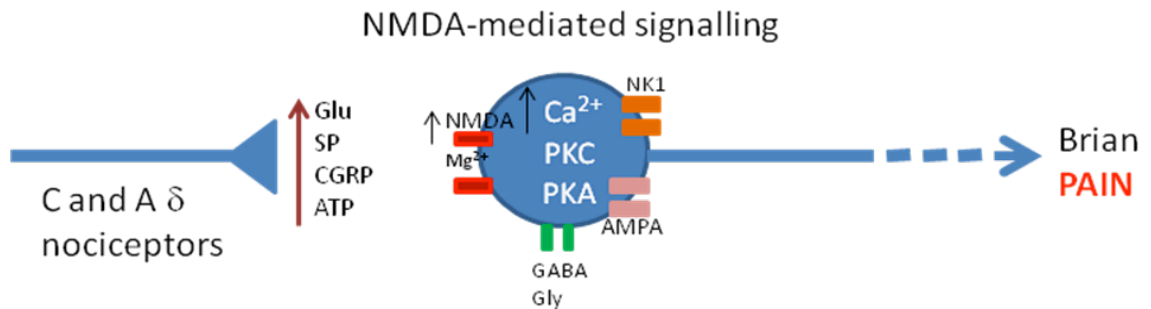
Figure 1.3: Central Sensitisation - Glutamate/NMDA receptor-mediated

After injury, activated C and Aδ nociceptors release a variety of neurotransmitters into the synapse, thus activating NMDA receptors, which release Mg^{2+} leading to increase intracellular calcium. This activates calcium-dependent signalling pathways and second messengers causing an increase in the excitability of the output neuron and facilitating the transmission of pain messages to the brain.
Glu - glutamate, SP - substance P, CGRP - calcitonin-gene related peptide, ATP – adenosine triphosphate, PKC - protein kinase C, PKA - protein kinase A, GABA - Gamma-Aminobutyric acid, Gly - Glycine
Reprinted and adapted from Cell, Vol 139(2), Basbaum, A. I., et al, "Cellular and molecular mechanisms of pain." Pages 267-284, Copyright (2009), with permission from Elsevier

1.2.5.1 Central sensitisation in pathological states of the CNS

Central sensitisation contributes to the longer-lasting, and sometimes persistent, pain hypersensitivity present in pathological damage to the nervous system. In chronic pain, due to either peripheral nerve or CNS damage, there can be recruiting of central hypersensitivity pathways, even in the absence of peripheral contributions. Large Aδ sensory fibre damage may lead to allodynia where gentle touch may be perceived as painful. Furthermore, prolonged sensory input to the dorsal horn of the spinal cord may cause excitotoxic death of inhibitory interneurons, thus further increasing the excitability of nociceptive transmission (Hill 2001). Sensory neurons in the DRG and dorsal horn undergo changes in transcription that alter their growth, membrane properties and transmitter function (Figure 1.4). Peripheral nerve injury leads to a degeneration of C fibre terminals in lamina II causing loss of presynaptic input and triggering intrinsic axonal growth capacity as part of the regenerative response thus providing a chance for myelinated Aβ fibres to sprout from laminae III-IV into laminae I-II and make contact with nociceptive specific neurons (Latremoliere and Woolf 2009). There is a reduction in the release, activity and synthesis of the inhibitory transmitters which leads to a state of disinhibition (Sivilotti

and Woolf 1994). In neuropathic pain, after peripheral nerve injury, damaged and non-damaged A and C fibres begin to generate spontaneous action potentials. In neuropathy, apoptosis of inhibitory interneurons could be attributed to the disinhibition in the superficial dorsal horn with loss of GABA and a reduction in glycinergic inhibitory currents (Moore et al. 2002). The decending pathways also exacerbate this dorsal horn excitation after peripheral nerve injury as there is an increase in descending excitatory activity from the brainstem, as well as a reduction of descending inhibitory controls (D'Mello and Dickenson 2008).

Changes in the brain also occur. This has been demonstrated in rodents after partial peripheral nerve injury where sensitised neurons are detected in the thalamus and primary somatosensory cortex (Guilbaud et al. 1992). Activation of the brain during neuropathic pain compared to nociceptive pain can be classified by two fundamental phenomena. Firstly, there is increased activity in primary nociceptive areas, classical areas of the pain matrix (more details of the pain matrix in section 1.4.7.2), and secondly, there is recruitment of cortical areas beyond the classical pain matrix (Seifert and Maihofner 2009). In chronic pain, there are also changes occurring in the brain volume, mainly being reported in anterior cingulate cortex, orbitofrontal cortex, insular cortex and dorsal pons which appears to be partially reversible when the pain is treated (Kuner 2010). Neuroimaging has demonstrated changes in the somatosensory cortical representation and excitability in patients with phantom limb pain (Flor et al. 1995; Willoch et al. 2000) and in complex regional pain syndrome (Maihofner et al. 2005).
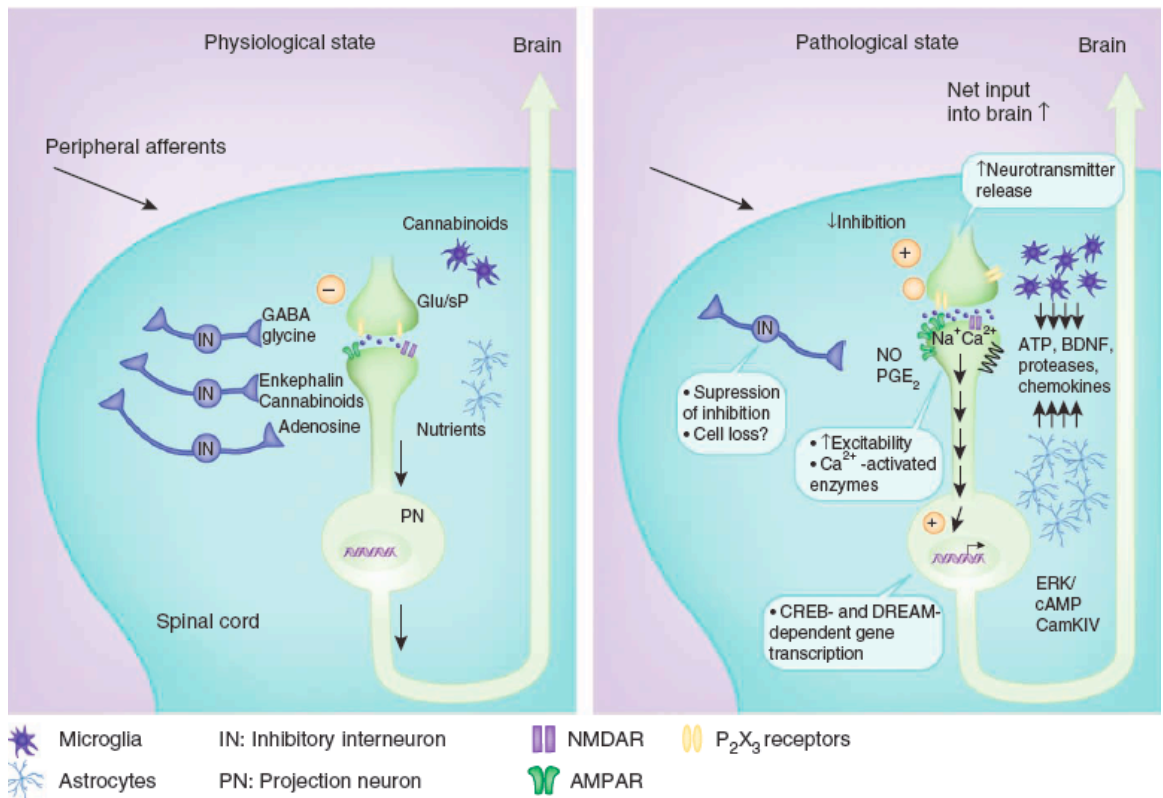
Figure 1.4: Mechanisms during physiological and pathological state in the spinal cord dorsal horn.

In the pathological state, changes occur including suppression of inhibition, potentiation of presynaptic release and postsynaptic excitability, gene transcription, release of neuromodulators from activated microglia and astrocytes and a net increase in nociceptive input onto higher brain structures.

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Activation of the glia in the spinal cord has been suggested to lead to pathological pain such as neuropathy. This occurs by the release of neuronal signaling molecules such as cytokines and chemokines thus causing neurotoxicity, neuronal hyperexcitability and chronic inflammation (Milligan and Watkins 2009).

1.2.6 Neuropathic pain

Neuropathic pain has been described by the special interest group on neuropathic pain of the International Association for the Study of Pain as 'pain arising as a direct consequence of a lesion or disease affecting the somatosensory system' (Treede et al. 2008). Neuropathic pain is a syndrome caused by a variety of different lesions and diseases, which manifests as a range of signs and symptoms and there are multiple underlying mechanisms of these different conditions (Jensen et al. 2011). The clinical key findings in neuropathic types of pain are spontaneous, continuous and paroxysmal pains with allodynia or hyperalgesia (Figure 1.5). Patients with chronic pain may express different combinations of these symptoms. Since neuropathic pain arises due to a disease or a lesion affecting the somatosensory system, its diagnosis is based on the demonstration of abnormal function in such systems, including negative (hypoesthesia and hypoalgesia) and positive sensory phenomena (allodynia and hyperalgesia) (Moisset and Bouhassira 2007).
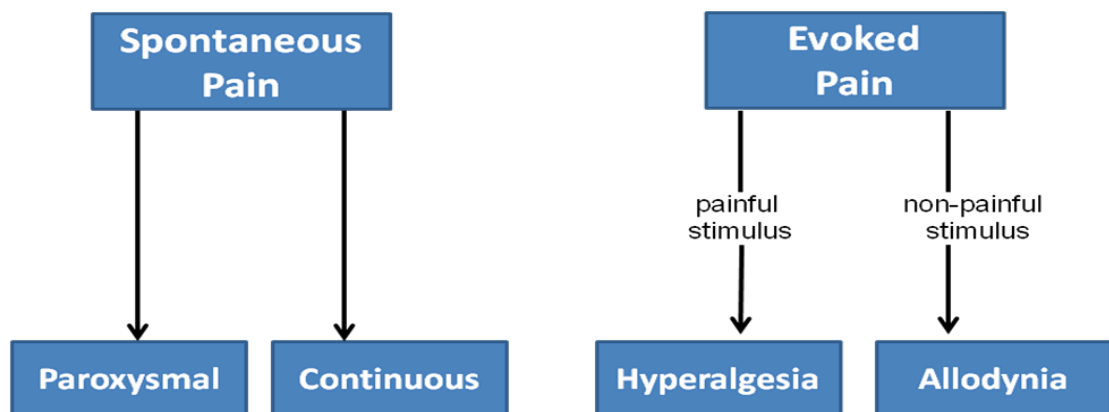
Figure 1.5: Symptoms in neuropathic pain

Reprinted from NeuroImage, Vol 37, Suppl 1, Xavier Moisset and Didier Bouhassira, Brain imaging of neuropathic pain, S80-88, Copyright (2007), with permission from Elsevier. (Moisset and Bouhassira 2007).

Epidemiological surveys indicate that 6-8% of the general population report chronic pain with neuropathic characteristics and these patients usually report poorer physical and mental health compared with patients with other types of chronic pain, even when adjusting for pain intensity (Freynhagen and Bennett 2009). In a large European study, it was shown that moderate to severe chronic pain occurs in 19%

of adults; nearly half do not receive proper pain management and their pain is very detrimental to their social functioning and working abilities (Breivik et al. 2006). In a postal survey of the general population carried in France, the reported prevalence of neuropathic characteristics in chronic pain respondents was 6.9% (95%CI: 6.6‑7.2), which was moderate to severe in 5.1% (95%CI: 4.8‑5.4) (Bouhassira et al. 2008). In a similar postal survey of the general population in the UK, the prevalence of pain, which was predominantly neuropathic in origin, was 8% and these people had significantly greater pain intensity, higher levels of expressed need, and a longer duration of pain (Torrance et al. 2006). In both UK and French surveys, the results demonstrated that the people who had chronic pain of neuropathic origin showed impairments relating to QoL and sleep and had higher anxiety and depression scores as assessed by the SF36 and SF12 (Smith et al. 2007; Attal et al. 2011). A recent systematic review showed that that the prevalence of cancer patients with neuropathic pain varied from about 20% to approximately 40% when mixed pain as well as pure neuropathic pain were included (Bennett et al. 2011). Neuropathic pain is often not diagnosed and when it is it often inadequately treated, and is accompanied by disability, poor QoL, distress and increased cost to the healthcare system (Haanpää and Treede 2010).

To diagnose neuropathic pain, assessment of the pain is required by undertaking a detailed history taking and clinical examination (and including bedside tests of somatosensory functions: touch, vibration, cold, warmth and pain sensibility as well as motor and autonomic signs). It is also important to localise the lesion by performing a neurological examination. The International Association for the Study of Pain developed a grading system for a clinical diagnosis of neuropathic pain (Haanpaa et al. 2011) (Figure 1.6).

Hypothesis: Is it likely that the pain is neuropathic in nature?
(Does the distribution of the pain relate to an anatomical site; does the history propose an appropriate disease or lesion?)

↓

Tests to assess and confirm

A: Sensory signs (negative or positive), in the region of the damaged nervous structure (either in bedside sensory examination or in quantitative sensory testing)

B: Diagnostic test confirming a lesion or disease explaining neuropathic pain (peripheral and central nervous system)

→ *Absence of A and B* Unconfirmed as neuropathic pain
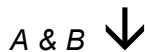
A & B ↓   *A or B* ↓
Confirmed neuropathic pain   Probable neuropathic pain
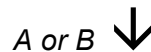
Figure 1.6: Flow chart of a grading system for neuropathic pain
Adapted from (Haanpaa et al. 2011)

Screening tools are used to identify patients with possible neuropathic pain, particularly when used by non-specialists. They are easy to use both by the patients and the health care professionals in clinics or via the telephone. These provide immediate information. About 10–20% of patients with a clinical diagnosis made by a physician of neuropathic pain are not identified when screening tools are used and therefore, screening tools may offer guidance for further diagnostic evaluation and pain management but cannot replace clinical judgment (Haanpaa et al. 2011). Screening tools will be discussed later in section 1.3.6.

Neuropathic pain is usually long lasting; however, some patients recover from this pain completely, and others may learn coping skills and might find relief with medications. This next section will now focus specifically on neuropathy caused by chemotherapy.

1.3 Chemotherapy-induced peripheral neuropathy

Chemotherapy-induced peripheral neuropathy (CIPN) constitutes major dose-limiting side effects of chemotherapy and is one of the most severe and unpredictable side effects of modern anticancer treatment. The incidence of CIPN varies considerably from 10 to 100% and the characteristics of CIPN are related to the type of chemotherapy used, the treatment schedules, dose intensity and cumulative dose (Balayssac et al. 2011). Another reason for such variability in the incidence is the way data is collected in studies when looking for neurotoxicity, as there is no standard measurement with regards to tools or sensory testing for CIPN (Dunlap and Paice 2006). Studies using detailed neurophysiological testing, or scoring systems report higher incidence of CIPN compared to studies that only use routine evaluation (Delforge et al. 2010).

With the use of haematopoietic factors that limit haematotoxicity, higher doses of anticancer drugs can be given in combination therapy, which increases the risk of further neurotoxicity (Chaudhry et al. 1994). Different chemotherapy classes target different components of the peripheral nervous system (PNS) including neuronal cell bodies in the DRG, mitochondria, axonal transport pathways, axonal membrane ion channels and calcium regulation (Park et al. 2008) and can cause pure sensory, mixed sensory motor or autonomic neuropathies. The difference of the anti-cancer drug sensitivity between the central and the peripheral nervous system relates to the CNS having a less permeable blood brain barrier compared to the blood-nerve barrier protecting the PNS.(Balayssac et al. 2005). The PNS is therefore more likely than the CNS to be affected by drug neurotoxicity. The incidence of neuropathy increases if certain co-morbidities such as diabetes, vitamin deficiencies or excessive alcohol intake already exist.

Chemotherapy-induced peripheral neuropathy may occur either early in the course of chemotherapy or after repeated courses. Sometimes, CIPN severity can progress months after the discontinuation of anti-myeloma therapy, a phenomenon known as coasting (Cavaletti et al. 2011); up to 8% of MM patients exhibit coasting when treated with bortezomib (Farquhar-Smith and Wigmore 2011).

In the next section, specific classes of anticancer drugs specifically related to MM will be discussed and the aforementioned three common drugs that are used: thalidomide, bortezomib and vincristine (figures 1.7 – 1.9).
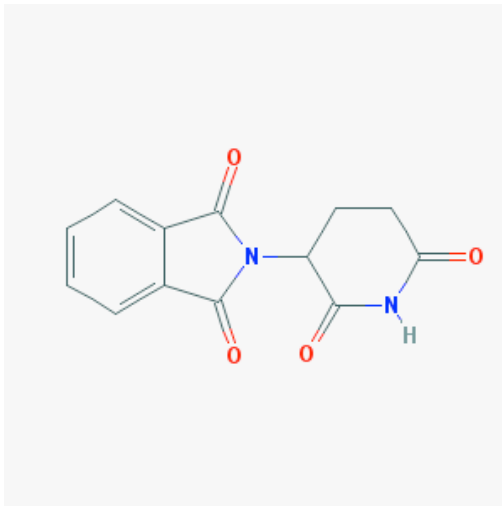
Figure 1.7: Schematic diagram showing the chemical structure of thalidomide

Thalidomide is an immunomodulatory agent. The empirical formula for thalidomide is $C_{13}H_{10}N_2O_4$ and the gram molecular weight is 258.2.

Image from Pub Chem (NIH)
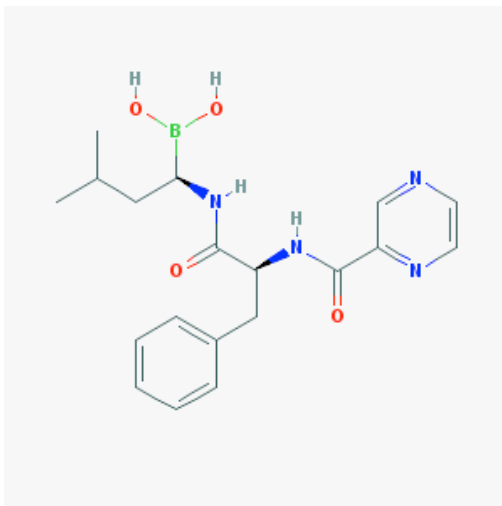
Figure 1.8: Schematic diagram showing the chemical structure of bortezomib

Bortezomib is an anti-neoplastic agent. The molecular formula is $C_{19}H_{25}BN_4O_4$ and the molecular weight is 384.24.

Image from Pub Chem (NIH)
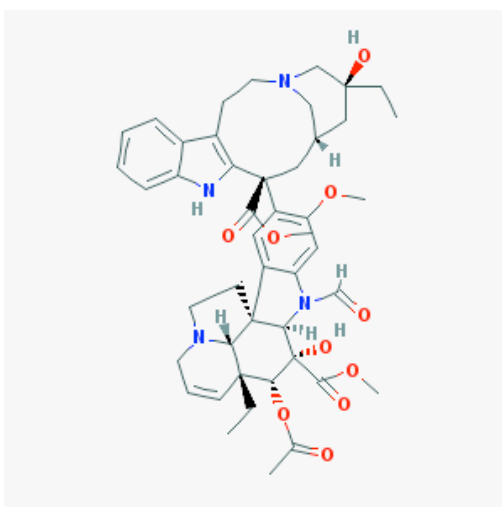
Figure 1.9: Schematic diagram showing the chemical structure of vincrisitine

Vincrisitine is the salt of an alkaloid obtained from a common flowering herb, the periwinkle plant. The molecular formula is $C_{46}H_{56}N_4O_{10} \cdot H_2SO_4$ with a molecular weight of 923.04

Image from Pub Chem (NIH)

1.3.1 CIPN in multiple myeloma

Peripheral neuropathy at the time of presentation in patients with MM is relatively infrequent, however those who present with established amyloidosis, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes) or following a nerve compression might present with neuropathy (Smith et al. 2006). Following the administration of modern anti-myeloma therapies, including thalidomide and bortezomib, an accumulating burden of peripheral neuropathy has been recognised (Smith et al. 2006; Richardson et al. 2009) and large clinical trials of novel agents using grading tools for screening CIPN have recognised peripheral neuropathy as a dose-limiting toxicity .

It has been shown that 70% of patients treated with thalidomide for 12 months will develop neuropathy (Mohty et al. 2010). By 14 months, 50% of patients who were receiving low doses of thalidomide (25-50mg daily) developed sensory neuropathy (Schiff et al. 2009). Clinically this usually presents as bilateral symmetrical sensory disorders and rarely as motor disorders. The sensory symptoms that patients experience includes stinging sensations or numbness that initially affect the toes, occasionally the fingers, and may also extend proximally. Later, the deep vibration sensitivity and proprioception may be affected, leading to progressive ataxia, difficulty in walking and trembling when posture is maintained. Whether there is a relationship between cumulative dose (Chaudhry et al. 2002; Cavaletti et al. 2004; Glasmacher et al. 2006) and duration of treatment with thalidomide (Tosi et al. 2005; Mileshkin et al. 2006) remains controversial and there are conflicting studies in the literature (Mohty et al. 2010).

With regards to bortezomib-induced peripheral neuropathy, the overall incidence ranges between 30% and 47%, with approximately 15% of cases having severe neuropathy (grade 3 to 4) (Bang et al. 2006). If CIPN occurs, this is usually by the fifth cycle of bortezomib treatment, and it tends to plateau by the eighth cycle (Schiff et al. 2009) however it can occur within the first treatment cycle.

CIPN is thus a common, potentially severe and dose-limiting side effect of treatment. Although some reversibility may be possible after dose reduction or cessation of specific agents, there is increasing recognition of the chronicity of peripheral neuropathy in many patients, not only compromising the administration of further anti-myeloma therapies but also generating long term and irreversible pain and disability. In addition, this frequently leads to the need for long-term administration of analgesic drugs with their associated side effect profiles. Overall

the result of developing peripheral neuropathy is reduced QoL, which significantly counters the recent achievements of improved life expectancy in this disease.

1.3.1.1 Current understanding of the pathophysiology of CIPN in multiple myeloma

The pathophysiology of CIPN is incompletely understood but might be caused by drug-induced damage to the dorsal nerve root ganglia neurons, axons, and/or Schwann cells of the peripheral nervous system. Structural damage to the peripheral nervous system results in abnormal somatosensory processing in the peripheral or central nervous system (Bhagra and Rao 2007). The concepts regarding the pathophysiology of neuropathic pain derived from experimental work in animal models and extrapolation to humans will be dependent upon model suitability. C and Aδ primary afferent neurons become abnormally sensitive and develop pathological spontaneous activity following a nerve injury, which leads to peripheral sensitisation. This triggers expression of sodium channels, the release of various receptor proteins, and growth factors from degenerating nerve fibres. This activity provokes secondary changes in central sensory processing, leading to spinal cord hyper-excitability and central sensitisation (Baron 2006).

Pain impulses, beyond the peripheral nociceptor and dorsal horn, ascends in the contralateral spinothalamic tract; there are connections to the medulla and brain stem via the spinoreticular and spinomesencephalic tracts and to the hypothalamus via the spinohypothalamic tract (Brooks and Tracey 2005). The spinal and intracranial pathways have so far not been thoroughly investigated in CIPN.

Chemotherapy-induced peripheral neuropathy arising from thalidomide is believed to be the result of axonal injury with progressive loss of large diameter myelinated fibres (Figure 1.10). In nerve electrophysiological studies (NES), the fundamental sign is a 50% decrease in the sensory nerve action potential (SNAP) amplitude, with relative conservation of nerve conduction velocities (Table 1.2) and this was shown in a study by Mileshkin et al who screened patients with relapsed/refractory MM in a trial of dose-escalating thalidomide with or without interferon and showed that patients had motor NES changes and often developed concurrent sensory changes, suggesting that thalidomide frequently causes a sensorimotor axonal neuropathy (Mileshkin et al. 2006). There is evidence to demonstrate that wallerian degeneration of the nerve fibres contributes to the development of neuropathic pain via production of cytokines like TNF-α and nerve growth factors (Xu et al. 2006).

Other mechanisms may be involved that include a decrease in nerve blood supply due to the anti-angiogeneic properties of thalidomide, direct toxic effects of thalidomide on neurons of the posterior root ganglia or dysregulation of neurotrophin activity through effects of thalidomide on nuclear factor-kappa B transcription factor (Mohty et al. 2010). Genetic variations in genes involved in drugs neurotoxicity have been shown to have a likely impact on whether the patient develops neuropathy (Johnson et al. 2008).

DORSAL ROOT GANGLION
Vincristine, bortezomib, thalidomide

MYELIN
Vincrisitine, bortezomib thalidomide

SMALL NERVE FIBRES
Vincristine, bortezomib

MICROTUBULES
Vincrisitine, bortezomib

MITOCHONDRION
Vincrisitine, bortezomib
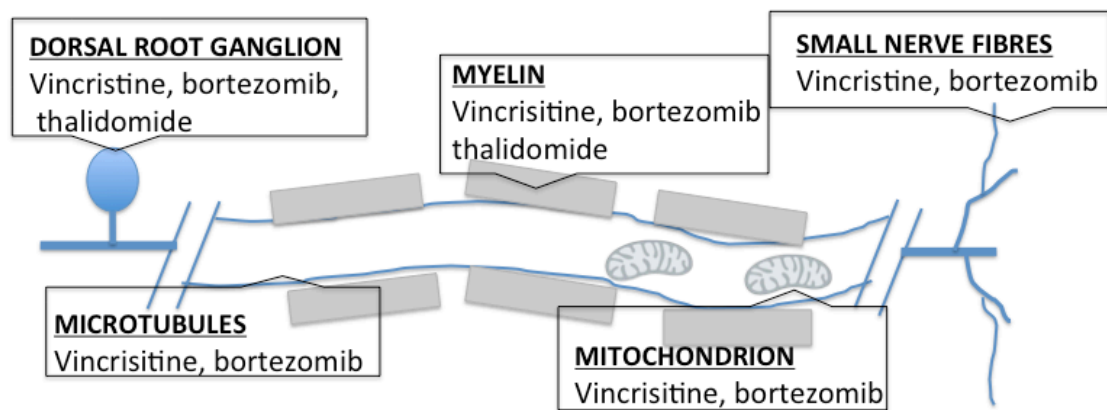
Figure 1.10: Chemotherapy-induced toxicity in the peripheral nervous system.

These are the specific sites where chemotherapy causes injury leading to neuropathy. Reprinted and adapted from Cytokine, Vol 59, Issue 1. Wang X. M., T. J. Lehky, et al, Discovering cytokines as targets for chemotherapy-induced painful peripheral neuropathy, Pages No. 3-9, Copyright (2012), with permission from Elsevier

Bortezomib, a highly selective reversible inhibitor of the 26S proteasome, shows antineoplastic effects against a variety of lymphoid disorders, including MM. It causes more sensory rather than a motor peripheral neuropathy (numbness, paresthesia, burning sensation, sensory loss, dysesthesia and pain) and affects the feet more than the hands (Mohty et al. 2010). A quantitative sensory study in patients with bortezomib-induced pain has shown these patients have significantly elevated touch detection threshold, impaired sharpness detection, and elevated thresholds for the detection of skin warming and heat pain; patients also had increased reports of cold pain. (Cata et al. 2007). This study also gives us insight to the refractory nature of chronic bortezomib-related neuropathic pain in that many of these patients had only partial pain relief despite different analgesics such as opioids and calcium channel blockers (Cata et al. 2007). Bortezomib-induced neuropathy is therefore associated with deficits in Aβ, Aδ and C primary afferent fibres. The present knowledge of the pathological features of bortezomib-induced

peripheral neurotoxicity is very limited. In a study by Cavaletti et al 2007, the effect of chronic bortezomib administration on the peripheral nervous system and on the spinal cord of rats was evaluated. Sciatic nerve examination and morphometric determinations demonstrated mild to moderate pathological changes, involving predominantly the Schwann cells and myelin, although axonal degeneration was also observed. Bortezomib-induced changes were also observed in DRG (Figure 1.10). Spinal cord was morphologically normal (Cavaletti et al. 2007). Another mechanism proposed is that there could potentially be a dysregulation of the neurotrophic factors since bortezomib inhibits the activation of the transcription factor, nuclear factor-kappa B and blocks the transcription of the trophic nerve growth factor (Mohty et al. 2010).

Vincristine acts by binding on intracellular tubulin, which interferes with axonal transport. It does not cross the blood brain barrier. It is thought that it induces alterations in the cellular microtubule structure in the peripheral nervous system (Figure 1.10) and this might be a mechanism for neuropathy (Polomano and Bennett 2001; Quasthoff and Hartung 2002). In an attempt to understand the potential mechanism of vincristine-induced pain in humans undergoing chemotherapy Aley et al, established a model of vincristine-induced hyperalgesia in rat. They injected rats with different doses of vincristine (20, 100 or 200µg/kg intravenous into a tail vein) and found that vincristine produced both acute and chronic effects on pain sensitivity (Aley et al. 1996). A study of patients receiving vincristine (two different dose intensities) for lymphoma (n=114) showed neuropathic changes in both groups; symptoms can begin two weeks after the first dose of vincristine, and the cumulative risk of long-term therapy was >75% (Verstappen et al. 2005).

Table 1.2: Electrophysiological findings following anti-myeloma therapy

Drug	Nerve conduction	Autonomic function tests
Thalidomide	SNAP & CMAP reduced; Sensory & motor NCV distally slowed (mild)	Rare autonomic involvement
Bortezomib	SNAP & CMAP reduced; Sensory & motor NCV distally slowed (mild)	Rare autonomic involvement
Vincristine	SNAP & CMAP reduced; Sensory & motor NCV distally slowed	Postganglionic sympathetic & parasympathetic loss of function with gastrointestinal dysmotility & orthostatic hypotension, urogenital dysfunction

SNAP, sensory nerve action potential; CMAP, compound muscle action potential; NCV, nerve conduction velocity

1.3.2 Predisposing factors for CIPN

It is not clear what the predisposing factors for developing neuropathy in patients receiving neurotoxic agents are and studies have shown contradictory results partly because the toxicity was measured using different assessment scales. In trials using bortezomib, the consistent risk factor for development of neuropathy was a previous history of peripheral neuropathy. On the other hand, age, history of diabetes, disease stage and creatinine clearance did not affect the incidence whilst in the relapsed patients, prior treatment with agents known to cause neuropathy did not appear to affect the incidence of neuropathy (Richardson et al. 2009; Dimopoulos et al. 2011). The myeloma itself may be an important causative factor as patients with newly diagnosed MM may have signs and symptoms of neuropathy and these patients may develop more severe neuropathy with neurotoxic agents (Richardson et al. 2006; Badros et al. 2007; Richardson et al. 2009). In the SUMMIT and CREST trials, prolonged exposure to bortezomib did not increase the incidence or severity of the neuropathy (Richardson et al. 2006). With thalidomide, in the Total Therapy 2 study, the incidence of neuropathy was higher in patients over the age of 65 (41% vs. 17%, $p<0.001$) (Barlogie et al. 2006). In other studies, the risk was not influenced by age, sex and prior therapy including the use of vincristine (Tosi et al. 2005; Mileshkin et al. 2006). Factors that seem to increase the incidence and degree of neuropathy with vincristine include large and frequent doses of the drug,

age (infants and adults appear to be mostly affected), patients with obstructive liver disease (might be potentiated by delayed biliary excretion as this is the main route of vincristine elimination) together with underlying neuropathy and malnutrition (Gidding et al. 1999).

1.3.3 Reversibility of CIPN

When bortezomib induced peripheral neuropathy develops, it is at least partially reversible in >50% of patients, particularly if the dose adjustment recommendations are adhered to (Mohty et al. 2010). In the VISTA trial, 60% of neuropathy due to bortezomib was completely resolved within a median of 5.7 months whilst in the APEX trial, 64% of patients with grade ≥2 bortezomib induced peripheral neuropathy experienced improvement or resolution within a median of 3.6 months (Richardson et al. 2011). There are mixed reports for the reversibility of thalidomide induced peripheral neuropathy. If sensory neuropathy develops with thalidomide, it is suggested that thalidomide is either stopped or there is a dose reduction as the symptoms can often worsen and become irreversible (Mohty et al. 2010). Neurotoxic symptoms and signs disappear weeks to months after vincristine therapy has been discontinued however reports that in one third of retrospectively studied adult patients, sensory neuropathy was found up to 77 months after therapy was stopped and subjective complaints existed for up to 40 months [100].

1.3.4 Current diagnosis and assessment of CIPN in myeloma

Currently the diagnosis of CIPN in MM patients is largely clinically based on history and physical examination. Blood tests are aimed largely at excluding other causes of neuropathy. Diagnosis of neuropathic pain is made when the distribution of pain and the associated sensory abnormalities, together with a history of chemotherapy known to cause neuropathy are all present.

1.3.5 Quantitative measures of CIPN: Laboratory testing

Laboratory testing mainly includes *quantitative sensory testing* (QST): a psychophysical technique requiring co-operation from the patient measuring:

- warmth: a C fibre–mediated sensation,

- cooling: an Aδ-mediated sensation,

- vibration: a sensation mediated by large, myelinated Aβ afferents.

The sensory stimulus is constant and the same stimulus is delivered to all subjects, however the response is subjective. If the result is abnormal, it may imply that there is a signal dysfunction along the sensory pathways anywhere between the receptors, the sensory or associated cortices (Shy et al. 2003). Individual modalities tested will provide clues to the type of nerve fibres affected. For example impairment of vibration perception (128Hz tuning fork) and joint position sense reflect large fibre neuropathy whereas defective warm and cold thermal discrimination is in keeping with small nerve fibre involvement.

Another test includes *nerve conduction studies* (NCS): measure the latency, velocity and amplitude of impulses travelling along a nerve. These tests focus on establishing that symptoms and signs are due to peripheral neuropathy and that the anatomical characteristics of the neuropathy are compatible with the known toxicity of the agent being used (Table 1.3).

Table 1.3: Quantitative evaluation of CIPN

History & Questionnaires	Symptoms and distribution
Physical examination	Sensory – tactile, pinprick, thermal, vibration, joint position sense Motor including tendon reflexes
Laboratory testing	Glycosylated haemoglobin, thyroid testing, Vitamin B12 & folate level
Nerve conduction studies	Sensory and motor nerve latency, velocity & amplitude
Electromyography	Distinguish neuropathies
Quantitative sensory testing	Detection threshold of accurately calibrated sensory stimuli (vibratory, thermal and cooling)

However, no adequate controlled studies have been published using quantitative measurements as the primary endpoint for CIPN. Also a correlation between quantitative measurements and clinical symptoms has not yet been established (Cleeland et al. 2010). Several limiting factors exist in the use of these tests including availability of instruments, their cost, and the variability in results obtained with different devices. These methods depend on the patient's co-operation and compliance and on the standard neurologic examination, which could be a major potential pitfall in the assessment of sensory threshold.

Physician based assessments are usually performed to grade CIPN with common toxicity scales, such as the National Cancer Institute-Common Toxicity Criteria, but several non-validated composite (i.e., clinical and instrumental) scales have also been used. The reason composites are used is to provide a more detailed analysis and also provides different aspects of CIPN that can be frequently missed by simple common toxicity scales (Cavaletti et al. 2007). Moreover, the common toxicity scales combine signs and symptoms of peripheral nerve impairment with subjective function limitations and QoL impairment. The Total Neuropathy Score in its complete version was originally developed and validated for diabetic neuropathy (Cornblath et al. 1999). The Total Neuropathy Score and its reduced versions appear to be the most effective of such scales and have been repeatedly tested in patients receiving chemotherapy. Highly significant correlation have been found between versions of the Total Neuropathy Score in patients with CIPN and several common terminology criteria scales, but the evaluation of CIPN has been deemed more accurate using the Total Neuropathy Score (Cavaletti et al. 2006; Cavaletti et al. 2007).

1.3.6 Qualitative measures of CIPN: Questionnaires

A variety of assessment tools exist that are based on the patient's perception and self-report of pain. To ensure accurate and reliable reporting of the HRQoL and pain, several assessments should be used. At the bedside a simple way of assessing pain is by using the verbal rating scale (mild, moderate, severe) (Jensen et al. 1986) or a numeric rating scale where the patient grades their pain on a 0–10 scale (0 = no pain, 10 = worst pain imaginable) (Paice and Cohen 1997). For neuropathic pain, several tools exist to identify pain of predominantly neuropathic origin, as distinct from nociceptive pain, without the need for clinical examination (Bennett et al. 2005). When a more comprehensive pain evaluation is needed, a multidimensional pain assessment is utilised. One widely used assessment tool is

the Brief Pain Inventory (Cleeland and Ryan 1994) which is a simple and easy to use tool which has been translated into many different languages. These are more commonly used in research settings. Pain is a multidimensional phenomenon with sensory-discriminative, affective-motivational, motor, and autonomic components. Therefore, these aspects are important to consider when looking at a patient's pain experience. Quality of life questionnaires refer to an individual's emotional, social, and physical wellbeing together with their functional ability. These are useful measures for evaluating progress in health goals and measuring the effectiveness of a clinical intervention as an important outcome is the change in the patient's own perceived state of health. Overall, these assessments are subjective and therefore these scales are based on the individual's perception.

Both in research and in clinics, patients are often asked to fill in questionnaires, however, it is important that the patients are able to understand and complete these assessments. Therefore the choice of the assessment tool to be given to the patient to complete has relevance as studies have shown that poor compliance, visual impairments, poor physical condition, and illiteracy are influencing factors in patients not completing these assessment tools (Stahl et al. 2003). No matter which assessment tool is used, the ultimate goals are in making the right diagnosis, giving the appropriate treatment, and following-up the patient. It is thus important that the physician checks the questionnaires as otherwise these will not impact on the pain control (Trowbridge et al. 1997).

1.3.7 Current management of CIPN

As the exact pathophysiology of CIPN is not completely understood, this has limited tge development of evidence-based neuroprotective strategies. Studies have been carried out to prevent and treat neuropathy secondary to chemotherapy using antioxidants, neurotrophic growth factors and detoxicants; unfortunately none of these were successful and management of CIPN patients is still an unmet clinical need (Cavaletti et al. 2011). To date, the dose modification of the chemotherapy is the only way to limit the neuropathy in most patients and this should be considered as soon as symptoms and signs develop and affect the patient's activities of daily living. It has been recommended that if peripheral neuropathy, grade 1 with pain or grade 2 neuropathy interfering with function but not with daily activities, develops (evaluated according to the National Cancer Institute's Common Terminology Criteria for Adverse Events), then the dose of bortezomib is reduced to $1.0mg/m^2$

from 1.5mg/m^2 and thalidomide reduced to 50% or suspended until disappearance of toxicity and then re-initiated at 50% dose. If patients were to develop grade 2 with pain or grade 3 neuropathy interfering with daily activities then both bortezomib and thalidomide will be suspended until disappearance of toxicity and re-initiated at a lower dose with bortezomib administered once weekly and if grade 4 develops then bortezomib and thalidomide will be discontinued (Mohty et al. 2010; Bird et al. 2011).

The possibility of a delayed worsening of CIPN after treatment withdrawal (coasting) should always be considered. Standard analgesic regimes are not very effective in treating neuropathic pain and there has been very little research specifically in the management of painful CIPN. A multimodal practice with the use of opioids together with other pain modulating drugs is now recommended (Ossipov and Porreca 2005; Raphael et al. 2010; Bird et al. 2011). A systematic approach for evaluating pain includes a thorough history followed by a pain assessment tool, physical examination, and any relevant investigations.

1.4 Neuroimaging

Neuroimaging has revolutionised the way we understand how the brain works. Nowadays, the main techniques available for imaging of brain function include: fMRI, magnetoencephalography and positron emission tomography (PET). These techniques markedly differ in their invasiveness and technical properties. Functional MRI and PET measure the effect of neuronal activity (metabolic and vascular changes) rather than assess the activity of neurons directly. Neuronal and synaptic activity leads to increased regional cerebral blood flow and this link in known as neurovascular coupling.

1.4.1 Functional Magnetic Resonance Imaging

Functional MRI is one of the most recently developed forms of neuroimaging and is used to determine the haemodynamic response related to neural activity in the brain. First discovered in the 1990 by Seiji Ogawa and Ken Kwong, the importance of the blood oxygen level dependent (BOLD) technique is the MRI contrast, which is used to infer brain activity. Functional MRI has become a popular tool for studying brain function especially since it has several advantages: there is no need to inject radioactive isotopes as it is non-invasive and uses non-ionising electromagnetic radiation; it produces good spatial resolution (2-4mm); scanning time can be short (depending on the paradigm) and due to overall diagnostic efficacy of MRI, it potentially has relatively wide availability. Magnetic field strengths are measured in units of Tesla (T). One Tesla is about to 20,000 times the strength of the earth's magnetic field. A typical magnet is created by a large electric current flowing through wires that are formed into a loop in the magnet structure. Static magnetic fields of the order of 1.5T-7T are used for in-vivo fMRI. To create such a large static magnetic field requires a high current-density and this is achieved by the use of superconducting magnet windings. In a high field-strength clinical research system operating at 3T, the wires need to be superconducting and immersed in liquid helium as a cryogenic liquid to keep it near absolute zero and in the superconducting state.

1.4.1.1 Brain navigation

The cerebrum is divided into two hemispheres each consisting of four lobes: frontal, parietal, occipital and temporal. The brain consists of grey matter containing closely packed neuron cell bodies and white matter mostly constituted by myelinated axons. The brain's structure is usually most aptly visualised in three dimensions however, conventionally, for MRI the brain is projected in isometric planes; sagittal plane: obtained by a vertical cut parallel to the ears, a coronal plane: parallel to the face and, a transversal plane: obtained by a horizontal cut as illustrated in Figure 1.11.
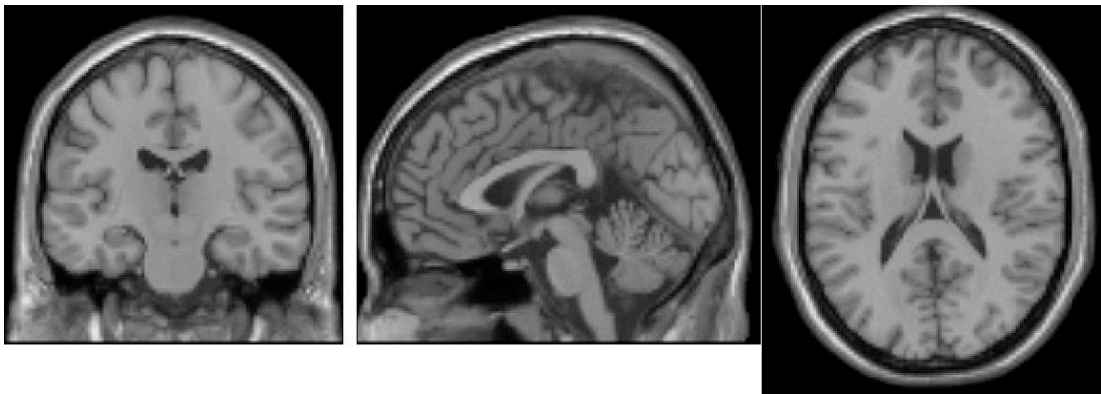
Figure 1.11: Visualisation of the brain showing MRI images of coronal, sagittal and transverse (axial) planes

1.4.1.2 Basic MR Physics

MRI is a 'spin'–phenomenon, where the spinning momentum of a nuclear charge produces a small magnetic field (like a tiny magnet with north and south poles). This behaves like a spinning top, which spins about its axis. Atomic nuclei consist of unpaired positively charged protons and uncharged neutrons. MRI uses in particular the properties of hydrogen atoms in water molecules, as water is the biggest source of protons in the body, followed by fat. This allows discrimination between white and grey matter, and cerebral spinal fluid in structural images of the brain. Under normal circumstances these nuclei have no fixed orientation so there is no overall magnetic field (Liney 2005). However, when nuclei are placed in an external magnetic field, this causes the nuclei to precess about the magnetic field direction, at a precession frequency equal to what is termed the Larmor frequency and they begin to align in given directions dictated by the laws of quantum physics (Figure 1.12). For a hydrogen nucleus, this can be in one of 2 directions: either parallel or anti-parallel to the applied static magnetic field (B_o). Those that are parallel to B_o are in a lower energy state than those that are anti-parallel are in a higher energy state. Above

absolute zero (-273°C), there is a slight overall difference between the populations in these 2 states. This difference depends on temperature and the magnitude of B_o. Even at body temperature (37°C) and at 3T, the difference is small and this is why sensitive equipment is required for a MRI scanner.

B$_0$

z

x

y

magnetic

field

No external magnetic

field
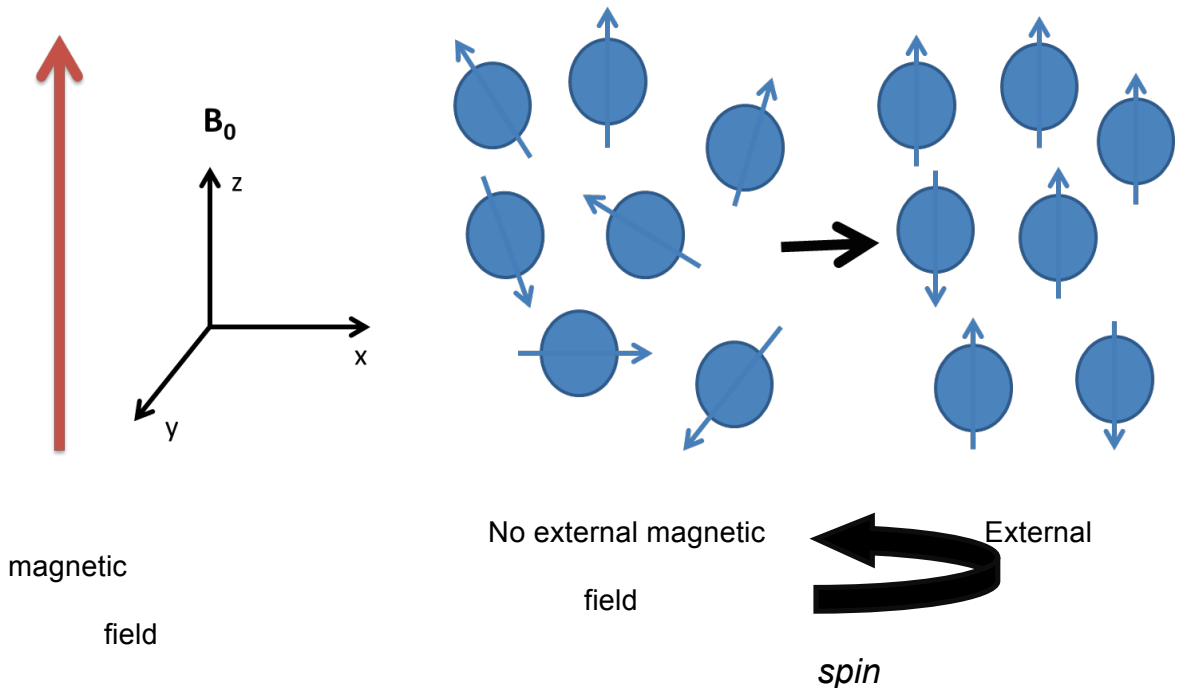
External

spin

Figure 1.12: External magnetic field, B$_o$ (in red) applied produces a small net magnetisation due to the net number of spins which align in the direction of the applied field

z axis = longitudinal direction often aligned with the main magnetic field.
y, x axis = transverse plane, perpendicular to the direction.

This net magnetisation (M_o) becomes the source of the MR signal - once it has been perturbed away from its equilibrium state. This process is how a signal is produced. For a patient who is lying supine with their head first in a superconducting magnet, the x direction is often chosen to be the left-right direction of the patient and the y direction is often chosen to be the anterior-posterior direction (Pooley 2005). A radiofrequency (RF) pulse is required to perturb M_o and change the net alignment of the nuclei. This is delivered via a RF transmitter coil. When the RF pulse is applied, the net magnetisation rotates through what is termed a flip angle. The angle that M_o has been 'flipped through' depends on the strength and the duration of the RF pulse. Immediately after the RF pulse, the protons begin to return to their equilibrium state and in so-doing the net magnetization alters mainly due to two effects:

- The hydrogen nuclei that are 'excited' gradually transfer their energy to the surrounding environment or lattice. As this happens, M_o returns towards B_o. This is termed longitudinal recovery or the spin-lattice interaction which is governed by a time-constant T1. Liquids have a long T1 whereas solids have a short T1.

- The excited hydrogen nuclei also exchange energy with each other and as this happens, their net magnetization spreads out or dephases and this is termed spin-spin interaction. The rate of dephasing is governed by a time-constant T2. T2 time constant describes the decay of the component of the net magnetisation. As with T1, the more mobile the sample, the longer the T2.

Once the energy has been dissipated to the lattice it has gone and thus T1 is always longer than (or equal to) T2.

The quality or homogeneity of the magnetic field plays an important role in the final image. Due to inhomogeneity in the main magnetic field, protons resonate at slightly different frequencies and therefore get out of phase and lose their transverse magnetization coherence. This is termed T2* decay. The T2* time constant governs the overall signal decay in the transverse plane and comprises both spin-spin interactions (T2 decay) and additional loss of phase due to imperfections in the external magnetic field, and magnetic susceptibility effects. Therefore, a magnet with good field homogeneity will allow T2* values to be closer to the true T2 values of tissues. Alterations from the 'true' T2 depend on local inhomogeneities. T2* is always shorter than T2. Thus, following a 90° RF pulse, a signal is produced by the rotating transverse magnetization and this is termed free induction decay (FID) which rapidly dephases due to T2* effects.

1.4.1.3 Pulse Sequence

An MR image is produced using a pulse sequence which contains such a RF pulse and a gradient pulse to spatially encode the resultant signal, which has controlled timings and durations. There are 2 principle types of pulse sequence: spin echo (SE) and gradient echo (GE). Spin echo sequences generally use 2 RF pulses (90° and 180°) to create an echo which is spatially encoded to form an image (Figure 1.13). The purpose of the 180° pulse after the 90° pulse is to refocus the protons by rotating the magnetization through 180° about the X axis, causing the magnetization to partially rephase, producing a signal. This eliminates the effects of the external causes of dephasing and the size of the echo signal depends only on the intrinsic T2 of the tissue and its molecular characteristics. Application of a 180° RF pulse will allow formation of an echo at a time TE (echo time).
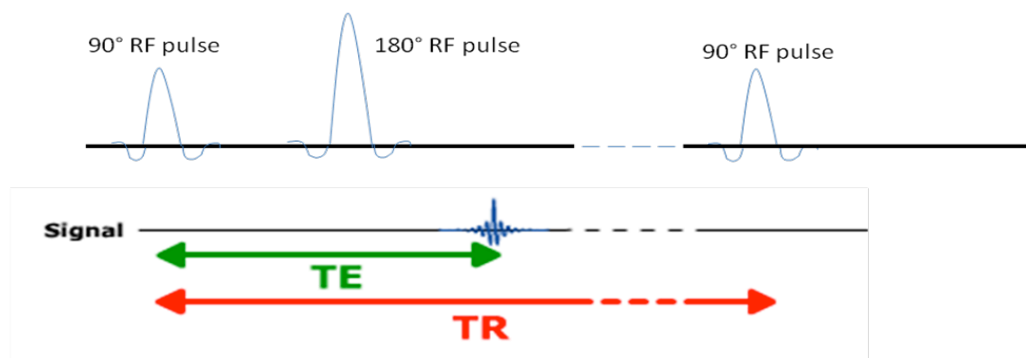
Figure 1.13: Spin Echo

Echo time (TE) is the time between the 90° RF pulse and MR signal sampling, corresponding to maximum of echo. The 180° RF pulse is applied at time TE/2. Repetition time (TR) is the time between two 90° excitations pulses

A GE sequences uses a single RF pulse followed by a gradient pulse to create the echo (Figure 1.14). The gradient echo sequence differs from the spin echo sequence in that there is no 180° RF rephasing pulse. It is also worth noting that the excitation flip angle is usually below 90° (partial flip angle). This effects signal contrast and the minimum TE that can be attained.
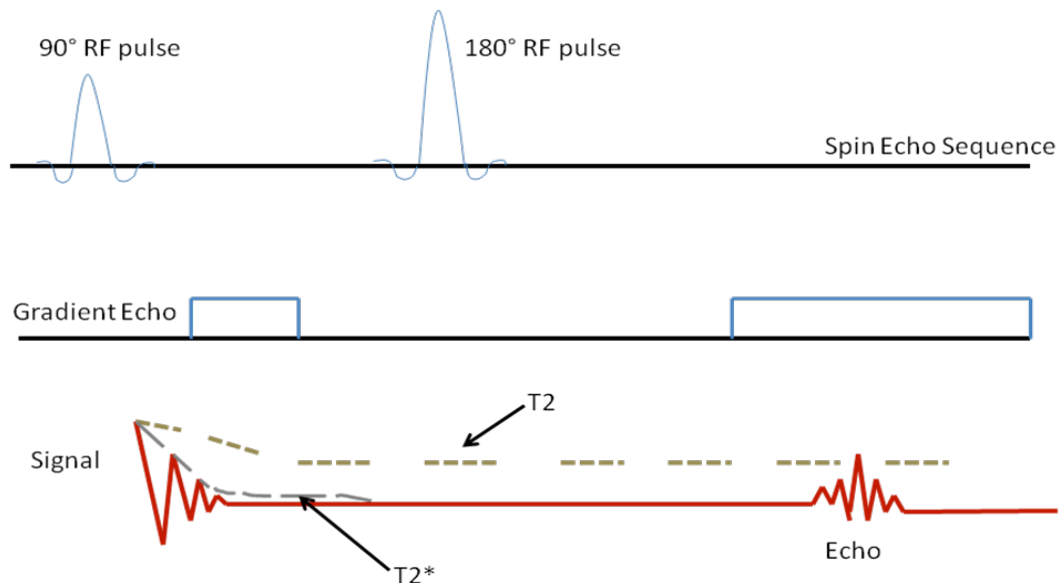
Figure 1.14: Differences between spin echo and gradient echo.

T2* weighted scans use a GE sequence. Gradient echo is subject to additional losses above the intrinsic T2 decay and makes it more prone to susceptibility losses at air/tissue boundaries, but can increase contrast for certain types of tissue, such as venous blood

Gradients alter the magnetic field resulting in a change in resonance frequency or a change in phase. Gradient fields are located in the x, y, and z directions. Gradient coils located within the magnet are designed to produce a desired gradient magnetic field and are used for overall spatial localistion (building an image).

There are many different types of pulse sequence, and by altering the TR and TE this can produce many different image contrasts. Contrast in needed in order to distinguish between different tissues. Contrast is due to differences in the MR signal, which depend on the T1, T2 and proton density of the tissues and sequence parameters used. The higher the signal is, the brighter it will appear on the MR image.

Each tissue has a specific proton density, T1 and T2 relaxation time. MR images display tissue contrasts that depend on proton density, T1 and T2. By setting the TR to short values, tissue contrast will depend on differences in longitudinal magnetization recovery (T1) (Hoa 2009). If TE is long enough, differences in transverse relaxation will alter tissue contrast (the T2 effect). However, the signal will disappear if TE is too long. In other words, a short TR and short TE sequence is usually called T1-weighted, a long TR and long TE sequence is usually called T2-weighted and a long TR and short TE sequence is usually called proton density – weighted. Cerebro-spinal fluid (CSF) is dark in a T1-weighted image (long T1 and

T2) whilst it is bright in a T2-weighted image. However, when the CSF is forced out in the periventricular matter as oedema T1 is shorter and this is due to the fact that in the CSF the protons are freer to move around whilst are more tightly packed in oedema. On the other hand, fat is bright in a T1-weighted image and grey in the T2-weighted image (short T1 and long T2).

It is possible to measure many echoes after each excitation pulse as long as the intrinsic spin-spin interaction (given by T2) in the xy plane has not decayed away. Echo planar imaging (EPI) can be imagined as an "add on" to a pulse sequence, to acquire more signals from each excitation pulse. Echo planar imaging is a unique imaging method because it can collect an MR image, from a single FID, in about 40-100ms. The advantages of this rapid imaging technique are that it helps to reduce motion-related artifacts and gives us a unique insight into the dynamic processes of the brain and this is very good for BOLD studies. The disadvantages are that EPI is very demanding on the imaging hardware because large field gradients have to be generated and switched rapidly and also the formation of artifacts due to factors related to the imaging sample (physical characteristics and nuclear magnetic resonance properties and the chemical shift components in tissue samples generate a chemical shift artifact), the hardware (gradient coils and amplifiers and field inhomogeneity) and the type of experiment. To overcome some of these limitations, modifications of the EPI pulse sequence and per-subject implementation can be implemented (eg. volume-selective shimming and the use of fat-suppression pulses).

1.4.1.4 Quality of the MR image

The quality of an MR image depends on the spatial resolution, the image contrast, the signal to noise ratio (SNR) and contrast to noise ratio and the presence of any artifacts. An MR experiment is a compromise between scan time and image quality and this is optimized according to the function of the organs and pathology. Spatial resolution corresponds to the size of the smallest detectable information. The smaller the voxels are, the higher the potential spatial resolution will be. In the context of BOLD fMRI this means that the voxels with BOLD-related signal changes can be closer to active neuronal areas. The matrix size determines the voxel volume and different sizes exist (e.g: 128 x 128 or 256 x 256 or 512 x 512) (Figure 1.15). Also the field of view and slice thickness play a role in the voxel volume.
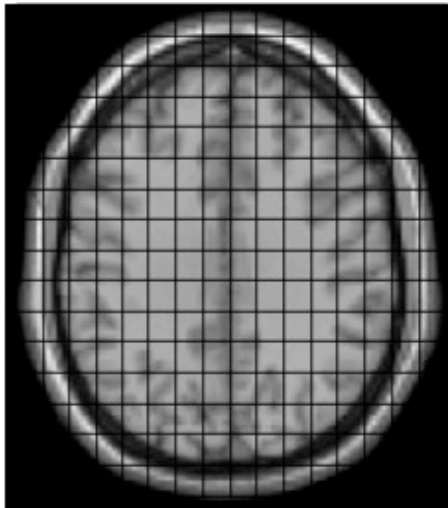
Matrix size: 512 pixels   256 pixels
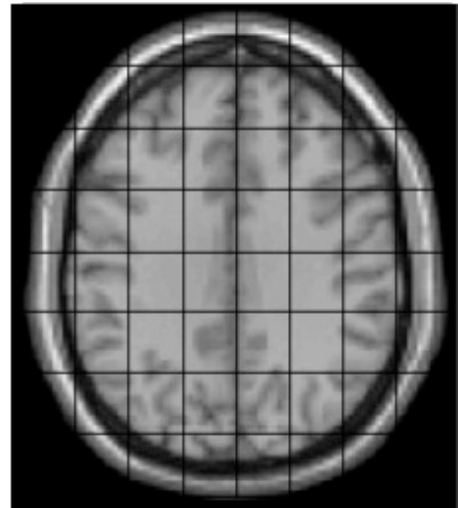
Figure 1.15: Different sizes of the matrix

Varying the matrix size, varies the details of the images scanned (NB: not the actual matrix sizes are depicted, rather a visual representation).

In a MR image, the voxels that make up the image contain a mixture of contrast (different signals) and noise. The SNR is a measure of signal strength relative to background noise. The signal is the voxel brightness. Incoherent noise appears on the image as irregular grainy patterns, which can degrade image details. The sources of noise in the image are from the MR coils and the patient's body due to thermal motion. The operator can control factors that produce noise, such as which RF coil is used (the smaller the sensitive volume of a coil, the lower the noise from the adjacent structures which it can detect, and the better the SNR will be), using the narrowest possible receiver bandwidth and also deciding on the voxel size. Low SNR may result in missing small levels of information or the obscuring of subtle contrast. The relative differences in signal from different tissues compared to the amount of noise present are termed contrast to noise ratio (CNR).

Changing acquisition parameters result in changes in the SNR, CNR and spatial resolution, which affect the final outcome or quality of the MR image. It is advised that to produce a good image, the contrast is first set by choosing the appropriate pulse sequence and timings, followed by the smallest field of view that fits the anatomy scanned and finally the matrix for the spatial resolution. However, the choice of these parameters together with how many times a signal is averaged, all affect how 'noisy' the image appears and thus what can be reliably detected.

1.4.1.4.1 Artifacts

Artifacts are features in an image that are not present in the subject (object) being scanned and which may lead to false interpretation. Artifacts are often due to *subject motion.* These could relate to random movements such as coughing, swallowing or eye movements. Also a periodic motion associated with respiration, cardiac beats, arterial or CSF pulsation can lead to the formation of artifacts, most notably ghosting. These ghost images could be reduced by synchronising the TR and the periodic motion, so that the measurements are always performed with the subject in the same spatial position. Reducing the patient's movements sometimes requires the use of physical restraint, sedation or general anesthetic. Flowing blood or CSF causes *flow artifacts* in the body. When blood flows through a slice it can receive the RF pulse, but flows out of the slice by the time the signal is recorded. Therefore, by the time the echo is recorded the slice has only blood in it, which has not experienced the 90° or the 180° pulse thus resulting in no signal in the blood vessel. To overcome this, a multi-slice sequence is used so that the slices could be positioned such that blood experiencing a 90° pulse in one slice can flow into another slice and experience 180° rotation and into a third and contribute to the echo (Hornak 1996). Therefore, the vessel will have high signal intensity. Another source of artifact can be due to the magnetic *susceptibility* of a material (which is the tendency for that material to alter the magnetic flux density when placed in a magnetic field). This can create local disturbances (eg. from dental work or tattoos) in the applied magnetic field resulting in image distortion and phase disruption / signal changes. In the context of this thesis, the acquisition sequence (T2*-weighted EPI), which is used to monitor BOLD fMRI contrast, can also be hampered by dropout of signal in the orbitofrontal and parietal brain regions due to the close proximity of magnetic field gradients near air-tissue interfaces (eg: sinuses). *Chemical shift* is apparent as a mis-registration between water and fat. The protons on water and fat molecules resonate at slightly different frequencies and this can interfere with the frequency-encoding process, leading to the formation of bright or dark bands. Performing imaging at low magnetic field strength, by decreasing voxel size and by increasing receiver bandwidth, can reduce this effect. Another kind of chemical shift artifact (phase cancellation artifact) occurs with gradient echo sequences as the absence of a 180° RF pulse causes a phase shift between protons of fat and water when the echo is formed. This phase shift depends on their resonance frequency shift. When fat and water spins, there will be Tes and this will be totally in phase and totally out of phase. If the selected TE is when the spins are

out of phase, then a black boundary will be seen around organs surrounded by fat. There are specific TEs in GE imaging which give the phase cancellation artifact. Therefore, choosing the right TE is very important to avoid this artifact. *Partial volume artifacts* occur whenever a voxel contains a mixture of tissue types and this can easily happen when scanning the body. This is resolved by using a smaller voxel, however this may result in poorer SNRs in the image. A *wraparound artifact* is the appearance of a part of the imaged anatomy, which is located outside of the field of view, inside of the field of view (Hornak 1996). An example is if the head is being scanned, the nose might be outside the field of view. However, the nose appears at the back of the head in the image, and this happens because objects located outside the field of view appear at the opposite side of the image. This occurs when the selected field of view is smaller than the size of the imaged object. Overall, artifacts are common in MRI and primarily lead to image degradation, although they can occasionally mimic pathological lesions. However, artifacts have helped to develop new technologies. In early reports of the development of MRI, flowing blood was simply a nuisance, producing artifacts. However, nowadays sequences are used to visualise rather than suppress flowing blood in the clinically useful technique of MR angiography. Of great importance to this work, a difference in magnetic susceptibility that, as outlined above, can lead to the production of signal distortions and dropout artifacts, can also be used in the form of the BOLD contrast effect, to yield differences in signal resulting from changes in the relative amounts of oxy- and de-oxyhaemoglobin which change on neuronal / synaptic activity (Ogawa and Lee 1990).

1.4.2 Neurovascular coupling

This refers to the relationship between local neural activity and subsequent changes in cerebral blood flow (CBF), that is, constriction/dilation of blood vessels to decrease/increase of cerebral blood flow to the brain region. The magnitude and spatial location of blood flow changes are assumed to be tightly linked to changes in neural activity through a complex sequence of coordinated events involving neurons, glia, and vascular cells that collectively adjust delivery of energy substrates to meet the local adenosine triphosphate demand (Riera et al. 2008). Brain activation is accompanied by a complex sequence of cellular, metabolic, and vascular processes. Neuronal activity is metabolically demanding. Increases in neuronal activity cause an increase in oxygen and glucose consumption (Hyder et

al. 1997), and timely delivery of energy substrates from blood to support neural activity is essential (Paulson et al. 2010). Whereas the fractional increases in CBF and glucose consumption are similar in magnitude, oxygen consumption increases much less than CBF, leading to a net increase in the amount of oxygen present in the blood and tissue that are local to the neuronal / synaptic activity. This oversupply of oxygen due to the mismatch between CBF and oxygen consumption is the basis of BOLD fMRI, which detects alterations in levels of deoxygenated haemoglobin and cerebral blood volume (Pasley and Freeman 2008).

1.4.3 Blood oxygen level dependent fMRI

The technique BOLD fMRI assesses neural activity. In the normal resting state, a high concentration of de-oxyhaemoglobin diminishes the MR signal due to its paramagnetic nature. When an area of the brain is being activated, there is increased blood flow and increased oxygen consumption. The surrounding neurons convert oxygenated haemoglobin, which is diamagnetic to deoxygenated haemoglobin. This overall difference in magnetic susceptibility (due to some oxygen consumption leading to an overall increase in oxygenated blood-delivery) results in BOLD signal changes on T2*-weighted imaging. Therefore changes in the BOLD signal can be used to detect changes in neural activity.

In a typical experiment the subject completes a series of task and rest intervals during which T2*-weighted MR images are acquired repeatedly. The signal changes during this time course are examined on a voxel-by-voxel basis to test how well they correlate with a model of the haemodynamic response to the task. In order to visualise the resultant functional anatomy, voxels that demonstrate a statistically significant correlation between measured signal and model can be highlighted and over-layed onto a greyscale anatomical image to create a BOLD-response or 'activation' map of the brain. The location and extent of activation is linked to the type of task or stimulus performed, for example a simple finger movement task will produce activation in the primary motor cortex (Liney 2005).

With neural activation, assuming a constant rate of blood flow to the functional area, the oxygenated haemoglobin level would be expected to decrease. Indeed, after brain activity there is a brief drop in blood oxygenation in the haemodynamic response (initial dip). However, there is a subsequent reactive increase in blood flow to the area, causing a net increase in the level of oxygenated haemoglobin (Figure

1.16). Once neural activity has returned to 'resting' baseline, localised arterial blood flow, volume and relative oxy-/deoxy-haemoglobin ratio also return to pre-activation (and hence T2*-weighted MR signal) levels. A post-stimulus undershoot (Fransson et al. 1998; Attwell and Iadecola 2002) is known to often precede the return to the baseline state.
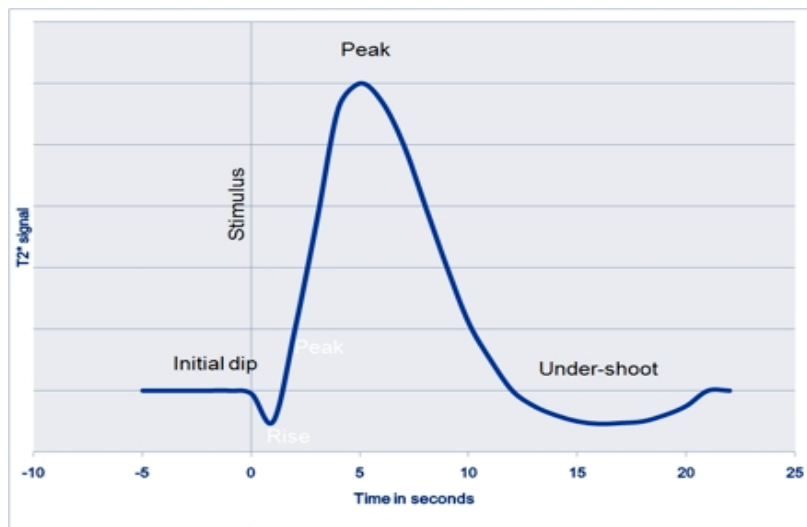
Figure 1.16: Typical haemodynamic response to neural activity and its effect on fMRI signal intensity.

Following stimulus: the BOLD response shows a small post-stimulus dip, then reaches a peak activation at 4–6 seconds, and often undershoots the baseline slightly before returning to prestimulus (10 seconds or more following the stimulus) (Mohamed et al. 2000).

A temporal resolution on the order of 100 milliseconds and a spatial resolution of 1-2 millimeters for fMRI is much greater than that of PET scanning (Posse et al. 1996). This allows for neuroanatomical structures to be readily visible and for transient cognitive events to potentially be imaged. The temporal resolution is determined by how often 'single-shot' images can be acquired (given by the repetition time) which is often limited by scanner gradient specifications and how large the volume to be measured is (field of view (FOV) and number of 2-D slices). The BOLD signal changes seen in fMRI are relatively small and depend on the field strength of the scanner (~ 2-3% at 1.5T and 4-6% at 3T 3T for typical motor or visual activation).

Due to MRI's non-ionising nature, each subject cab be scanned several times during one imaging session (ie. different 'runs') and on different occasions. Within one run a lot of images are acquired, due to the small contrast-to-noise ratio of the BOLD response, scanning the volume of the brain over and over. This volume consists of

several slices. Each slice consists of small volume elements, called voxels, which are three-dimensional pixels (Figure 1.17). The anatomical prescriptiuon of slice position and orientation can be varied for a 'run', depending which areas are of interest. A slice has a certain thickness and the in-plane resolution is given by the extent of the image (FOV) and the acquisition matrix size. Thus for a FOV=25.6cm and a matrix of 256 x 256, the in-plane spatial resolution would be 1mm.
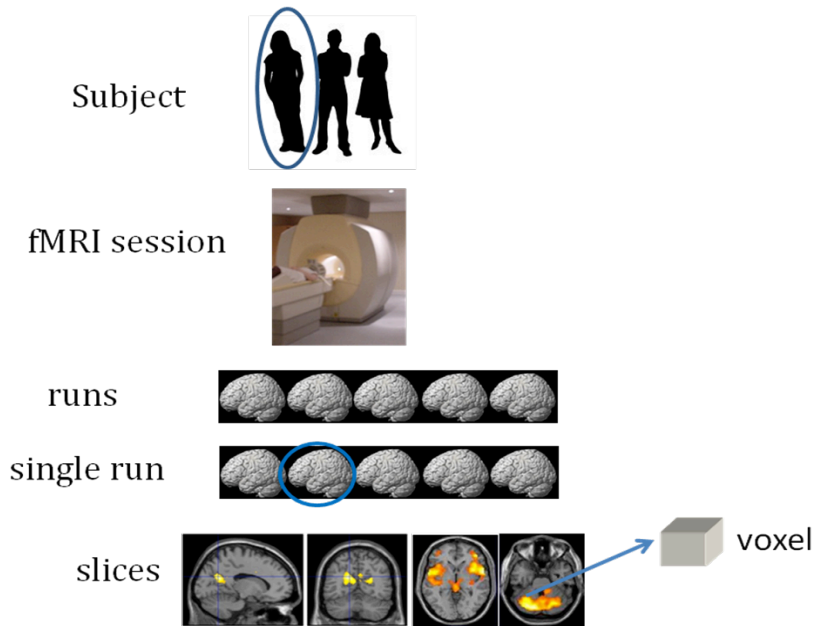
Figure 1.17: MRI process of what happens during a visit for a scan.

A subject attends for an MRI scan where fMRI data is acquired whilst scanning the subject. From each run, slices are obtained and each and the MRI signal is represented as a voxel, a small volume of tissue within the patient's body.

1.4.4 fMRI experimental designs

BOLD fMRI does not measure absolute neural activity and study designs are of paramount importance in order to statistically contrast the neuronal activity of interest with a suitable rest or background condition. Over recent years, different presentation schemes or 'paradigms' have been employed to assess the haemodynamic response to many types of stimuli. Stimulus paradigms have been block, event-related or of mixed designs. Normally, one stimulus would evoke a BOLD response as seen in figure 1.18.
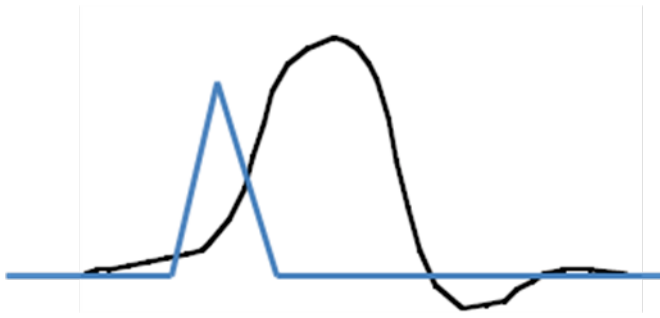
Figure 1.18: The haemodynamic response in relation to a stimulus

Blue = stimulus; Black = BOLD signal responding to a single stimulus. This demonstrates a delay between the stimulus and the brain's haemodynamic response.

1.4.4.1 Block design

Epoch-based design uses blocks of stimulation (boxcar designs with alternating activation and rest yielding on and off conditions, Figure 1.19). When the subject is given a series of categorically similar stimuli one after another then the BOLD response can increase to a measurable level; the chances of measurement when the subject is being scanned increases. The BOLD signal is made up of individual haemodynamic responses from each stimulus.
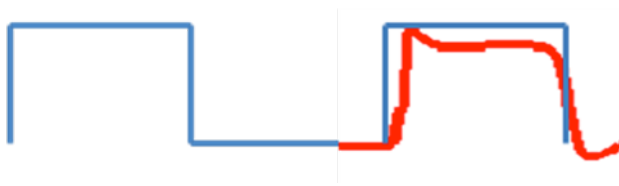
Figure 1.19: Block design

Alternate between stimuli of tasks and controls and measures fMRI response to each block. (Blue = on and off stimulus, Red = BOLD haemodynamic brain response).

The advantages of a block design are that this allows considerable experimental flexibility and is statistically powerful and (relatively) straightforward to analyse. However, this design has its own limitations. Potential confounds can be present such as habituation and anticipation and the BOLD signal may not remain constant across the epoch of interest. Within a block, the underlying haemodynamic response can change from the first trial in the block to the last trial within the block.

1.4.4.2 Event-related design

This paradigm allows detection of the brain's response to brief events or stimuli. This allows randomly intermixed events of different types and thus means that the response to any one event is not systematically influenced by prior events (Figure 1.20). This type of design is useful for behavioural studies.
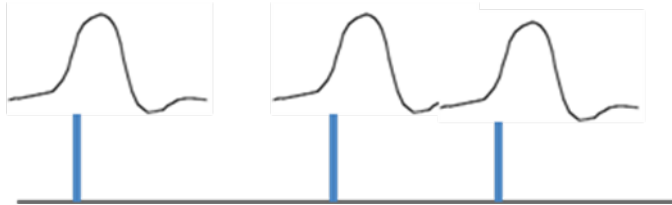
Figure 1.20: Event-related design

Measures brain's responses to brief events/stimuli repeated many times. The blue line represents the stimulus and the black 'wave' repesents the brain's haemodynamic response.

Event related designs allow for stimulus events from various experiment conditions to be presented randomly in one run therefore more randomisation is possible and this leads to a less predictable experimental nature. For each event there is a single haemodynamic response function which can be identified. The disadvantages related to event related design are that it is more complex both in the design and in the statistical analysis and afforded statistical power.

1.4.4.3 Mixed designs

A combination of block and event-related designs is a mixture of the characteristic block design measurement of repetitive sets of stimuli and the transient responses detected by event-related designs (Amaro and Barker 2006).

1.4.5 Analysis of functional neuroimaging data

The analysis of imaging data in this work was carried out using statistical parametric mapping software designed in University College London (SPM5) – see Methods Chapter. A commonly used method for detection of neural activity and the variability in the data of experimental and confounding effects in fMRI is using the general linear modelling together with the Gaussian random field theory (GRF). A model is set-up according to the stimulus applied and this model will be fitted to the data

obtained, and therefore a good fit between this model and the data means that the data has a high probability that it resulted from the specified stimulation. General linear model (GLM) estimates parameters by analysing each voxel using any statistical parametric test and the resulting statistics are assembled into an image (Friston et al. 1994). The GRF is important as it allows detection of an effect or activation at an unknown spatial location. Data from individual subjects can be pre-processed independently. Analysis of functional neuroimaging data involves several steps (figure 1.21):

Spatial pre-processing: the aim is to reduce unwanted variance components in the voxel time-series that are induced by movement or shape differences among a series of scans. The process involves realignment, spatial normalisation and spatial smoothing. A Gaussian model was used to eliminate type 1 and type 2 errors. SPM5 combines the GLM and GRF and allows statistical inference to be made in reference to deviations in the BOLD response from the null hypothesis.

Realignment: The aim is to remove movement artifact in fMRI time-series. The SPM programme does this by realigning a time-series of images to the first image in the series, acquired from the same subject using a least squares approach and a six parameter (rigid body) spatial transformation (Friston et al. 1996). The six translational and rotational corrections are displayed as a function of scan number providing an indicator of subject spatial stability.

Normalisation: This module spatially (stereotactically) normalises images into a standard space defined by a template image thus making the results from different studies and individualscomparable by aligning them to a standard space. The template images supplied with SPM conform to the space described by the atlas of Talairach and Tournoux (Talairach and Tournoux 1988). Generally these algorithms work by minimizing the sum of squares differences between the acquired image and the template.

Smoothing: This is for smoothing or convolving image volumes with a Gaussian kernel of a specified width (Friston et al. 2000). It is used as a preprocessing step to suppress noise and effects due to residual differences in functional and gyral anatomy during inter-subject averaging.

Anatomical localisation: The anatomical location presented in this thesis has been identified in two ways. All images are normalised to a standardised anatomic space (Talairach and Tournoux 1988) using the Montreal Neurological Institute (MNI) template.
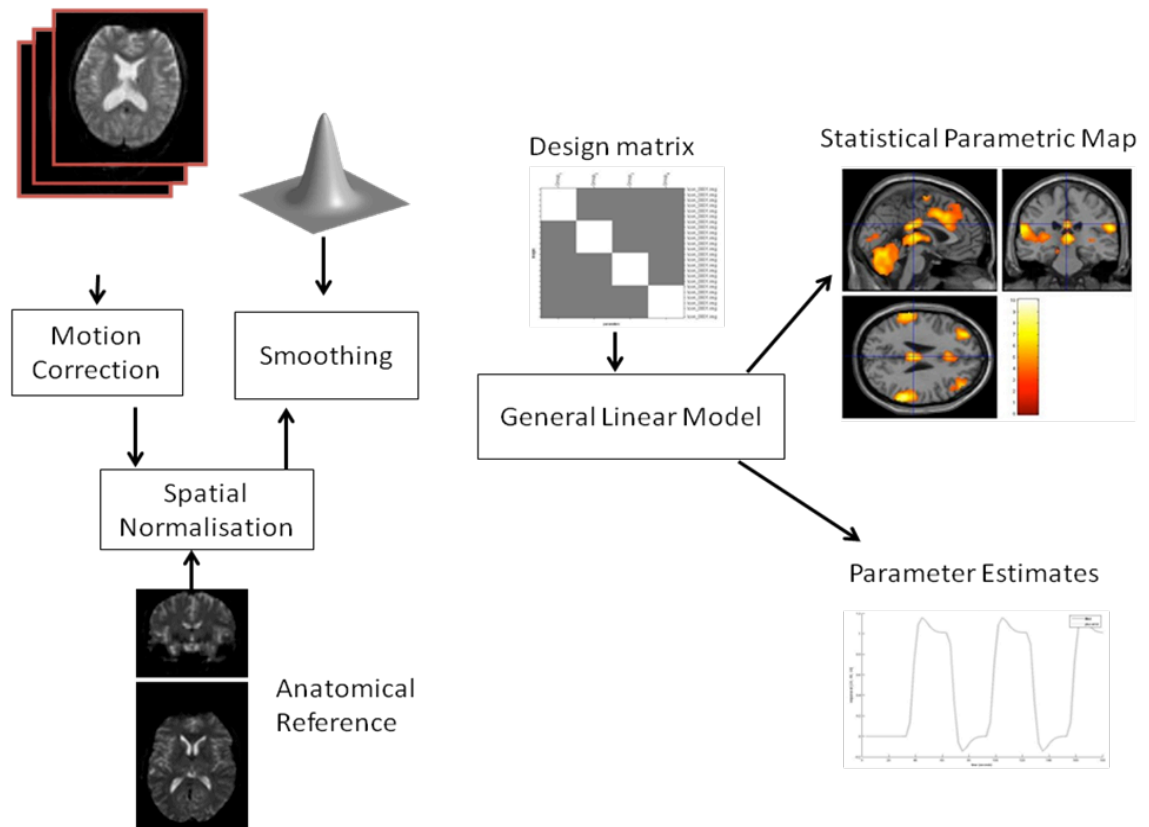
Figure 1.21: Overview of SPM analysis

Negative x coordinates denote the left side of the brain; and positive x coordinates give regions in the right side of the brain. The SPM5 display utility displays images in neurological convention, which means that the left side of the image corresponds to the left side of the brain.

1.4.6 Statistical analysis of data

Statistical parametric mapping refers to the construction of spatially extended statistical processes to test hypotheses about regionally specific effects. Statistical parametric maps (SPMs) are image processes with voxel values that have, under the null hypothesis, a known distributional approximation (usually Gaussian) (Frackowiak 1997). SPMs show the location, spatial extent, and relative magnitude

of statistically significant activations to an experiment. When SPM estimates a model, the software analyse the data obtained across the experiment and sees if it fits the hypothesis, which would have been described in the design matrix. The design matrix specified how factors of the model change over time. The SPM automatically convolves the effects of the hemodynamic response function with the stimulus vectors, as well as filtering changes in the data that are not relevant to the conditions. The design matrix contains the explanatory variables. Each column of the design matrix corresponds to a condition associated with the experiment. The formulation of a design matrix appropriate to the study is important and inferences are sought. After completion of the model estimation, a set of data is obtained showing the effect each condition of the experiment had at each voxel. The importance to know is if one condition made a significantly greater contribution than another condition and this is specifically done by contrast analysis. Each voxel is assigned with a T-statistic and SPM could be programmed at a certain p-threshold to find only the voxels whose t-statistics fit above that probability threshold.

Experimental designs in functional neuroimaging can be broadly divided into subtractive, parametric and factorial. In designing the experiment, the task might be designed to be identical, however the brain is likely to show changes in activity between tasks due to confounding factors as the brain is involved in other parallel physiological processes unrelated to the experimental task. Therefore, to highlight areas of the brain related to the task, statistics are employed to look for the most significant difference above the background brain activity and this is known as the general linear model.

Subtractive designs apply the idea of subtracting images acquired when the subject was performing the active condition from images when the subject was performing a control condition. Here, there is the assumption that the two conditions can be cognitively added, a principle known as pure insertion, implying no interactions among the cognitive components of a task. The images would be analysed assuming that any BOLD signal difference, above the statistical level chosen, would represent all brain regions involved in the performance of that task. This design is used for cognitive or sensorimotor activation studies (Friston et al. 1996). However the assumption of pure insertion in subtraction logic is often false.

Parametric designs include studies where some physiological, clinical, cognitive or sensorimotor parameter is correlated with physiology to produce a SPM of the significance of the correlation or regression. In this design, a range of different levels

of parameters are introduced and then the relationships are identified between the imaging signal and the values that the parameter assumes (Moonen et al. 1999).

Factorial designs provide for interactions between each component. An interaction represents a change in a change and is associated with factorial designs where two or more factors are combined in the same experiment (Frackowiak 1997). The effect of one factor, on the effect of the other, is assessed by the interaction term. Factorial designs have a wide range of applications and are important for clinical studies. The effect of a disease process on sensorimotor or cognitive activation is simply an interaction and involves replicating a subtraction experiment in subjects with and without the pathophysiology studied (Friston et al. 1996). The parameters used in the study detailed in Chapter 2 were included as a flexible factorial model and will be discussed later.

1.4.7 Brain activation

1.4.7.1 Finger movement task

Finger tapping is a simple motor control task and it involves the control of movements in the spatial domain and movement timing. In functional neuroimaging studies, finger-tapping tasks are commonly used to study the human motor system. In such tasks, subjects are asked to tap their fingers in synchrony either at their own pace or with a series of pacing tones separated by a constant interval. Pacing stimuli are used to make sure that all subjects perform uniformly the finger tapping task uniformly at a predetermined rate. The stimuli are usually in the form of regularly paced, repetitive auditory or visual cues. Complex finger tapping tasks such as bimanual tapping tasks or multi-finger sequential are often used together with pacing stimuli and are employed to elicit neural activation that is more representative of what would be observed in an typical manual movement. A meta-analysis on finger-tapping tasks showed that clusters of concordance in regions commonly associated with the performance of motor tasks including the primary sensorimotor cortex, supplementary motor area (SMA) located in the medial part of the superior frontal gyrus, basal ganglia and cerebellum (Witt et al. 2008). Areas of the cerebral cortex are involved in the planning, control and execution of voluntary motor tasks. There are different areas in the motor cortex, which have different functional roles (Figure 1.22). The primary motor cortex has a somatotropic representation, known as the motor homunculus, where the surface area devoted to

control a particular movement of each body part varies in direct proportion to the accuracy of the movements that can be made by that part. The hand and arm motor area is the largest.

a)   b)
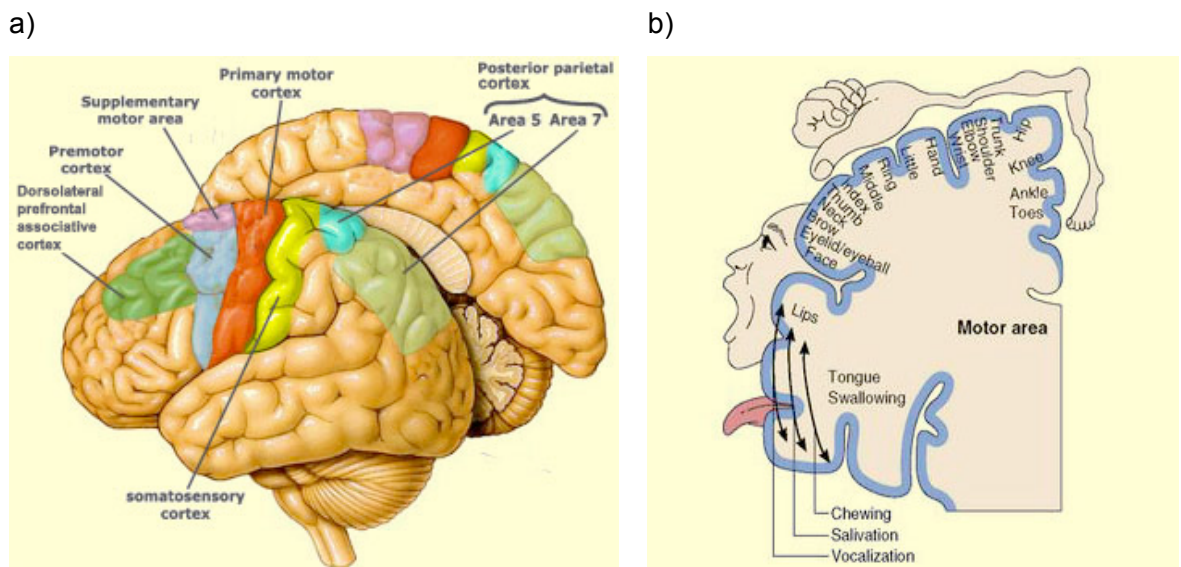
Figure 1.22: a) Motor cortex typically divided in primary motor cortex, pre-motor area and supplementary motor area; b) Somatotropic representation on the primary motor cortex.

Reproduced with permission from www.thebrain.mcgill.ca

The primary sensorimotor cortex activation has been associated with simple voluntary movements and also involved in the processing of complex sequential tapping tasks as well as the processing of bimanual movements. The SMA processes simple voluntary movements together with higher motor processing functions (movement initiation, motor planning, programming and learning, bimanual coordination, responsiveness to cueing of movement and selection of movement). Basal ganglia play a part with the performance of simple repetitive and complex sequential movements. The cerebellum is activated during preparation, execution, and timing of both simple and complex movements (Witt et al. 2008). The cerebellum stores learned sequences of movements; it contributes to fine-tuning and co-ordination of movements and sustains close communications with the cortex. The primary motor cortex, SMA, basal ganglia and cerebellum are not the only parts of the cortex that are involved in generating voluntary movements. The posterior parietal cortex also plays a crucial role in relation to motor planning and the prefrontal cortex is important for executive function, judgement and higher level processing (Leff et al. 2011).

1.4.7.2 Experimental pain stimulation in healthy volunteers

Changes in brain activity in response to painful stimuli have been studied using fMRI. Over the last twenty years, imaging studies have examined the perception and modulation of experimental stimuli including pain. This has led to the characterisation of a network of brain areas that consistently activate in response to pain, which has been designated as the "pain matrix" (Melzack 1999). These brain areas are primarily responsible for discriminating location and intensity of painful stimuli together with affective pain processing (Rainville et al. 1997; Bushnell et al. 1999; Kanda et al. 2000; Vogt et al. 2003). Disruption of the pain matrix and the associated pathways are thought to have implications for the pathogenesis and persistence of neuropathic pain (Tracey 2005). Mainly these studies have been carried out on healthy volunteers following acute pain stimulation and so largely remain to be tested in the context of pathological neuropathies. In a meta-analysis by Apkarian et al, haemodynamic studies of acute pain in normal subjects using PET and fMRI showed the six most commonly reported areas: anterior cingulate gyrus, primary and secondary somatosensory cortex, insular cortex, thalamus and prefrontal cortex (Apkarian et al. 2005) (Figure 1.23). These authors then compared incidences of significant activation of several brain areas across chronic clinical conditions (where the brain activity was specifically related to the condition) and acute pain. The comparison showed that chronic clinical pain conditions (like migraine, complex regional pain syndrome, fibromyalgia, irritable bowel syndrome, cardiac pain) more frequently involve prefrontal cortex, whilst in normal subjects, perception of experimental pain more frequently involves somatosensory cortex S1 and S2, thalamus and anterior cingulate gyrus (Apkarian et al. 2005).
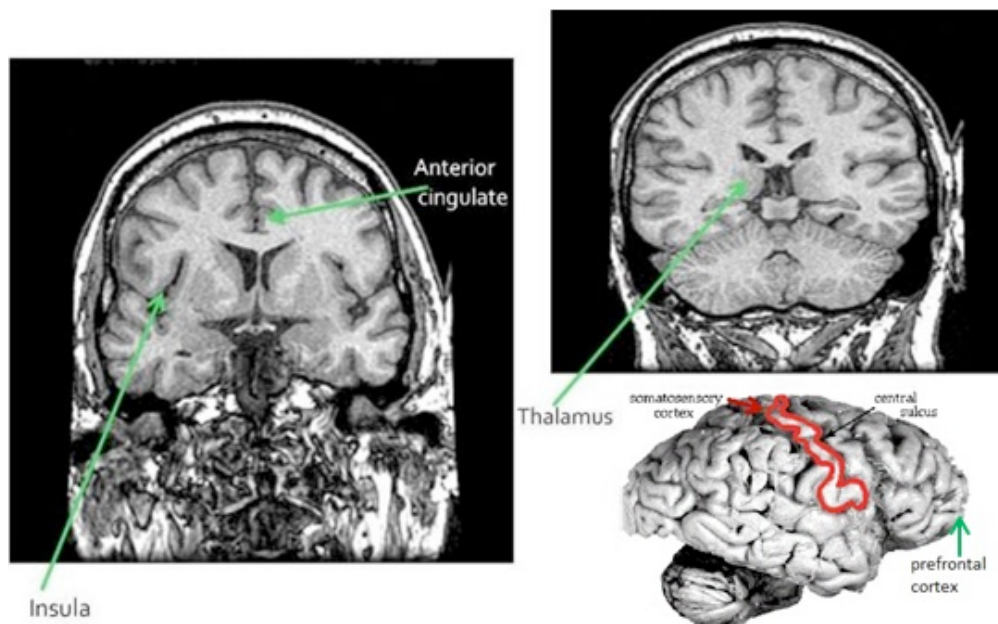
Figure 1.23: The 'pain matrix' showing areas where there is brain activation with painful stimuli

The ACC is part of the forebrain and plays a pivotal role in controlling the sensory, attentional, motivational and emotional elements of pain (Mohr et al. 2005). The insular cortex is folded deep within the lateral fissure between the temporal lobe and the frontal lobe and regulates emotion, self-awareness, cognitive functioning and interpersonal experience (Jaggi and Singh 2011). The prefrontal cortex is important for the social, emotional and executive functioning in humans (Teffer and Semendeferi 2012) and has extensive neural connections in the brain. The primary and secondary somatosensory cortices receive noxious and innocuous input from the thalamus and processes temporal and spatial aspects of somatosensory stimuli (Hofbauer et al. 2001). The thalamus forms part of the lateral wall of the third ventricle and is composed of multiple nuclei, which receive input from many cortical and subcortical structures; it is the sensory relay station to the cortex and is also part of the limbic system (McCormick and Bal 1994). Other brain areas, including the cerebellum, amygdale, basal ganglia, and hippocampus can also be activated depending on individual factors (Tracey and Mantyh 2007).

Pain is a subjective experience, and influenced by the individual's personality and complex multi-factorial networks involving emotions, memory and cognition, pathological and genetic factors. Also the response to pain is based on the particular situation and the appropriate response. In one study using fMRI (Ploner et al. 2010), 16 healthy human subjects were recruited and received brief radiant heat pulses to

the dorsal aspect of the subject's right foot (adjusted to pain threshold). Stimulus intensity was adjusted to pain threshold so that about half of the stimuli were perceived as painful and half were not. Stronger activation of the cerebral pain network including thalamus and somatosensory, mid-cingulate, and insular cortices resulted from stimuli perceived as pain compared to activation that resulted from identical stimuli that were not perceived as painful. There was also activation in dorsolateral prefrontal cortex and putamen (probably related to perceptual decision and motor processes). Neural activity in brain areas related to pain and pain modulation three seconds before pain and no pain stimuli was reported to be in the left anterior insular cortex where stronger activity was present not only in response to but before pain and no pain stimuli. This likely reflects the susceptibility to a sensory event that then determines the subsequent perception of pain. When cerebral connectivity before pain and no pain stimuli was compared, the anterior insular cortex exhibited the strongest response indicating that this brain region is particularly linked to the subjective perception of pain. The pre-stimulus functional connectivity of the bilateral anterior insular cortex to the brainstem in the PAG (an area of importance in the pain modulatory system), reflected the susceptibility to pain and affected subsequent stimulus perception. The authors concluded that variations in functional connectivity underlie personality-related differences in individual susceptibility to pain (Ploner et al. 2010).

1.4.7.3 Experimental pain stimulation in chronic conditions

Abnormalities in pain processing, rather than just damage or inflammation to the peripheral nerves, seem to play a part in chronic painful conditions like fibromyalgia syndrome, chronic low back pain and irritable bowel syndrome (Verne et al. 2003; Giesecke et al. 2004). Chronic neuropathic pain and changes in the brain have been less thoroughly investigated (Moisset and Bouhassira 2007). However, recent studies in another, much more common cause of peripheral neuropathy like diabetes, may identify a means of systematically investigating CIPN in MM.

1.4.7.3.1 Extrapolation of MRI studies from diabetic peripheral neuropathic pain

The Sheffield Diabetes/Radiology team reported evidence for the involvement of the CNS in diabetic peripheral neuropathy (DPN). Eaton et al, using non-invasive in-vivo MR imaging, measured the spinal cord cross-sectional area three different levels (lower cervical, upper and lower thoracic regions) of nineteen patients with DPN, ten diabetic patients with no DPN and ten healthy volunteers. They demonstrated a lower cross-sectional spinal cord area in the cervical and upper thoracic regions in patients with DPN compared to healthy volunteers, indicating extensive disease (Eaton et al. 2001). The theories postulated by this result are that the peripheral nerve damage causes secondary shrinkage of the spinal cord due to degeneration or atrophy or that the primary insult may be to the CNS, with the peripheral changes occurring as secondary phenomena and it is also plausible that both CNS and peripheral involvement occur concomitantly (Selvarajah et al. 2011). In another study, 98 subjects with type 1 diabetes were subdivided into three subgroups (no DPN, subclinical DPN, and established DPN) and Selvarajah et al were able to demonstrate decreased cross-sectional spinal cord area in patients with early subclinical DPN and also a significant trend of lower cord cross-sectional area with more severe neuropathy occurred across diabetic groups, indicating a continuing loss of cord area as the disease progresses (Selvarajah and Tesfaye 2006). Brain neurochemical and blood flow abnormalities are also reported within the thalamus, known to be a principle gateway to the brain's sensory cortex. Eighteen male subjects with type 1 diabetes (eight no DPN, ten established DPN) and six age and sex-matched non diabetic healthy controls underwent proton magnetic resonance spectroscopy of the thalamus and the main finding was a significantly thalamic neuronal dysfunction in the group of patients with DPN compared to patients with no DPN and healthy volunteer controls (Selvarajah et al. 2008). One possible explanation for thalamic neuronal dysfunction in DPN may be that the damage to the peripheral nerves led to loss of afferent input thus leading to changes occurring at progressively higher levels in the CNS. In another study, eighteen subjects with type 1 diabetes (no DPN=6, painful DPN=5, painless DPN=7) and five healthy volunteers had MR perfusion imaging at 1.5T; exogenous perfusion contrast (intravenous bolus of gadolinium chelate) was used to examine the microvascular perfusion characteristics of both the thalamus and caudate nucleus (control region). Group comparisons showed that painless DPN had lower thalamic regional cerebral blood volume compared with healthy volunteers and no DPN, whereas painful DPN had higher thalamic regional cerebral blood volume (p=0.04). Thus, in painful DPN there

was increased thalamic vascularity, whereas in painless DPN, greater thalamic microvascular impairment was present. Similar changes were not demonstrated in the caudate nucleus, which was a control area (not involved in somatosensory perception) (Selvarajah et al. 2011). In a preliminary study, 18 type 1 diabetic subjects (6 with no DPN, 6 with painful DPN, and 6 with painless DPN) were studied to test the feasibility of monitoring the brain's response to the presentation of heat-pain in the context of DPN; all participants had fMRI at 3T and a thermode device to the dorsum of the right foot delivered heat pain stimulation. This analysis showed that subjects with no DPN had greater BOLD response than those with painless DPN. Subjects with painful DPN showed significantly greater response than those with painless DPN. The neuro-anatomical areas involved in the BOLD response to heat pain include the primary somatosensory cortex, lateral frontal and cerebellar regions (Figure 2.24) (Wilkinson et al. 2007). This may be explained by reduced ascending nociceptive input as a result of diabetic neuropathy.
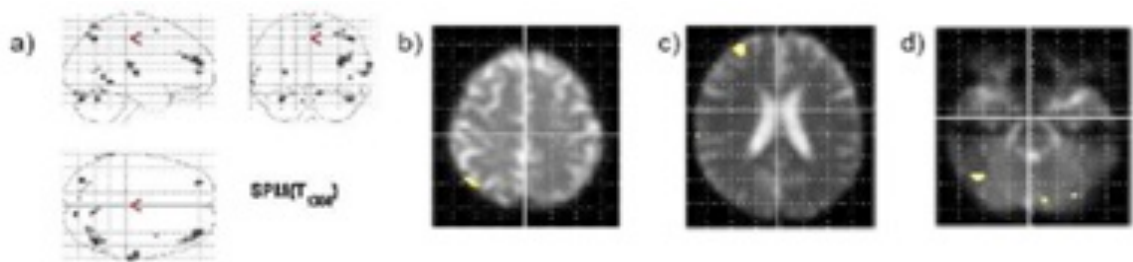
Figure 1.24: Statistical Parametric Map

a) (neurological co-ordinates) showing sagittal, coronal and axial projections of anatomical areas of significantly greater BOLD response (P<0.05 corrected for multiple comparisons) in type 1 diabetic subjects with DPNP than those with painless DPN; b, c & d) differences overlaid on the base EPI images within the b) somatosensory cortex, c) middle gyrus of the frontal lobe and d) cerebellum.
Adapted from (Wilkinson et al 2007).

1.4.7.3.2 Extrapolation of MRI studies from other chronic painful neuropathic conditions

Abnormalities in pain processing as shown on neuroimaging rather that just damage or inflammation to the peripheral nerves seem to play an important part in chronic painful conditions like trigeminal neuralgia, post-herpetic neuralgia and chronic low back pain (Giesecke et al. 2004; Geha et al. 2007; Moisset et al. 2011). Post-herpetic neuralgia is an example of a human chronic neuropathic condition, since patients display multiple central and peripheral signs of neuropathy. Changes that occur include loss of cells in the DRG and of myelin and axons in the dorsal horn, decreased innervation by nociceptive afferents, as well as brainstem and spinal cord lesions on MRI (Geha et al. 2007). In a fMRI study by Geha et al, the ratings of fluctuations of spontaneous pain during fMRI were analysed against the ratings of fluctuations of a bar observed during scanning, the overall brain activity for spontaneous pain of post-herpetic neuralgia involved affective and sensory-discriminative areas mainly including the primary and secondary somatosensory, thalamus, insula and anterior cingulate cortices. Areas that play a part in emotion, hedonics, reward, and punishment were also activated including the ventral striatum, orbital frontal cortex, amygdala, and ventral tegmental area. Trigeminal neuralgia is a unique neuropathic pain syndrome combining paroxysmal pain and evoked pain. An fMRI study in fifteen patients with classic trigeminal neuralgia in the second or third division of the nerve were studied, painful stimuli of the cutaneous trigger zone were associated with significantly increased activity in the spinal trigeminal nucleus, thalamus, primary and secondary somatosensory cortices, anterior cingulate cortex, insula, premotor/motor cortex, prefrontal areas, putamen, hippocampus and brainstem whilst non painful stimuli activated the same areas except the spinal trigeminal nucleus, brainstem and anterior cingulate cortex (Moisset et al. 2011). In this study, a large number of areas of the 'pain matrix' were activated during non-painful stimulation of the trigger zone and this could reflect the state of maintained sensitisation of the trigeminal nociceptive systems in trigeminal neuralgia.

Another area of interest that activates to painful stimuli is the cerebellum. Noxious thermal heat and brush stimuli were applied to the right side of the face in a group of patients with neuropathic pain involving the maxillary region of the trigeminal nerve and in a group of healthy subjects and this showed that different cerebellar regions were activated in acute and chronic pain processing (Borsook et al. 2008). Painful stimuli trigger cerebellar lobules activation which are believed to be involved in

cognitive and emotional processing both in patients with neuropathic pain and healthy subjects whilst the cerebellar sensorimotor regions are activated by touch and thus it is proposed that the cerebellum modulates the cognitive and emotional experience that differentiates the pain awareness from the appreciation of innocuous stimulation (Moulton et al. 2010). In a study looking at patients with chronic back pain, there were significant differences in the CNS grey matter (decrease of grey matter in the brainstem and the somatosensory cortex and increase in grey matter bilaterally in the basal ganglia and the left thalamus) between chronic back pain patients and healthy controls using voxel-based morphometry suggesting a cortical and subcortical reorganisation on a structural level (Schmidt-Wilcke et al. 2006).

Chronic neuropathic pain and possible changes in the brain have been less thoroughly investigated (Moisset and Bouhassira 2007). Our understanding of the pathological mechanisms underlying CIPN and possible future treatment target areas for this debilitating complication may be significantly enhanced by applying the fMRI techniques. To our knowledge, there have been no fMRI studies in MM patients who have developed treatment emergent CIPN.

1.4.8 Opioids use and the effect on the CNS

It is well known from preclinical studies that chronic opioid exposure has an effect on the nervous system and that clinical changes in the brain structure and function seem to occur (Robinson and Kolb 1999; Cunha-Oliveira et al. 2007). Studies administering acute opioid in opioid-naive volunteers revealed changes in BOLD signal in several areas of the CNS; mainly morphine and remifentanil decreased the brain activity in the somatosensory cortex and nucleus accumbens which is contrary to what happens after a painful stimulus and increased signal change in areas rich in opioid receptors mainly anterior and posterior cingulate cortex and frontal gyri (Becerra et al. 2006; Leppa et al. 2006). An fMRI study on ten chronic opioid-dependent patients showed changes in the structure and the function areas of the brain associated with affect and impulse control and motivation and reward including volumetric changes in amygdala, white matter tract abnormalities in the internal and external capsules and alterations in functional connectivity involving the nucleus accumbens, anterior insula and amygdala (Upadhyay et al. 2010). In another study, ten chronic low back pain patients were administered oral morphine daily for one month and MRI showed reduced grey matter in the right amygdala and dosage-

correlated volumetric increase in the right hypothalamus, left inferior frontal gyrus, right posterior cingulate, and right caudal pons which are known to have high mu-opioid receptor density and binding capacity (Younger et al. 2011). After an average of 4.7 months of stopping the opioids, an MRI was performed and demonstrated that many of the morphine-induced brain changes were still present (Younger et al. 2011).

1.4.9 Thermal stimulation in fMRI studies

Noxious thermal stimulation has been delivered by a thermode in different studies (Disbrow et al. 1998; Peyron et al. 1999; Tracey et al. 2002; Valet et al. 2004; Apkarian et al. 2005; Freund et al. 2009) or by a laser (Bornhovd et al. 2002; Garcia-Larrea et al. 2003) Babiloni et al.2007). Noxious stimulation selectively activate nociceptive-specific transduction mechanisms in afferents located in the superficial layers of the skin (Julius and Basbaum 2001). Aδ and C nociceptors are activated with thermal stimulation. Thermal stimuli can be applied via a contact heat evoked potential device (CHEPS), which delivers heat pulses rapidly with adjustable peak temperatures. The advantages over laser evoked potentials (LEPs) is that C fibre afferents (which confer the slow, long-lasting aspect of pain) are consistently activated; it stimulates a larger area than LEPs thus evoking brain responses of a higher signal-to noise ratio (Granovsky et al. 2008) and is generally much safer in that repetitive use does not cause erythema (Chen et al. 2001). Also LEPs usually have a very short stimulus duration, which may be a disadvantage in a clinical study where the stimulus is somewhat unnatural in comparison to the general real life experience of thermal pain. However, contact thermodes unavoidably activate non-nociceptive Aβ fibre afferents, because contact with the skin activates slowly adapting mechanoreceptors. In a study by Warbrick et al, event-related brain potentials using electroencephalography (EEG) were measured in response to contact heat stimuli applied to the arm and leg in ten healthy subjects (Warbrick et al. 2009). They used a single trial analysis using a multiple linear regression approach and found that single trial averaging resulted in larger amplitude measurements for fixed location stimulation. In a study, 16 healthy volunteers had a contact thermode applied to the right forearm either at a fixed location or at variable locations after each pulse with an inter-stimulus intervals 8–10seconds. The results showed that with fixed stimulus location, perceived pain intensity and contact-heat evoked potentials exhibited signs of rapid habituation reflecting fatigue of peripheral

nociceptive neurons and in such conditions, nociceptive input to the spinal cord progressively diminishes due to peripheral fatigue of Aδ and C fibre nociceptors (Greffrath et al. 2007). In my study, the effect of coupling neuronal activation and the BOLD response had to be taken in account and that the modelled BOLD response is applicable to the general linear model.

Another way of selectively activating nociceptive fibres is by intra-epidermal electrical needle electrodes where the free nerve endings of Aδ and C fibres lie in the epidermal layer of the skin. Although this is more accurate, it has the disadvantage of being invasive (Mulert and Lemieux 2010).

There have been various design studies using noxious heat stimulation with varying temperatures, stimuli and durations. A study conducted in the Academic Unit of Radiology in Sheffield, measured the brain's response to heat pain stimulation in diabetic peripheral neuropathy and the heat pain stimulation was 30 seconds with an inter-stimulus duration of 150 seconds (Wilkinson et al. 2007). This showed clear group differences in neuronal activity between painful and painless neuropathy. An fMRI study conducted by Tracey et al. utilised a shorter stimulus duration of 12 seconds to investigate the attention modulation of pain, eliciting significant PAG activation with this design (Tracey et al. 2002). In another BOLD fMRI study to evaluate attention in pain, a short 5 seconds stimulus with a 40 second inter-stimulus duration was applied (Bantick et al. 2002). A 10 second design to evoke heat-pain stimulation was assessed by Upadhyay et al and demonstrated the presence of distinct temporal profiles in BOLD fMRI responses in healthy subjects (Upadhyay et al. 2010). A study using noxious heat (46°C for 29 seconds alternating with a baseline temperature of 35°C for 36 seconds) showed activation in the somatosensory pathways involved in pain discrimination (somatosensory cortex and thalamus); in motivational and affective areas (cingulate gyrus); in motor control (cingulate gyrus, precentral gyrus, and cerebellum); in the memory of pain (insula); and in regions implicated in the modulation of pain (PAG) (Becerra et al. 1999).

This stimulus diversity suggests that the optimum duration of heat-pain stimulation and the inter-stimulus interval duration are not known; therefore a protocol for the CIPN pilot study was developed to estimate the most effective duration and inter-stimulus interval to evoke stimulation and BOLD fMRI response (See chapter 2).

1.4.9.1 Habituation to thermal stimulation

Habituation, a decrease in pain and pain-related responses with continuous or repetitive thermal painful stimulation has been demonstrated in the neuroimaging literature. In a fMRI study, six male healthy volunteers were given four noxious (46°C) stimuli lasting 29 seconds and when all four stimuli were analysed together, a decrement of statistical significance was observed relative to the combination of the first two stimuli and in fact no significant activation was seen for the last two stimuli in any anatomical region in both averaged and individual data sets (Becerra et al. 1999; Greffrath et al. 2006).

In one session single trial event related design, using six painful laser stimulation on the left hand in 32 healthy subjects, fMRI BOLD habituation was assessed by comparing the first half of the experiment against the second half; the site of the stimulation was manually moved after each trial and the interval between stimuli was pseudo-randomised between eight and twelve seconds (Mobascher et al. 2010). Areas of the brain that showed habituation were found in primary and secondary somatosensory cortices, the insula and the anterior cingulate cortex (ACC). Also in those subjects with faster habituation, there was increased activation in the rostral ACC and the PAG and these subjects provided lower pain ratings (Mobascher et al. 2010).

In another fMRI study investigating habituation to pain in twenty healthy volunteers, repeated painful stimulation over several days with a 20 minute pain paradigm to the left volar forearm (10 blocks of thermode stimuli with each block containing a series of six 48°C stimuli, each lasting six seconds), for eight consecutive days was delivered, and fMRI performed on days 1, 8 and 22. This resulted in significantly decreased pain ratings to identical painful stimuli and this was also reflected in the MRI results where over time, there was a decrease in the BOLD response to the painful stimuli in the pain matrix. On the other hand, there was increased activation in the subgenual ACC over time where this region plays an important role in endogenous pain control thus suggesting that habituation to pain may be centrally mediated by increased antinociceptive activity (Bingel et al. 2007).

However, in another study, 24 healthy volunteers were exposed to a 20-minute session of painful stimulation once a day to the left volar forearm over eight days. Half of the participants received the opioid-receptor antagonist naloxone on days one and eight (Rennefeld et al. 2010). The results demonstrated that the naloxone administration did not affect the development of pain habituation and that painful

stimulation repetitively over several days resulted in a significant habituation to pain at the site of stimulation and also significant pain attenuation at the non-stimulated limbs. This could indicate that there is a contribution of a central mechanism involving the supraspinal CNS which could involve a change in cognitive or affective processing of pain (Rennefeld et al. 2010).

1.4.10   Reproducibility of fMRI data

The test-retest reliability of fMRI BOLD signal changes has been investigated especially over the last few years. Motor tasks such as finger tapping and other paradigms such as learning tasks have been used for reproducibility studies (Wagner et al. 2005; Gountouna et al. 2010). In the literature, studies have shown that areas of activation are qualitatively repeatable when using the same scanner but are quantitatively of high inter and intra subject variability (McGonigle et al. 2000; Marshall et al. 2004; Gountouna et al. 2010).

1.5 Summary

Cancer care recognises that, as well as reducing tumour size and prolonging life, it is important to take into account the burden of treatment side effects (both acute and long-term), co-morbidities and the large range of associated psychological and social issues affecting patients and families (Ahmedzai and Walsh 2000). In addition, oncology has focused latterly on the problem faced by those in long-term remission; i.e. survivors. This interest has been focused on survivors of childhood cancer, but increasingly is being extended to the follow-up of adults, including those long-term survivors with controllable but incurable cancers such as MM (Demark-Wahnefried et al. 2006; Baker et al. 2007; Eiser et al. 2007; Greenfield et al. 2007). Multiple myeloma is an incurable disease, but treatment of active symptomatic disease may result in repeated phases of control. Delivery of more intensive treatment, including haematopoietic stem cell transplantation and introduction of novel agents, has resulted in significant improvements in life expectancy. Despite enhanced disease control, none of the current novel agents are free of significant toxicity, which frequently persists after completing treatment. Myeloma is thus increasingly being considered as a chronic disease state in which progressive damage from MM is potentially compounded by cumulative treatment related toxicities.(Brenner et al. 2009; Johnsen et al. 2009; Bird et al. 2011; Snowden et al. 2011). However, compared with some other cancers with protracted survival, (Mols et al. 2005) relatively little is known in MM about patients' psychosocial and broader holistic needs, particularly with modern clinical management strategies.

Pain is described as an unpleasant sensory and emotional experience associated with actual or potential tissue damage and is subjective. Whilst the experience of acute pain has a protective role, chronic persistent pain has no biological advantage and causes suffering and misery. Chronic pain creates physical, emotional and financial issues to patients, their families and society (Breivik et al. 2006). The incidence of chemotherapy-induced neuropathy can be variable with different chemotherapeutic regimes. Neurotoxicity can be so severe that it can limit the dose of chemotherapy and even potentially stop curative cancer treatment thus having an impact on survival. More cancer patients are experiencing better outcomes with chemotherapy and prolonged survival. Long-term management of CIPN is therefore, becoming one of the most challenging aspects of treatment in this disease. There is still no prophylactic treatment to deal with it and current neuropathy treatments are poor, owing to a lack of knowledge regarding pathological mechanisms.

Thus, there is an urgent need for better understanding of the mechanisms of CIPN to prevent or treat this adverse event so that more patients are treated with the therapeutic full dose and for the correct length of time. Neuroimaging methods can potentially play an important part in this and have made a huge impact within brain research. A significant amount of research has been conducted with pain models in healthy volunteers and the non-invasive identification of pain mechanisms has led scientists and clinicians to reconsider issues related to the pathophysiology and diagnosis of certain painful diseases. Neuroimaging is becoming an increasingly important tool in the study of clinical painful scenarios; both in the acute and chronic conditions.

This thesis is divided into two studies.

The aims of first study are:

-        To determine whether differences exist in central pain processing pathways as assessed by fMRI during noxious thermal stimulation between MM patients with chemotherapy-induced peripheral neuropathy and healthy volunteers.

-        To determine the degree to which quantitative sensory testing predicts presence and severity of CIPN.

-        To describe HRQoL in MM patients with CIPN, using qualitative assessments (questionnaires).

The aims of the second study are:

-        To characterise the late effects in patients with advanced but stable MM, clinically and in terms of HRQoL

-        To investigate the relationship between symptoms and functioning and cytokine levels relevant to MM.

-        To compare HRQoL in MM patients with neuropathy and those without according to s-LANSS.

Chapter 2

Central pain processing in multiple myeloma patients with chemotherapy-induced peripheral neuropathy

2 Introduction

A high incidence of CIPN has evolved due to modern cancer treatments. One such cancer is MM, a haematological malignancy, which is characterised by abnormal proliferation of plasma cells primarily found in the bone marrow. Following the administration of modern anti-myeloma therapies, an accumulating burden of peripheral neuropathy has been recognised (Richardson et al. 2009; Bird et al. 2011). Chemotherapy-induced peripheral neuropathy is thus a common, potentially severe and dose limiting side effect of treatment. Mohty et al reported that the incidence of CIPN is very common, >70% in certain trials of thalidomide and around 40% with bortezomib (Mohty et al. 2010). Although some reversibility may be possible after dose reduction or cessation of the causative agents, there is increasing recognition of the developing chronicity of peripheral neuropathy in many patients. This chronic nature not only potentially compromises the administration of further anti-myeloma therapies but can also generate long term and irreversible pain and disability. In addition, this frequently leads to the need for long-term administration of analgesic drugs with their attendant side effect profiles. In the latter scenario, therapeutic response is often very poor. Overall, the result can be one of reduced QoL and survival, which significantly counters the recent achievements of improved life expectancy in this disease.

Peripheral neuropathy is often considered a disease of the peripheral nervous system, however early studies also report pathology within the spinal cord (i.e. the CNS). Although peripheral abnormalities are often reported, no great advancements in treatment have emerged. Studies using MRI have shown abnormalities in spinal cord in diabetic peripheral neuropathy (Eaton et al. 2001; Selvarajah et al. 2006) even at a sub-clinical stage (Selvarajah et al. 2008). Brain neurochemical and blood flow abnormalities are also reported within the thalamus (Selvarajah et al. 2008; Selvarajah et al. 2011) known to be a principle gateway to the brain's sensory cortex. In diabetic peripheral neuropathy, both peripheral and CNS dysfunction are implicated.

Changes in brain haemodynamic response to painful stimuli have been studied using fMRI. Abnormalities in pain processing as shown on neuroimaging rather than just damage or inflammation to the peripheral nerves, seem to play an important part in chronic painful conditions such as trigeminal neuralgia, post-herpetic neuralgia and chronic low back pain (Giesecke et al. 2004; Geha et al. 2007; Moisset et al. 2011). Our understanding of the pathological mechanisms underlying CIPN and identification of possible future treatment target areas for this debilitating complication may be significantly enhanced by applying fMRI in this context. To our knowledge, there have been no reported fMRI studies in patients with MM who have developed treatment emergent CIPN.

In this study, we sought to determine whether differences exist in central pain processing as assessed by BOLD fMRI, during noxious thermal stimulation between MM patients who develop CIPN and health volunteers. We hypothesised that nerve damage caused by chemotherapy results in the alteration of the cortical and sub-cortical pain matrix, and that in turn contributes to altered pain perception in CIPN-myeloma patients.

2.1 Methods and materials

2.1.1 Pilot Study for the fMRI design

Before proceeding to the main study, a pilot study was performed with healthy volunteers to design and test an fMRI protocol during heat-pain stimulus delivered by a contact heat evoked potential thermode device. Ethics approval was obtained (see section 2.4).

Five healthy volunteers (2 male, 3 female; mean age 36 ± 15 years) were studied to investigate the fMRI technique used for the main study. The volunteers were identified by members of the team (mainly members of staff) and given a healthy volunteer information sheet. Potential volunteers were screened and if willing to undergo this study a written consent form and MRI screening forms were signed prior to scanning.

Inclusion Criteria

- Subjects above the age of 18

- Able to provide informed written consent

- Able and willing to comply to study requirements

Exclusion Criteria

- The presence of any major psychiatric or neurological disorder on examination or medical history

- Claustrophobia

- Pre-existing neuropathy due to other aetiology

- Medications that could affect pain perception, e.g. non-steroidal anti-inflammatory drugs

- Standard MRI exclusion criteria: heart pacemaker, electrical implants.

2.1.1.1   Thermal Stimuli

A contact heat evoked potential device (CHEPS) (Medoc Ltd, Ramat Yishai, Israel) was used for contact thermal stimulation (Figure 2.1). It has a contact diameter of 27mm and uses heating foil technology in combination with a Peltier element. The CHEPS heating rate was set to its maximum 70°C/s, the cooling rate was set to 40°C/s and the baseline temperature was fixed at 32°C. The pathway unit was PC-controlled, enabling the programming of specific thermal stimulation protocols which were delivered by the MR-compatible CHEPS thermode.

Figure 2.1: Medoc Pathway unit (left) and CHEPS thermode used for thermal stimulation (right).

Two experimental protocols for thermal stimulation were tested (Figures 2.2a and 2.2b) and the CHEPS thermode was applied to the dorsum of the right foot.
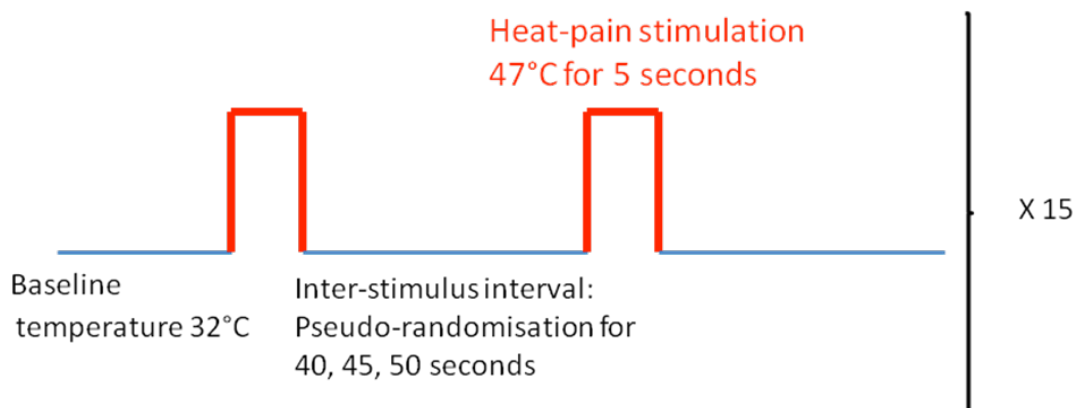
Figure 2.2a: Protocol 1 - Heat-pain stimulation for 5 seconds

A 5-second thermal stimulus (47°C) was applied to the right foot with a random pain-free interval (32°C) of 40,45 and 50 seconds

Starting with 10 seconds at baseline temperature of 32°C, a five second thermal stimulation was delivered at 47°C. This was then followed by a pseudo-randomised inter-stimulus interval of 40, 45 or 50 seconds where the temperature returned to 32°C. This sequence was repeated 15 times. The timings of onset for the heat pain stimuli for this protocol were 10, 55, 105, 160, 205, 260, 310, 355, 405, 460, 505, 555, 600, 655 and 705 seconds.
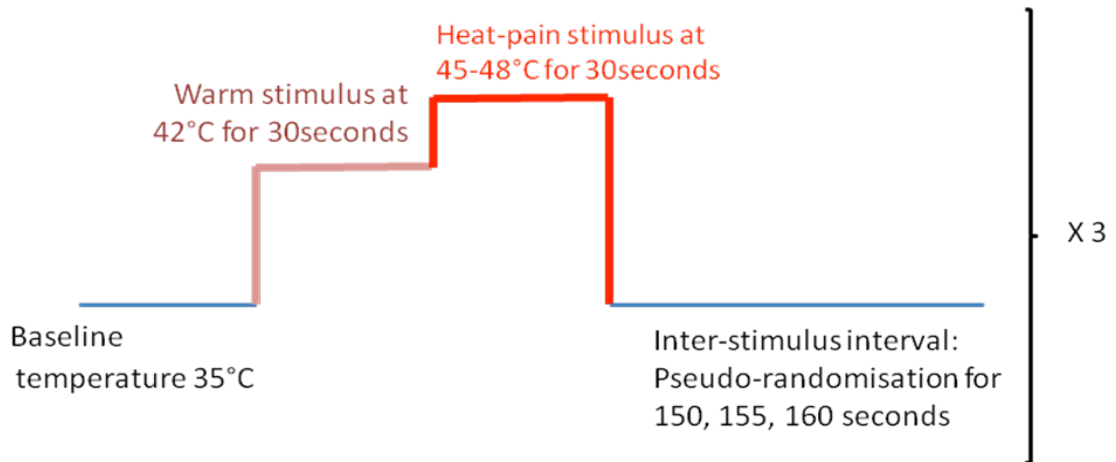
Figure 2.2b: Protocol 2 – Heat-pain stimulation for 30 seconds

A warm stimulation (42°C) for 30 seconds followed by a thermal stimulation (45-48°C) for 30 seconds was delivered with a random pain-free interval (35°C) of 150, 155 or 160 seconds

The protocol 2 sequences started with 30 seconds at a baseline of 35°C and were followed by a warm stimulation sequence of 42°C for 30 seconds and a noxious sequence at the pre-determined temperature (45-48°C) for 30 seconds. The inter-stimulus interval was pseudo-randomised to 150, 155 or 160 seconds where the temperature returned back to 35°C. This sequence was repeated 3 times. The timings of the onset of the heat pain stimuli (noxious) for this protocol were at 60, 300 and 540 seconds.

2.1.1.2 Heat-Pain stimulation

To determine the threshold of thermal stimulation to be delivered during scanning, volunteers received a five second duration stimulation starting at 47°C. They were asked to verbally rate the intensity of the pain on a numerical rating scale (NRS) from zero (no pain) to ten (maximum imaginable pain).

In this pilot study we wanted to achieve a pain rating of ≥ 7/10. The stimulation threshold was increased or decreased accordingly by steps of 1°C, until the verbal

pain rating was ≥ 7 and the volunteers could tolerate this painful stimulus when used inside the scanner. The maximum temperature used was 48°C and the minimum temperature was 45°C.

After the fMRI scan, volunteers were asked to verbally rate the overall intensity of pain experienced inside the scanner on the same NRS and also asked if they were able to anticipate the onsets of the thermal stimulus in the two protocols to give an appreciation which inter-stimulus interval could best remove anticipation.

2.1.1.3 fMRI Acquisition

Data was acquired using a 3.0 Tesla (Achieva 3.0T, Philips Medical Systems, Holland) MRI scanner. The scanning protocol included a standard T1-Wighted, 3-Dimesional and T2-Weighted, 2-dimensionsal anatomical imaging scans prior to functional imaging sequences. The fMRI data sets comprised of single-shot T2* weighted gradient-recalled, echo planar imaging sequence. For each data set 35 slices of 4mm thickness, with an in-lane resolution of 1.8mm x 1.8mm at each imaging time-point or dynamic were captured.

Time to echo (TE) = 35ms; Time to repeat (TR) = 3000 ms; SENSE encoding factor = 1.5. T2* weighted functional imaging runs were carried out at rest (baseline scan), during an auditory-motor finger tapping task and during application of both stimulation protocols using CHEPS to the dorsum of right foot.

Synchronisation between the CHEPS protocol and fMRI acquisition was verified by the scanning operator (physicist) and a member of the team operating the CHEPS thermode.

2.1.1.4 Finger–tapping sequence

Inter-individual variation in non-pain related BOLD response was measured using a motor task sequence, which assessed brain regions known to have no association with the pain matrix. This allowed the assessment of neurovascular-coupling. A 180 second (60 dynamics) scanning sequence was used. A 30 second rest period (ten dynamics) was followed by finger tapping for 30 seconds. This was repeated three times.

The finger task movement consisted of the volunteers touching their thumbs to the index, ring, middle, and little fingers consecutively on both hands at the subjects' own pace at a steady rate. During the rest period, the subjects were asked to relax their hands at their sides. The scanning operator prompted the volunteers to start and stop using the intercom system via headphones. The successful initiation and ending of the finger-tapping sequence according to the auditory cue (start/stop) was monitored and recoded by a member of our team. The volunteers were taught the task and practised it before the fMRI scanning in order to make sure they understood the procedure.

2.1.1.5   fMRI data Analysis

The analysis of imaging data was carried out using statistical parametric mapping software (SPM5). Analysis of functional neuroimaging data involves several steps. Spatial pre-processing was performed and the aim was to reduce unwanted variance components in the voxel time-series that were induced by movement or shape differences among a series of scans.

The process involved realignment, spatial normalisation and spatial smoothing prior to the statistical analysis of the data. This was done for all the images obtained per subject.

The first step was slice time correction, which corrects differences in image acquisition time between slices and this was necessary to make the data on each slice correspond to the same point in time. Afterwards, the individual brains were displayed and reoriented to the anterior commissure as this area is approximately located in the centre of the brain and this alignment was chosen to minimise the influence of inter-individual anatomical differences (Figure 2.3).
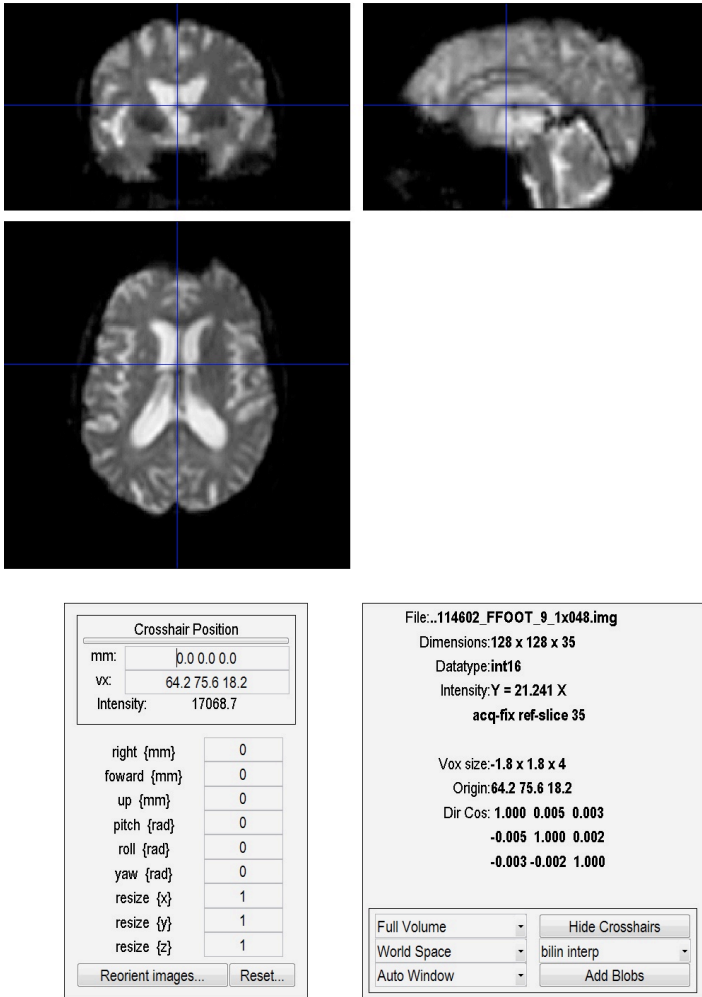
Figure 2.3: Alignment process in SPM displaying an image being aligned to the anterior commissure.

The top-left image is coronal with the top (superior) of the head displayed at the top and the left shown on the left, (as if the subject is viewed from behind).

The bottom-left image is axial with the front (anterior) of the head at the top and the left shown on the left, (as if the subject is viewed from above).

The top-right image is sagittal with the front (anterior) of the head at the left and the top of the head shown at the top, (as if the subject is viewed from the left).

The panel on the left gives information about the position of the cross-hair and the intensity of the image. The positions are reported in both mm and voxels. The coordinates in mm represent the orientation the MNI space. The vx coordinates indicate which voxel is being displayed. Below this are a number of boxes that allow the image to be re-oriented and shifted.

The panel on the right shows various details about the image being displayed including positional and voxel size information.

Realignment (estimate and reslice), also known as motion correction, was then carried out and this realigned a time-series of images acquired from the same subject and used an image (in this case, first slice was used in descending order) as a reference to which all subsequent scans were realigned. The reference scan was chosen as a representative scan. The aim was primarily to remove movement artefact in fMRI time-series. Normalising the images of the study afterwards was performed so that inter-individual averaging becomes a more reasonable statistical application and also so that activation sites can be reported according to their co-ordinates within a standard space and therefore have a precise characterisation of functional anatomy. This module spatially normalised the MRI images into a standard space defined by the template image (standard reference image) supplied by SPM. Spatial smoothing of the realigned and normalised images followed and the purpose of this was to deal with the functional anatomical variability that is not compensated by spatial normalisation and thus improving the ability of a statistical technique to detect true activations. When each image is spatially smoothed, this improves the SNR, but also reduces the resolution, thus a balance must be found between improving the SNR and maintaining the resolution of the functional image. A good estimate of the extent of such a smoothing was given by the full width at half maximum in mm of the Gaussian kernel (8 8 8 as the default in SPM). After finishing this process, the data could be analysed for statistics.

At this point, a statistical model for the data was designed. Firstly, statistics indicating evidence against a null hypothesis of no effect at each voxel were computed and an image was produced. Secondly, the image was then assessed, locating voxels where an effect was shown whilst limiting the possibility of false positives. A statistical model was designed to obtain model parameters which were then used to look for an effect of interest, in this study, the difference between pain-heat stimuli and baseline and to do this a statistic for each brain voxel that tests for the effect of interest in that voxel was calculated and this result was shown as a volume of statistic values. The next step was to decide if the volume showed any evidence of the effect. As it was not known beforehand where to look for an effect, we searched the whole brain and the question was detecting an effect or activation at an unknown spatial location. This presented statistical problems related to the problem of multiple comparisons as functional analyses involve a number of statistical comparisons (Nichols et al. 2005). However, by applying the random field theory, a threshold in a set of data was found and the Gaussian model was used to eliminate type 1 and type 2 errors (Figure 2.4). The SPM5 combines the GLM and

random field theory and allows statistical inference to be made in reference to deviations in the BOLD response from the null hypothesis. Estimation using the GLM of the condition-specific effects was created by convolving a boxcar sequence with the hemodynamic response function.
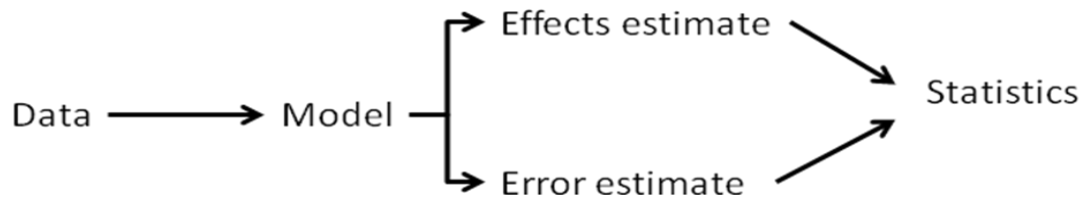
Figure 2.4: Modelling in SPM

The reason for modelling is to make inferences about effects of interest and to decompose data into effects and error and form statistic using estimates of effects and error.

In our experiment we used a block design, as this was adequate for our study since this was an early, exploratory research project and it allowed us to use multi-factorial designs. The parameters used in this study were included as a flexible factorial model. 1st level analysis specified the design matrix, the fMRI data files and filtering estimation of GLM parameters and finally was able to analyse the results using contrast vectors to produce statistical parametric maps of individual brains for each subject. In this step, the conditions were specified and these were different according to the protocol used.

A functional anatomical mask of voxels activated in response to all heat-pain stimuli (compared with baseline) in both groups of subjects (CIPN-myeloma and healthy volunteers) was created at a voxel-level statistical threshold $p < 0.001$, uncorrected. This mask was used as a volume-of-interest for correction for multiple comparisons in subsequent between-group contrasts.

In protocol 1, the conditions specified where:

The onset (seconds) of timing for the heat-pain stimuli were:
10 55 105 160 205 260 310 355 405 460 505 555 600 655 705
The duration of the heat-pain stimuli was 5 seconds.

The onset (seconds) of the timing at baseline (temperature set at 32°C) were:

0 15 60 110 165 210 265 315 360 410 465 510 560 605 660 710

And the duration (seconds) of the baseline were:

40 45 50 40 50 45 45 45 50 40 45 40 50 45 50

In protocol 2, there were 3 conditions specified:

The onset (seconds) of timing for the warm stimuli were:

30 240 480

The duration of the heat-pain stimuli was 30 seconds.

The onset (seconds) of the timing at baseline after the heat-pain stimuli (temperature set at 35°C) were:

90 330 570

The duration (seconds) of the baseline was 150 seconds.

The onset (seconds) of timing for the heat-pain stimuli were:

60 300 540

The duration of the heat-pain stimuli was 30 seconds.

For finger tapping,

The onset for each finger tapping (scans):

10 30 50

The duration was 10 scans.

The design matrix was then produced for each subject.

2nd level analysis was used for group analysis and the flexible factorial design matrix was produced for protocol 1 and 2 (Figure 2.5a and 2.5b).

Statistical analysis: Design
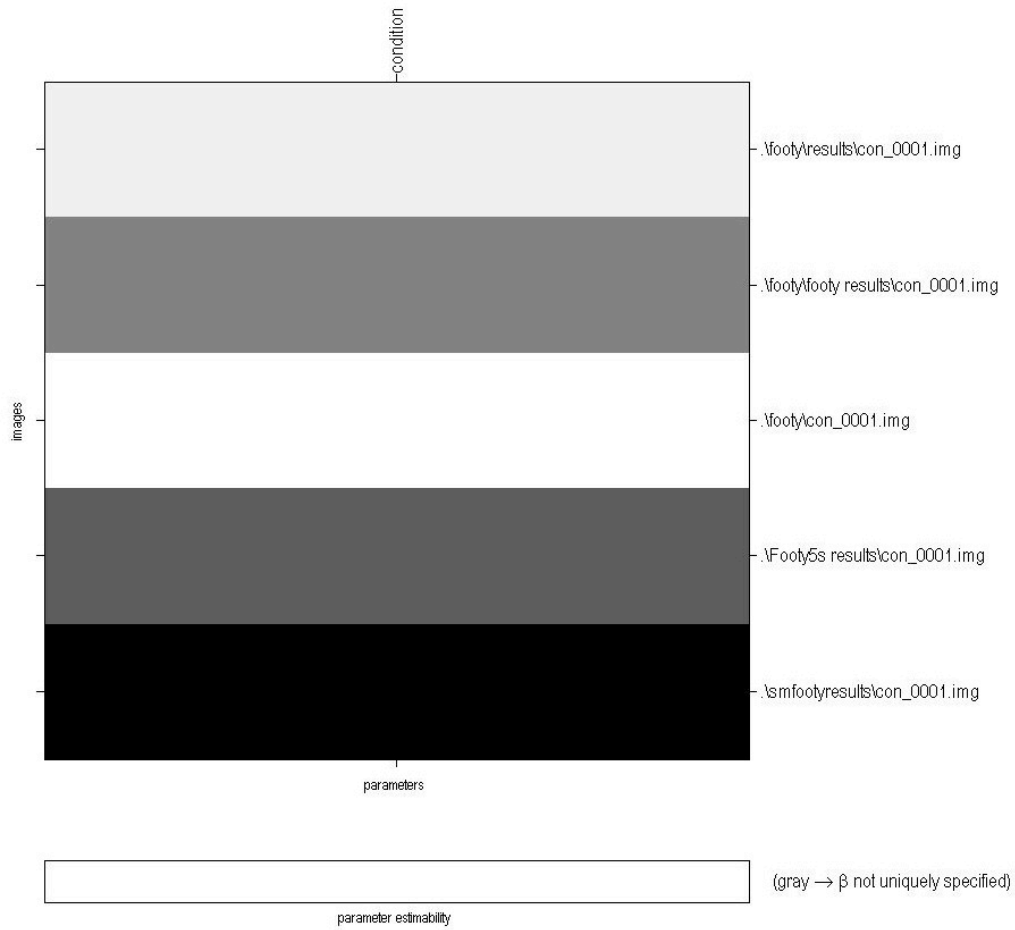
condition

images

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parameters

parameter estimability

(gray → β not uniquely specified)

Design description...

Design : Flexible factorial
Global calculation : omit
Grand mean scaling : <no grand Mean scaling>
Global normalisation : <no global normalisation>
Parameters : 1 condition, +0 covariate, +0 block, +0 nuisance
1 total, having 1 degrees of freedom
leaving 4 degrees of freedom from 5 images

a)

Statistical analysis: Design
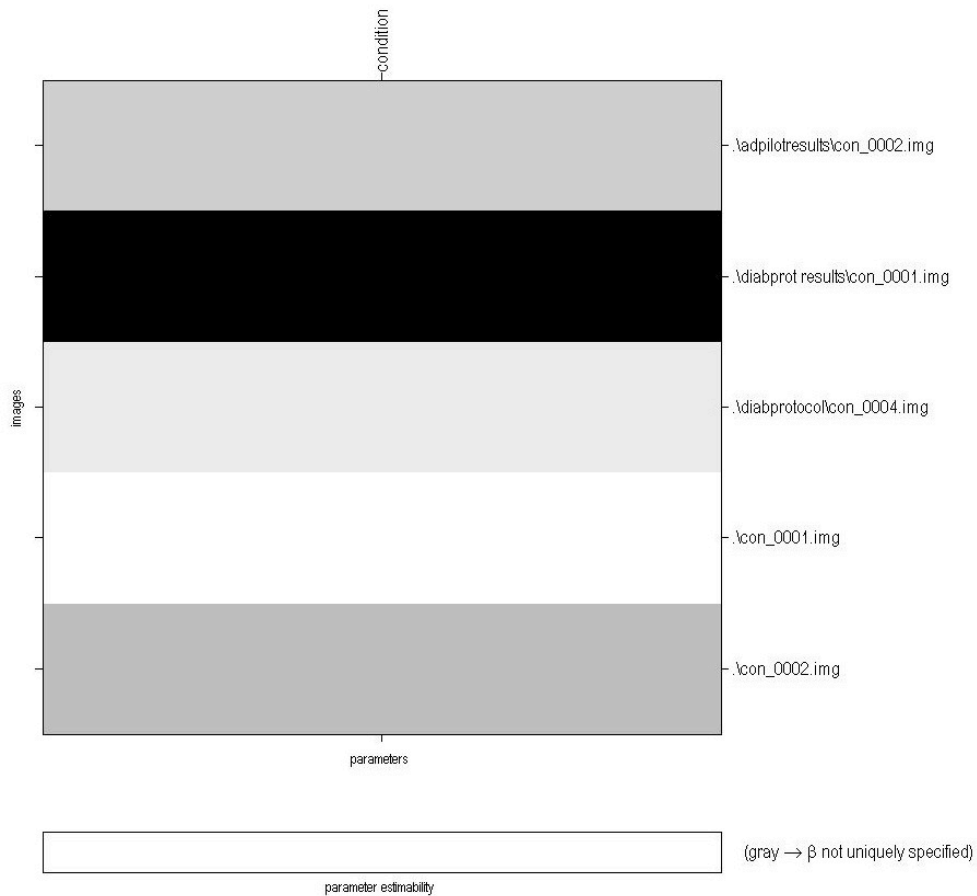
condition

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parameters

(gray → β not uniquely specified)

parameter estimability

Design description...

Design : Flexible factorial
Global calculation : omit
Grand mean scaling : <no grand Mean scaling>
Global normalisation : <no global normalisation>
Parameters : 1 condition, +0 covariate, +0 block, +0 nuisance
1 total, having 1 degrees of freedom
leaving 4 degrees of freedom from 5 images

b)

Figure 2.5: Design matrix after 1st level analysis

   a) For Protocol 1: 5 subjects with duration of heat-pain stimuli for 5 seconds
   b) For protocol 2: 5 subjects with duration of heat-pain stimuli for 30 seconds

The results were evaluated using contrast analysis where a contrast was specified in terms of weights for each condition. In the pilot study it was decided to use spatial extent thresholding (voxel threshold = 55) to exclude isolated or small groups, leaving significant clusters of brain activation behind. In view of such small numbers, the results were not corrected for multiple comparisons and significantly activated regions (p< 0.001, uncorrected) were superimposed onto a T1 average brain. A flexible factorial design was implemented to compare brain activation between groups.

For finger tapping, second level analysis was performed using one sample t-test as the five healthy volunteers data was grouped together and analysed.

All activation results were displayed in the anatomical space as defined by the MNI; however the convention in the neuroscience community is to report brain locations in the standard space of the Talairach and Tournoux atlas (Talairach and Tournoux 1988). The location of regions with BOLD activity was determined by converting MNI (x,y,z) co-ordinates as provided by SPM5 into Talairach co-ordinates, which were then interpreted using the Talairach and Tournoux atlas (Talairach and Tournoux 1988).

2.2 Results of pilot study

Five healthy volunteers were recruited for this pilot study. They all underwent an fMRI scan during which two different protocols were tested on the dorsum of the right foot. Average group activation results for each protocol were calculated and relative activation of the protocol measured.

2.2.1 Pain rating

Ratings for pain intensity were taken before and after MRI scans. The mean and standard deviation (SD) pre-scan rating was 7.6 ± SD 0.9; the mean post-scan rating was 7.8 ± SD 1.3. The mean temperature used for both the thermal stimulation protocols was 47.4 ± SD 1.8°C.

After the MRI scans, volunteers were asked about their pain experience. All volunteers felt that the stimulation during protocol 2 was more painful than for protocol 1 due to a greater unpleasantness associated with the longer stimulus duration.

2.2.2 Group analyses of experimental protocols

The group analysis of the two protocols revealed substantially more regions of activation within areas of the pain matrix with protocol 2 compared to protocol 1 (Figure 2.6). In particular there was extensive activation in the prefrontal cortex (PFC), ACC, insular and thalamic areas with protocol 2 at $p < 0.001$ (cluster threshold) (Table 2.1). On comparison to protocol 2, protocol 1 did not significantly activate any brain regions.

Table 2.1: Areas of BOLD fMRI activation after thermal stimulation for protocol 1 and 2

Neuroimaging Data	Talairach co-ordinates (x,y,z)	t Statistic
Group analysis for Protocol 1		
No significant brain areas activated		
Group analysis for Protocol 2		
Rt anterior cingulate	4, 18, 18	30.99
Lt superior frontal gyrus	-34, 46, 26	24.09
Lt paracentral lobule in SMA	-2, -30, 66	22.26
Rt Medial frontal gyrus	20, 44, 12	19.93
Rt Middle Frontal Gyrus	36, 40, 12	19.16
Rt sub-lobar Insula	26, 28, 6	19.16
Lt inferior frontal gyrus	-24, 32, -2	16.82
Lt anterior cingulate	-16, 40, 8	16.55
Rt Sub-lobar thalamus	4, -4, 4	14.39

Group average data for experimental thermal stimulation protocols are presented. Statistical thresholds were $P < 0.001$, uncorrected for both protocols.

a)
b)
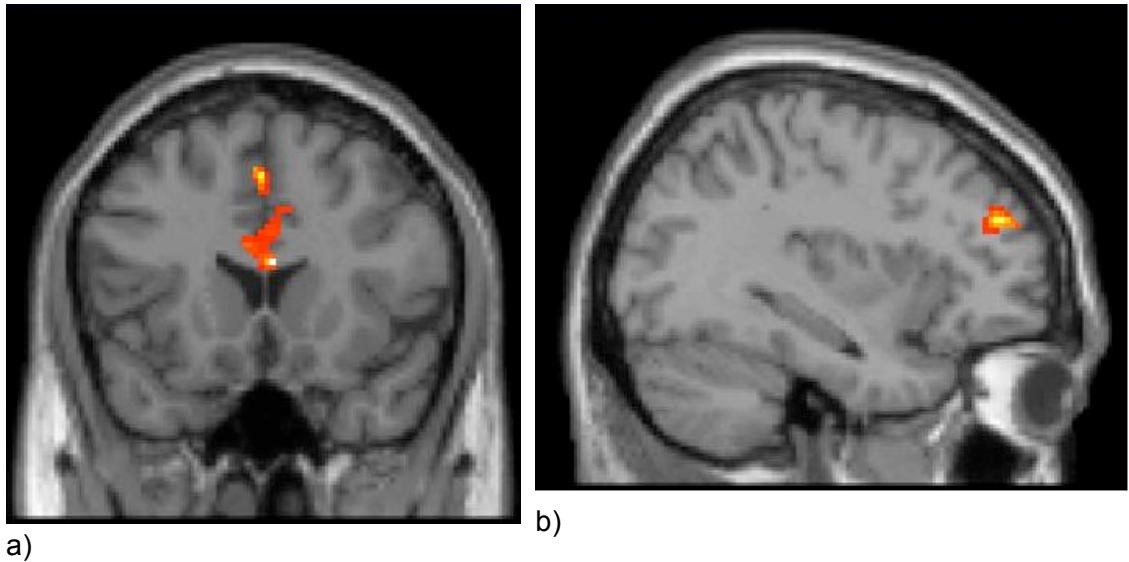
Figure 2.6: Brain activation in 'pain matrix' regions in protocol 2.

Functional image data is displayed against sections through a canonical single subject T1-weighted image showing a) coronal view of the right anterior cingulate gyrus and b) sagittal view of the left prefrontal cortex. For display purposes, the cluster-level threshold is p<0.001, uncorrected, at threshold level (k_E) of 369 and 67 respectively.

2.2.3 Finger-tapping

There was significant activation in the motor areas when the finger tapping data was analysed (Table 2.2). Although the primary motor cortex was only minimally activated, the premotor cortex and SMA were activated bilaterally and there was substantial activity in the cerebellum (Figure 2.7). Bilateral somatosensory cortex activation was present which reflects the sensory input from the feeling of fingers tapping.

Table 2.2: Areas of BOLD fMRI activation for finger tapping (group analysis)

Neuroimaging Data	Talairach co-ordinates (x,y,z)	t Statistic	Z Score
Lt post central gyrus	-42, -24, 52	301.34	6.16
Rt posterior cerebellum	4, -70, -14	118.63	5.54
Rt inferior parietal lobule	52, -36, 44	60.63	5.05
Lt superior temporal gyrus	-52, -2, -4	56.49	5.00
Rt post central gyrus	40, -24, 52	52.85	4.94
		44.89	4.81
Rt inferior frontal gyrus	48, 44, 10	27.45	4.41
Lt, sub-lobar insula	-46, 6, 4	21.91	4.21
Rt medial frontal gyrus	10, 8, 50	20.00	4.13
Lt medial frontal gyrus	-10, -8,58	8.56	3.28
Rt primary motor cortex	16 -26 56		

Group average data for the finger-tapping protocol are presented. The statistical threshold was set to P < 0.001, uncorrected.

(a)   (b)
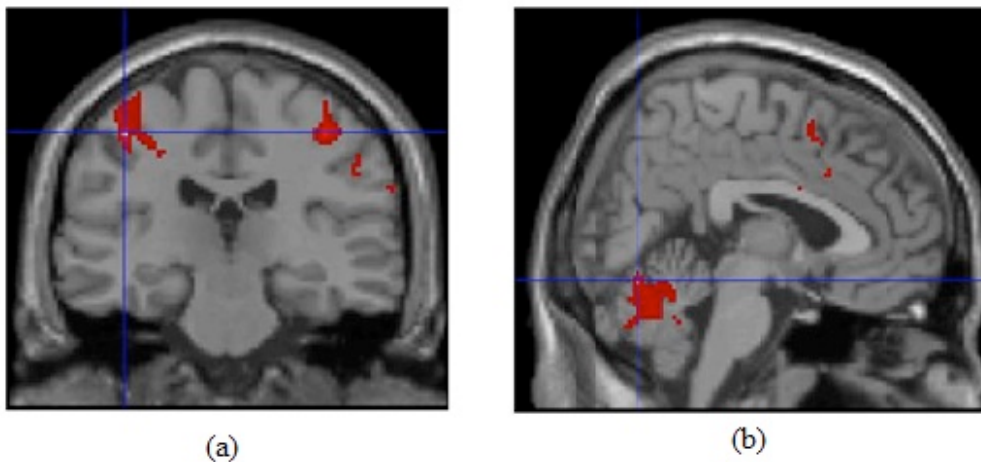
Figure 2.7: Group analysis of activation for finger tapping

Brain activation highlighted on functional image data displayed against sections through a canonical single subject T1-weighted image showing: (a) coronal view of bilateral S1 and pre-motor cortex activation and (b) sagittal view activation of cerebellar areas of activation.

2.3 Discussion of pilot study

In this pilot study, our aim was to test the feasibility and efficacy of the designs for fMRI related protocols. We have shown that it is feasible to capture brain activation in response to noxious thermal stimulation with a CHEPS device using BOLD fMRI. Similarly our finger tapping experiment successfully produced functional brain BOLD responses of the motor areas.

Pain rating

A verbally delivered 11-point numerical rating scale was used for pain rating in the pilot study. The reason for this choice is that the validity of NRS has been well documented and it is extremely easy to administer and score compared to the visual analogue scale as this has more practical difficulties (Rebollo 2008). For the main study, we decided to use the graphically 11-point numeric rating scale.

Noxious thermal stimulation paradigms

Group analysis of fMRI data for five volunteers during CHEPS thermal stimulation with protocols 1 and 2 showed distinct areas of activation that varied between the two protocols. Protocol 1, (5 second thermal pain stimulation durations) revealed no significant areas of BOLD activation in regions associated with pain processing. In contrast, protocol 2 (30 second thermal pain stimulation) produced significant (p<0.001) BOLD activation in many of the regions thought to be involved in the processing of painful stimuli. These brain areas included: ACC, PFC regions, the SMA and the right insula.

We concluded that the difference in mean activation between the two protocols was due to the duration of stimulation; the 5 second stimulation might be too short duration to evoke fMRI response. This pilot data suggested that the greater activation was observed with protocol 2; this could also be due to the consequence of a more unpleasant pain experience associated with a longer duration of pain stimulation. This was supported by the greater pain unpleasantness reported by the patients post-scan for protocol 2.

When we analysed the fMRI images, inter-group comparisons could not be effectively accomplished due to our small population size and no significant

activation with protocol 1. Based on the overall group neuronal activation produced in pain networks with protocol 2, we chose to base the final stimulation design on this protocol. It was decided that when our main study was conducted, a protocol with more stimulation epochs would be more advantageous in producing a more defined neural activation to painful stimulation. We settled on seven separate stimulation epochs, which is shown later on in figure 2.17.

Inter-stimulus Interval

In protocol 1, the inter-stimulus interval was pseudo-randomised to 40, 45 or 50 seconds, while in protocol 2 it varied between 150, 155 or 160 seconds. The purpose of testing varying durations was to try and minimise the ability of patients to anticipate pain. None of the volunteers were able to accurately anticipate the stimulus onsets in both protocols. We therefore decided to choose a shorter duration, which in-turn enabled us to carry out more stimulation epochs in the time available.

Finger tapping

Non-pain related BOLD activation was assessed in this pilot study using finger tapping. In the literature, areas of greatest activation, which have motor involvement, were shown to be bilateral in S1, cerebellum, bilateral in SMA, prefrontal cortex and pre-motor cortices. Similar results for brain activation were obtained in the five volunteers. This demonstrated the feasibility of performing a motor task to assess non-pain related BOLD activation. Instead of the radiographers telling the subject to start and stop finger tapping, we introduced an auditory beep for the main study protocol, to which subjects would tap their fingers in time with, stopping and starting with the audio cue. The aim was to ensure a more consistent rate and rhythm of finger tapping during the main study.

2.4 Overview of the main study

2.4.1 Hypothesis

Peripheral nerve damage caused by chemotherapy in MM results in the alteration of the cortical and sub-cortical pain matrix, and this in turn contributes to altered pain perception in CIPN.

2.4.2 Primary Aim

To determine whether differences exist in central pain processing pathways as assessed by fMRI during noxious thermal stimulation between MM patients with chemotherapy-induced peripheral neuropathy and healthy volunteers.

2.4.3 Secondary Aims

1) To determine the degree to which quantitative sensory testing predicts presence and severity of CIPN.

2) To describe the QoL of patients with MM and chemotherapy-induced peripheral neuropathy, using questionnaires.

The study was conducted in compliance with the EU GCP directive and the ICH Good Clinical Practice, and formally approved by Sheffield Teaching Hospitals NHS Foundation Trust R&D Department following local research ethics committee review.

2.5 Methods and Materials

The study sample comprised of patients with MM who developed CIPN (n=12) and healthy volunteers (n=12). Patients were identified from the haematology clinics and the haematologist explained this study. Afterwards, patient information sheets (Appendix 1) were sent to all subjects. This was followed up a week later where I called the patient to answer any questions and if willing to participate, arrange their study visit. On the day they attended, subjects completed written consent forms. Healthy volunteers were recruited by having posters across the Royal Hallamshire Hospital and on contact a healthy volunteer information sheet was sent out (Appendix 2). A week later this was followed by a phone call by myself to check if they were willing to participate and arrange their study visit. No incentive was given to patients or healthy volunteers except that travel expenses were paid for.

Criteria for subject inclusion for patients with MM

- Age less than 70 years

- Treated with one of the anti-myeloma therapies

    o Thalidomide

    o Bortezomib

    o Vincrisitine

- Neuropathic pain for at least six months duration

- Pain of ≥7/10 on a graphical numerical rating scale

- Willing to discontinue analgesics and co-analgesic (anti-depressants and anti-epileptics) medication 48 hours before fMRI if possible

Criteria for subject exclusion

- Presence of any major psychiatric disorder

- Standard MR exclusion criteria

- Claustrophobia

- Neuropathy caused by other medical conditions

2.5.1 Study visit for neuropathy assessment

2.5.1.1   Quantitative measures

All subjects underwent standard assessments of neuropathy. This included completion of a case report (Appendix 3) form encompassing a detailed clinical history and examination followed by comprehensive neurophysiological testing.

2.5.1.1.1 Neurophysiological testing

Quantitative sensory testing

Quantitative sensory testing refers to a set of methods that extend the traditional neurological examination of somatosensory function and the aim of QST is to provide parameters for sensory loss (small and large fibre functions) and sensory gain (hyperalgesia, allodynia, hyperpathia) (Rolke et al. 2006). The main advantages of QST over standard bedside examination would be greater precision in assessing the functionality of the somatosensory systems. The limitations of QST are that firstly there is no general agreement on standard procedures and every sensory function may be assessed in a variety of ways and even the same test instrument may give different outcomes due to a variation of stimulation parameters. Variability was limited/improved by standardising the technique Secondly, to perform all these tests take a long time and would not be possible to perform in a routine clinic.

The QST assembles a comprehensive list of robust and validated short form tests representing measures of all relevant sub-modalities of the somatosensory system, namely:

cold and warm detection thresholds; mechanical detection threshold; mechanical pain threshold and mechanical pain sensitivity; dynamic mechanical allodynia and vibration detection threshold.

The Aβ-fibre function is represented by the mechanical detection thresholds (MDT) and vibration detection thresholds (VDT). The Aδ-fibre function is represented by the cold detection threshold (CDT) and the mechanical pain threshold for pinprick stimuli. The C-fibre function is represented by the warm detection threshold (Rolke et al. 2006).

The thermal tests and vibration detection threshold were performed using Computer Assisted Sensory Evaluation IV (CASE IV) (W.R. Electronics, Stillwater, MN, USA) (Figure 2.8). Cold and warm detection thresholds were measured. Heat pain detection thresholds were assessed using a porcelain block device, through which pre-defined temperature stimuli were applied to the dorsum of the right foot. The thermal stimulator uses a 4-degree-per-second ramp up and down, and is typically operated in a range from 9 to 49°C, with accuracy of ± 0.25°C. Patients were instructed to rate the randomised heat pain intensities using a numeric rating scale. The NRS is a linear 11 point scale (0-10) which was used consistently in this study. The CDT of right foot was also assessed. Participants were asked to respond to whether randomised temperatures below baseline skin temperature were felt using a yes/no response buttons. Results were entered and recoded by the system.
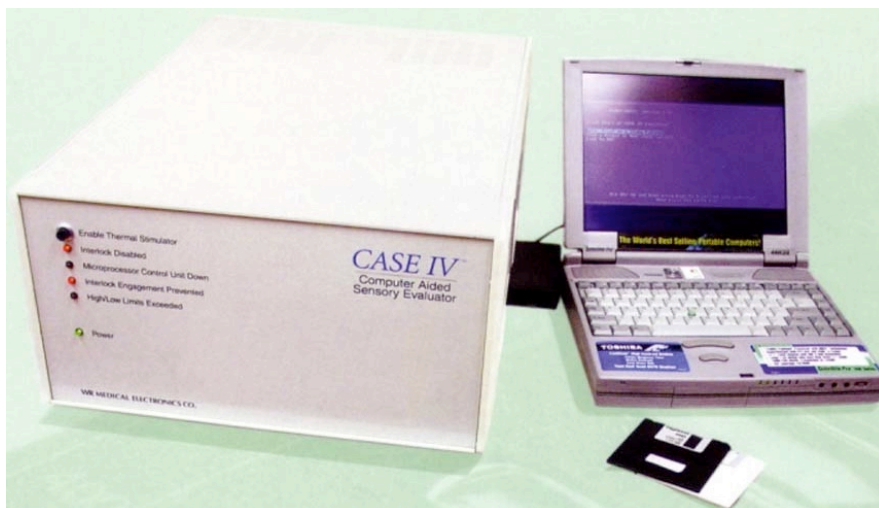
Figure 2.8: Computer Assisted Sensory Evaluation (CASE) IV system

With the CASE IV system, a statistically validated set of age adjusted normative data is provided. Several factors affect the length of testing: the time required to explain the test to the patient, the speed in which the patient makes decisions, the alertness of the patient and the number of stimuli needed to find the threshold (WR Medical Electronics Co 2005). For thermal testing the time also depends on the level of sensitivity because larger stimulus magnitudes (longer times) are needed for insensitive patients.

With regards to vibration detection threshold, an algorithm known as one time period with 4, 2, 1 stepping system was used. This was performed using a 30g preloading weight at the location of the vibrating stylus. This algorithm starts at a baseline level, which then increases in stimulus level if patients are insensitive. The VDT of the dorsum of right big toe proximal to the nail bed was assessed. Participants' responses to vibration frequencies were again recoded using yes/no response buttons.

The QST measurements using the CASE IV System were expressed in the form of percentiles. Values were expressed as percentiles, where >99th percentile was considered to be abnormal. The CASE IV system works by using 25 standardised stimulation levels, which are known as just noticeable differences (JND) for patient testing and analysis. The model of JND steps is based on the fact that a sensitive person can detect fine differences between two levels of stimulation, whereas an insensitive person cannot. These differences of <1 JND steps are difficult to distinguish, thus one JND step is the smallest difference presented to patients. For the vibration and cooling detection thresholds an algorithm known as one time period with 4, 2, 1 stepping is used by the system. This algorithm starts at a baseline level, which then increases in stimulus level if patients are insensitive. The increase/decrease in stimuli level alters, beginning with JND of 4, decreasing by JND of 2 and increasing by a step value of 1, until the individual's cooling and vibration thresholds are determined. Because the 4, 2, 1 rule eliminates additional trials at unnecessary steps, the test is accurate, reliable, and time-efficient. The test is started at a baseline level: for vibration stimuli, the baseline is 0 micrometres of displacement; for cooling stimuli, the baseline is set to 30°C; for heat-pain the baseline is set to 34°C. For warming stimuli, the maximum temperature is limited to 50°C. For cooling stimuli, the minimum temperature was 8°C. (WR Medical Electronics Co 2005).

2.5.1.1.2 Clinical Neuropathy Assessments

When testing with the monofilament, cotton wool and vibration fork, the first test was performed at the subject's chest (over sternum) so the subject could compare the quality of the sensation evoked by each test stimulus as the site of neuropathy was expected to have altered sensation.

The mechanical detection threshold was measured at the plantar surface of the big toe and the pad of the index finger using a standardised nylon Semmes-Weinstein monofilament. It delivers a 10-gram force when it applied perpendicularly to the skin's surface and buckles. The contact area with the skin is uniform to avoid sharp edges that would facilitate nociceptor activation. Each subject was instructed to close their eyes during this test and were asked if they could feel the stimulus and to identify the location of the filament (Ruppert M et al. 2004; Felix and Widerstrom-Noga 2009).

Mechanical pain sensitivity was assessed using a pinprick stimulus to obtain a stimulus response function for pinprick-evoked pain. Subjects were asked if they felt the pinprick and whether it was painful. This test was designed to detect pinprick hyperalgesia. Dynamic mechanical allodynia was assessed using a light tactile stimulator (innocuous stimulus), a cotton wool tip fixed to an elastic strip. The tactile stimulus was applied with a single stroke of approximately 2 cm in length over the skin.

The Neuropathy Impairment Score – Lower Limbs plus 7 tests (NIS (LL) +7), provides a composite measure of the neuropathic impairments of lower limbs. This was used and is discussed later under section 2.5.1.1.5.

2.5.1.1.3 Nerve Conduction Studies (NCS)

The use of NCS is to help localise the site or level of the lesion; determining if the pathology involves the peripheral nerve, neuromuscular junction, plexus, nerve root or anterior horn cells; help identify the pathophysiology, in particular distinguishing axonal loss from demyelination; diagnose mononeuropathies and diagnose more diffuse processes. Nerve conduction studies are also used to monitor nerve function over time to determine disease progression and to assess the complications of treatment (eg. chemotherapy). Nerve conduction studies correlate well with the morphological changes seen in nerve biopsies (Veves et al. 1991) and with clinical

testing and examination (Redmond et al. 1992; Feki and Lefaucheur 2001). These tests measure sensory and motor nerve function transmitted by impulses from large (myelinated) fibres that make up only 25% of the total nerve fibre population. Electrophysiological tests are therefore not sensitive to small fibre damage (Dyck et al. 2003). Therefore, NCS cannot be used as the only tests in the investigation of CIPN (Cleeland et al. 2010).

The parameters most commonly tested in NCS are peripheral nerve conduction velocity (NCV), summated amplitude, conduction latency and motor nerve F-Wave latency. In the upper limb, the median and ulnar nerves; sensory and motor are usually tested. In nerve fibre demyelination, there is a decreased latency and NCV whilst in nerve fibre loss there is a reduction in peripheral nerve summated amplitudes.

Several factors alter the rate of nerve conduction. Most important in a clinical laboratory are temperature of the tested nerve, normal variations among nerves and nerve segments, and patient age (Kimura 1984). It is best to conduct studies in a warm room with the temperature maintained between 21 and 23°C. If the skin temperature falls below 34°C, the limbs should be warmed. Sensory and motor conduction velocities are substantially slower in the legs than in the arms. Longer nerves may generally conduct more slowly than shorter nerves and conduction velocity is generally faster in the proximal than in the distal segments of a nerve.

Common sources of errors include the spread of the stimulating current to a nerve not under study, eliciting an unwanted potential from distant muscles; the presence of an anatomises between the nerves or anomalous innervations and errors inherent in the measurement of nerve length and conduction time (Kimura 1984).

I attended training sessions and performed all the nerve conduction studies. Training sessions included a regular attachment with a clinical consultant neurophysiologist at the Royal Hallamshire hospital and also an attachment to the Diabetes centre where nerve conduction studies were being carried out with a Consultant Diabetiologist (with neuropathy assessment expertise). Competency was established prior to assessing study subjects by performing repeat nerve conduction measurements on 20 normal controls to ensure reproducibility of methods precision of results and minimise intra-examiner variability had been achieved.

Nerve conduction equipment

The nerve conduction study was performed using the Medelec, Synergy Oxford Instruments, Oxford UK. The temperature was maintained to at least 31±1°C during the entire nerve conduction study. Room temperature was around 24±2°C. Skin preparation (cleaning the skin with alcohol and then abrading the skin with a mild dry abrasive material) was performed prior to starting nerve conduction studies to minimise shock artefact and improve the quality of the study. The ground electrode was placed between the active recording electrode and the stimulating electrode. The strength of the stimulus was increased in steps until a maximum response was obtained. When maximum response was obtained the stimulator was increased by 5-10% more to make sure the response was supramaximal.

Measurements

All distances were measured to the nearest 1.0mm. Extreme care was taken to use the same distance for each subsequent test. All waveform measurements were made at the sweep speed and display sensitivity at which the waveform was acquired.

Sensory NCS were performed by electrical stimulation of a peripheral nerve and recording from a purely sensory portion of the nerve. Sensory latencies were calculated in milliseconds (ms). Sensory amplitudes were much smaller than the motor amplitudes, usually in the microvolt (μV) range. The sensory NCV was calculated based upon the latency and the distance between the stimulating and recording electrode (Figure 2.9).
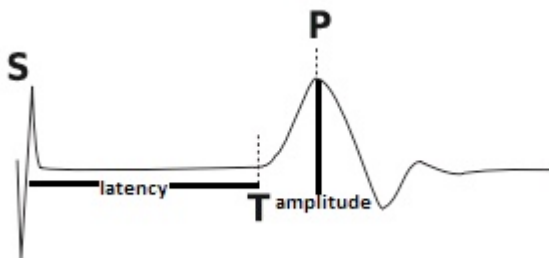
Figure 2.9: Sensory Wave Form

S= Stimulus point, T = Take off point, P = Peak. The time latency from S to T is typically about 3 milliseconds.

Motor NCS were performed by electrical stimulation of a peripheral nerve and recording from the muscle supplied by the stimulated nerve. The electrical impulse was measured from the time it travelled from the stimulation to the recording site; this is latency and was measured in ms. The amplitude (size of the response) was also measured in millivolts (mV). By stimulating two different locations along the same nerve, the NCV across different segments was determined. The distance between the different stimulating electrodes and the difference in latencies was calculated.

Sural Nerve Sensory Nerve Conduction Study

The sural nerve innervates the lateral and the posterior third of leg; the lateral aspect of foot & heel, and the lateral portion of the ankle. This nerve, formed by the junction of the medial sural cutaneous with the peroneal anastomotic branch, passes downward near the lateral margin of the tendo calcaneus, lying close to the small saphenous vein, to the interval between the lateral malleolus and the calcaneus. The sural nerve passes down to the posterolateral side of leg and onto the dorsal aspect of lateral side of foot, giving rise to lateral calcaneal branches (Gray and Standring 2005).

The right sural nerve was examined in the left side-lying position. The active recording electrode was placed immediately behind the lateral malleolus. The ground electrode was placed between the stimulating and recording electrodes. The sural nerve was stimulated 14.0cm from the active recording electrode in the midline of the posterior lower leg (Figure 2.10).

Figure 2.10: Stimulation of the right sural nerve

This shows the active recording electrode placed behind the lateral malleolus and the nerve was stimulated in the midline of the posterior lower leg

The sweep speed was set at 1.0ms/division. The amplifier gain/display sensitivity was set at 10μV/division. When a supramaximal response was obtained, this marked with the cursors. The distance between the active electrode and the site of the cathode stimulating electrode were measured and recorded.

Peroneal Motor Nerve conduction Study

The deep peroneal nerve begins at the bifurcation of the common peroneal nerve, courses anteriorly around fibula and passes obliquely forward beneath the extensor digitorum longus. It comes into relation with the anterior tibial artery and supplies anterior compartment muscles where it then descends to the front of the ankle joint and divides into a lateral and a medial terminal branch. The terminal lateral branch curves laterally and supplies the extensor digitorum brevis, extensor hallucis brevis, the adjacent tarsal and tarsometatarsal joints and occasionally, the second and third dorsal interosseous muscles. The medial terminal branch divides into two sensory nerves that supply the adjacent sides of the great and second toes with sensation (Gray and Standring 2005).

The nerve was stimulated at the ankle and the fibula head at the knee. The active electrode was placed over the centre of the palpable portion of the extensor digitorum brevis muscle on the lateral aspect of the dorsum of the foot. The reference electrode was placed over the fifth metatarsal-phalangeal joint. For the ankle stimulation site, the cathode was placed on the anterior aspect of the ankle, lateral to the tendon of the tibialis anterior muscle (8cm from the active electrode). For the fibular head stimulation site, the cathode was placed slightly posterior and inferior to the fibula. The distance between the active recording electrode and the cathode of the stimulator was measured. The distance between: 1) the active electrode at the ankle and the site of the stimulating cathode electrode; 2) the ankle and fibula head.

 Tibial motor nerve conduction study

The tibial nerve is the larger of the two divisions that form the sciatic nerve. It originates from L4 through S3 roots and travels as part of the sciatic through the posterior thigh under the long head of the bicep femoris. The tibial nerve passes through the popliteal fossa to pass below the arch of soleus. It supplies the motor

innervation of the posterior compartment of the leg and all foot muscles (except the extensor digitorum brevis). Its motor distribution in the leg includes the gastrocnemius, plantaris, soleus, popliteus, tibialis posterior, flexor digitorum langus and flexor hallucis longus. The nerve is superficial as it passes vertically down the middle of the popliteal fossa. It lies lateral then crosses superficially to lie medial to the popliteal artery. As it leaves the fossa, it gives off the sural nerve supplying the posterior leg. Its course then becomes deep as it passes between the gastrocnemius and soleus muscles, under the tendinous arch formed by the soleus, lying on the posterior surface of the tibialis posterior muscle and then on the tibia. It again becomes superficial at the upper end of the medial malleolus where it passes behind it and in front of the calcaneous tendon. Its terminal course takes it under the flexor retinaculum (posterior tarsal tunnel). The medial calcaneous branches perforate the flexor retinaculum and supply the skin of the heel and medial side of the sole of the foot. Under the retinaculum the nerve divides into medial and lateral plantar branches and innervates the plantar aspect of the foot (Gray and Standring 2005).

The active electrode was placed over the centre of the abductor hallucis muscle anterior and inferior to the navicular tubercle. For the distal stimulation site at the ankle, the cathode was placed on the medial aspect of the ankle 8.0cm proximal to the active recording electrode. For the proximal stimulation site, the cathode was placed in the middle of the popliteal fossa. The distance between the active recording electrode at the ankle and the site of the stimulating electrode were measured. The distance between the ankle and the knee were also measured.

2.5.1.1.4 Autonomic testing

There is a variety of tests performed for specific autonomic testing mainly including cardiovagal or parasympathetic test, adrenergic or sympathetic tests and sudomotor tests (Biaggioni 2011). Computer assisted autonomic function tests were performed in this study to assess if autonomic neuropathy was present in patients with CIPN. The simplest way to measure autonomic function is by studying the cardiovascular system. A 3–lead electrocardiogram (ECG) was recorded after the subject had rested supine for five minutes (Burdick Eclipse LE Electrocardiograph, Burdick Inc., Milton, Wisconsin USA) and the ECG leads were connected to an ATARI 360S microprocessor on a computer. Both were equipped with analogue to digital signal converters and QRS algorithm recognition software sampling at 600Hz.

Assessment of the parasympathetic and sympathetic autonomic function was conducted by measuring the heart rate variability (HRV). This involved measuring the beat-to-beat variation in heart rate and was accomplished by measuring the R-R interval variation (Figure 2.11):
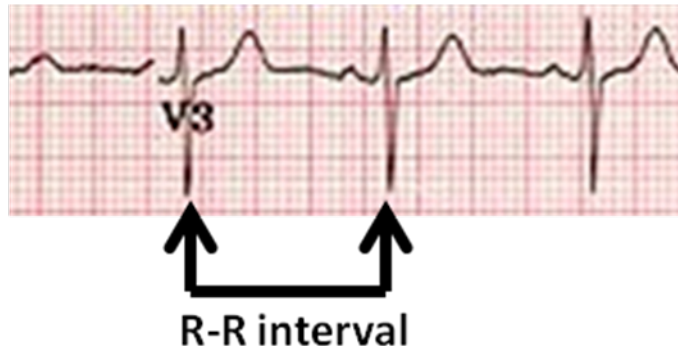
Figure 2.11: The R-R intervals on an ECG trace

i) At rest, heart rate was obtained after the subject had rested supine for five minutes,

ii) During deep breathing where patients were asked to take slow deep breaths at a rate of six cycles/min. Results were expressed as the ratio of the peak inspiratory heart rate to the lowest expiratory heart rate during a single cycle (O'Brien et al. 1986),

iii) During valsalva manoeuvre where patients were asked to exhale into a tube connected to a mercury sphygmomanometer and maintain a pressure of 40mmHg for 15 seconds. On relaxation, ECG recording continued for a further 45 seconds. The valsalva ratio was calculated as the ratio of the highest heart rate achieved during the manoeuvre to the lowest heart rate during the relaxation period (Ewing and Clarke 1982).

iv) Lying-standing. The peak heart rate (due to sympathetic activation) occurs at approximately the 15th beat after standing; as vasoconstriction occurs and blood pressure rises towards baseline, heart rate falls, reaching a nadir at approximately the 30th beat (Ewing and Clarke 1982). Therefore, the maximum and minimum R-R ratio is defined as the longest R-R interval during beats 20-40 divided by the shortest R-R interval during beats 5-25.

Orthostatic hypotension was assessed by measuring the postural fall in blood pressure using a mercury sphygmomanometer 60 seconds after standing and

sympathetic autonomic failure was diagnosed when the level fell by at least 20 mmHg.

Physiologically, heart rate variations should be present at rest. In parasympathetic denervation, these variations are lost. Normally, heart rate changes according to breathing, standing from the lying/sitting position, increased intra-thoracic pressure and sympathetic activation. O'Brien et al. have standardised a number of simple tests to evaluate autonomic nerve function (O'Brien et al. 1986) and age-related normative values have been established.

Autonomic neuropathy may also affect small fibres in the periphery, specifically those innervating blood vessels and sweat glands. Quantitative sudomotor axon reflex testing is a recognised test in the evaluation of small fibre neuropathy (England et al. 2009); however this was not performed in this study.

2.5.1.1.5 Assessment Tools

Total Neuropathy Score (reduced version)

The Total Neuropathy Score (reduced version) TNSr, has been used to stage neuropathy in MM patients and this was used in this study (Table 2.3) (Cavaletti et al. 2007). A composite score (0-32) is used and it evaluates motor and sensory symptoms and signs, quantitative determination of the pinprick and vibration perception threshold, and the neurophysiological examination of one motor and one sensory nerve in the leg.

The presence of neuropathy was defined as TNSr >2. A higher score indicates worse peripheral neuropathy.

Table 2.3: Total Neuropathy Score (reduced version)

Parameter	0	1	2	3	4
Sensory symptoms	None	Symptoms limited to finger & toes	Symptoms extend to ankle & wrist	Symptoms extend to knee & elbow	Symptoms above knee & elbow, functionally disabling
Motor symptoms	None	Slightly difficult	Moderately difficult	Require help/ assistance	Paralysis
Pin sensibility	Normal	Reduced in fingers & toes	Reduced in ankle & wrist	Reduced in knee & elbow	Reduced to above elbow & knee
Vibration sensibility	Normal	Reduced in fingers & toes	Reduced in ankle & wrist	Reduced in knee & elbow	Reduced to above elbow & knee
Strength	Normal	Mild weakness	Moderate weakness	Severe weakness	Paralysis
Tendon reflexes	Normal	Ankle reflex reduced	Ankle reflex absent	Ankle reflex absent, others reduced	All reflexes absent
Sural Nerve amplitude	Normal/ reduced to <5% LLN	76 to 95% of LLN	51 to 75% of LLN	26 to 50% of LLN	0 to 25% of LLN
Peroneal Nerve amplitude	Normal/ reduced to <5% LLN	76 to 95% of LLN	51 to 75% of LLN	26 to 50% of LLN	0 to 25% of LLN

ULN = Upper limit of normal; LLN = Lower limit of normal

Neuropathy Impairment Score (NIS)

A group of muscles both right and left sides were evaluated for weakness:

1 - 25% weak; 2 - 50% weak; 3 - 75% weak; 3.25 - movement against gravity; 3.5 - movement with gravity eliminated; 3.75 - muscle flicker without movement; and 4 - paralysis.

Muscle stretch reflexes were graded as:

normal, 0; decreased, 1; or absent, 2.

Touch-pressure, vibration, joint position and motion, and pinprick were graded on the dorsal surface at the base of the nail of the terminal phalanx of the index finger and great toe as:

 normal, 0; decreased, 1; or absent, 2 (Dyck et al. 1997).

Pinprick was assessed with a straight pin, vibration was also using 128Hz tuning fork at the ankle and first metatarsal-phalangeal joints. The tuning fork was struck and then held to the skin. The examiner timed how long the vibration was perceived. Less than 10 seconds was considered impaired. Joint motion was tested by moving the terminal phalanx of the index finger and great toe, evaluated by the responses to ten questions. The neurological examination from which the NIS of the lower limbs (Dyck and Thomas 1999) was derived was used to calculate the neuropathy composite score for the Dyck's score (Dyck et al. 1997; Dyck et al. 2003).

Dyck's score

There is no reliable marker that could be used to test for CIPN. The Dyck's neuropathy score, validated for diabetic peripheral neuropathy, provides an indication of neuropathic impairment in the form of a composite neuropathy score (Table 2.4). It looks at quantitative sensory testing including nerve conduction studies and autonomic nerve function together with clinical examination and reported symptoms (Dyck et al. 1997). The neuropathy impairment score for the lower limbs plus seven tests (NIS lower limbs+7) is considered to be the gold standard for the detection of diabetic neuropathy (Dyck and Thomas 1999). It measures muscle weakness, reflex loss, sensory loss and nerve conduction abnormalities in the lower limbs, and autonomic heart rate abnormalities during deep breathing. The Dyck's staging criteria for scoring neuropathy is: ≥4.5 = clinical neuropathy; 1 – 4.5 (with experimental evidence of nerve dysfunction) = sub-clinical neuropathy and 0 = no neuropathy.

Table 2.4: Calculating Dyck's neuropathy composite score
(Dyck et al. 1997)

1. Score items 17-24, 28-29 and 34-37 of NIS (lower limbs). The vibration score was substituted with percentile abnormality* of VDT for each great toe for the clinical vibration sensation point score (as calculated using CASE IV).
2. Add the transformed points for percentile abnormality HRV of deep breathing
3. Summate transformed points for the percentile abnormality* of five attributes of nerve conduction studies for lower limbs: - Peroneal nerve amplitude, velocity and latency   - Tibial nerve latency   - Sural nerve amplitude

*Transformed points for percentile abnormality are defined as the values corresponding to confidence intervals using the standard deviation of normal value < 95th = 0; ≥ 95th – 99th = 1; ≥ 99th – 99.9^{th} = 2; ≥ 99.9^{th} = 3 (≥5th = 0 to ≤0.1th = 3) (appendix 4).

For point 3: Summate transformed points for percentile abnormality of the five attributes of nerve conduction of lower limb: divided by the number of attributes with obtainable values (*motor nerve conduction velocity and distal latency cannot be estimated when compound muscle action potential is 0), multiply by five (the number of attributes), and add this number to the global score.

2.5.1.2 Qualitative measures: Questionnaires

Health-related quality of life is an important assessment of the impact of disease or the related treatment on the patient's physical, social and psychological functioning. Consistently, studies have reported reduced QoL in patients with neuropathic pain (Jensen et al. 2007). Ensuring accurate and reliable reporting of the health-related quality of life and pain, several assessments were used.

2.5.1.2.1 EORTC QLQ-C30 and the myeloma module QLQ-MY20

EORTC QLQ-C30 is a 30 item self-reporting questionnaire developed to assess the QoL of cancer patients. It contains five functional subscales (role, physical, cognitive, emotional and social functioning) as well as several symptom scales (Aaronson et al. 1993). The QLQ-MY20 module is used to gain extra information about health-related QoL in patients with MM, particularly issues around body image and future perspective. It is recommended that the QLQ-C30 and QLQ-MY20 are used together to measure QoL in trials in MM (Cocks et al. 2007). These health-related quality of life assessments are important for evaluating the benefits against

the toxic effects of the treatment and this is especially important in relapsed MM patients whose survival is limited.

2.5.1.2.2 Self-report Leeds Assessment of Neuropathic Symptoms and Signs (s-LANSS)

This is a self-reporting screening tool that aims to identify pain of predominantly neuropathic origin, as distinct from nociceptive pain (Bennett et al. 2005). The domains assessed are: prickling/tingling, mottled/red/pink skin, sensitive skin, bursting/sudden pain, hot/burning, allodynia, and numbness/tenderness. If a patient registers 12 or more on this scale then there is a strongly indicative that neuropathic pain is present to some degree.

2.5.1.2.3 Neuropathic Pain Scale (NPS)

This scale is designed to assess distinct pain qualities associated with neuropathic pain (Galer and Jensen 1997). The NPS is a self-report scale for measuring neuropathic pain. It consists of 10 distinct questions, which ask about the intensity and quality of the patient's pain: two global (intensity and unpleasantness) and eight specific ratings that assess both pain quality (sharp, dull, cold, hot, sensitive and itchy) and pain location (surface and deep). In validation studies, it has been found to have a good predictive power in discriminating between subgroups of patients with neuropathic pain (Jensen et al. 2006; Rog et al. 2007). The patient rates the dimensions on a numerical scale 0-10. The NPS has been used in several neuropathic pain double blind trials mainly as a secondary outcome, sometimes as a primary outcome and also used in reporting effects of treatments on specific items (Haanpaa et al. 2011).

2.5.1.2.4 Neuropathy Total Symptom Score (NTSS 6)

The NTSS-6 was designed to evaluate individual neuropathy sensory symptoms in patients with diabetes mellitus and diabetic peripheral neuropathy. The NTSS-6 showed internal consistency, test-retest reliability, and construct validity (Bastyr Iii et al. 2005). The dimensions are deep aching pain, burning pain, pickling/tingling sensation, numbness/dead feeling, lancinating/ electrical shock-like pain and allodynia. Each symptom is graded on intensity and frequency (Figure 2.12).

| | Symptom Intensity | | | |
Symptom Frequency	Not Present	Mild	Moderate	Severe
Never or occasional (normal amount)	0.00	0.00	0.00	0.00
Occasional but abnormal (less than 1/3 of the time)	0.00	1.00	2.00	3.00
Often (1/3 to 2/3 of the time)	0.00	1.33	2.33	3.33
Almost continuous (more than 2/3 of the time)	0.00	1.66	2.66	3.66

Figure 2.12: NTSS-6 Scoring sheet

The scores for the NTSS-6 range from 0 to 21.96. A score >0 indicates the presence of >1 sensory symptom. Clinically significant symptoms are defined as an NTSS-6 total score >6 points (either 6 symptoms with at least mild intensity and occasional frequency, or 3 symptoms with at least moderate intensity and occasional frequency, or 2 symptoms with severe intensity and occasional frequency). We used the NTSS-6 in our study to try and identify descriptions of sensory symptoms experienced by myeloma patients.

2.5.1.2.5 Chronic Pain Acceptance Questionnaire (CPAQ)

The CPAQ provides information on how chronic pain affects patients. It shows the level of acceptance of their chronic pain and their focus on participation in activities, the pursuit of personally relevant goals and the relative absence of attempts to control or avoid pain, respectively. The CPAQ was originally constructed as part of the development of an acceptance oriented treatment approach for pain patients. A revised 20-item version of the instrument with two subscales has shown adequate reliability and validity (Vowles et al. 2008). Data regarding the CPAQ indicated fully adequate internal consistency with Cronbach's alpha of 0:85. (McCracken et al. 2004). The two subscales describe: the degree to which one engages in life activities regardless of pain (activity engagement, 11 items), and willingness to experience pain, which is the inverse of engaging in behaviours to limit contact with pain (pain willingness, 9 items). A single total score was calculated on the basis of the nine reverse-keyed items and the remaining 11 items measuring activities engagement. The CPAQ is rated on a 0 (never true) to 6 (always true). The

CPAQ total score ranges from 0 to 120, with a higher score indicating higher acceptance of pain. Two components were derived for scoring: activities engagement and pain willingness. A single total score was calculated on the basis of the nine reverse-keyed items and the remaining 11 items measuring activities engagement.

Activity engagement (pursuit of life activities regardless of pain). Items – 1, 2, 3,5, 6, 8, 9, 10, 12, 15, 19.(2)

Pain willingness (recognition that avoidance and control are often unworkable methods of adapting to chronic pain) reverse score items – 4, 7, 11, 13, 14, 16, 17, 18, 20

2.5.1.2.6 Pain Catastrophizing Scale (PCS)

This is a 13-item self-report scale whose items are rated on a 5-point Likert-type scale ranging from 0 (not at all) to 4 (always) the degree to which they experienced each of 13 thoughts or feelings when in pain. This has three different categories to assess negative thinking styles related to pain: rumination, magnification, and helplessness. Coefficient alphas were .87, .60, and .79 for the rumination, magnification, and helplessness subscales, respectively and for the total PCS was .87(Sullivan and Bishop 1995). The PCS total score is computed by summing responses to all 13 items. The PCS total scores range from 0 – 52. The PCS subscales are computed by summing the responses to the following items:

Rumination: Sum of items 8, 9, 10, 11

Magnification: Sum of items 6, 7, 13

Helplessness: Sum of items 1, 2, 3, 4, 5, 12

The PCS proves to be a useful research instrument in efforts to understand the psychological processes that lead to heightened physical and emotional distress in response to aversive stimulation and it is a reliable and valid measure of catastrophising.

2.5.1.2.7 Hospital Anxiety and Depression Score (HADS)

To determine the presence of depression and anxiety, the HADS is frequently used (Zigmond and Snaith 1983). It is a validated 14-item questionnaire measuring anxiety and depression in two separate subscales. This provides a simple yet reliable tool and measures levels of psychological functions using two subscales, which can be added together to give a measure of overall psychological distress. Cronbach's alpha has ranged from 0.80 to 0.93 for the anxiety subscale and 0.81 to 0.90 for the depression subscale (Herrmann 1997).

Neurophysiological testing, total neuropathy scoring and qualitative assessment was performed to be able to grade, score and report neuropathic dysfunction, impairment and symptoms using standard and reproducible approaches so that results were consistent, quantitative and useful.

2.6 Study visit for functional Magnetic Resonance Imaging

Prior to imaging, the intensity of dynamic heat stimulus necessary to generate pain response of at least 7cm on a 10cm numerical rating scale (Figure 2.13) was identified at two anatomical sites: the dorsum of the foot and upper anterior aspect of the thigh. This was done due the high inter-individual differences of skin thickness and susceptibility to pain. Patients were asked to verbally rate their level of perceived pain intensity on a graphical numerical scale from 0 to 10.
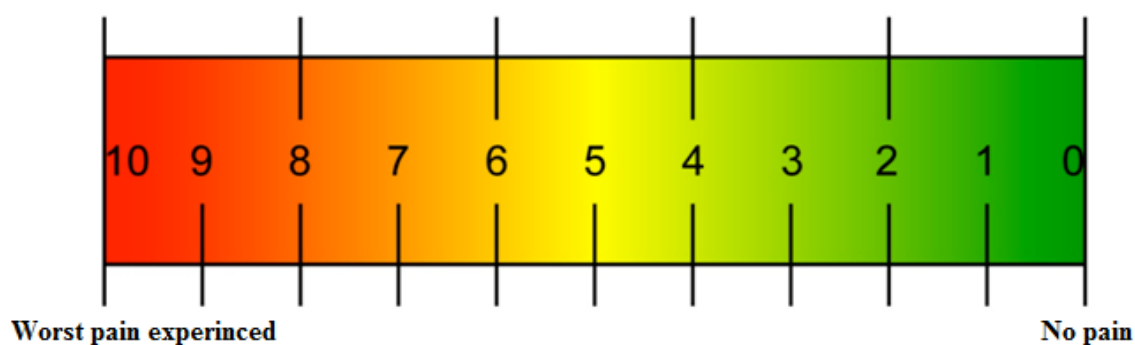
Figure 2.13: Graphical numerical rating scale

The foot was chosen as this was the area where the patients with chemotherapy-induced neuropathy had the pain; whilst the thigh was an area where patients had no pain as shown in figure 2.14, so this will be causing purely acute pain.

a) b)
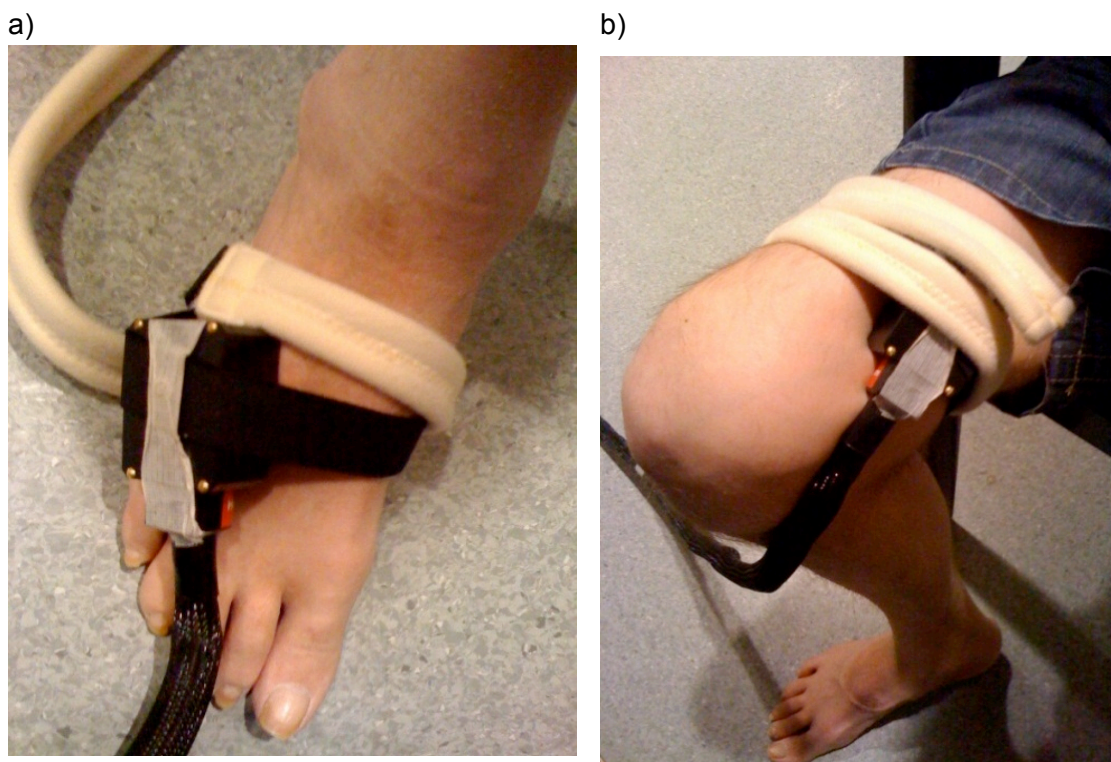
Figure 2.14: CHEPS thermode application to right foot and right thigh

All heat-pain stimulation was applied to the right hand side of the body for each participant. The experimental thermal stimulus was delivered using a contact heat evoked potential device (CHEPS), which rapidly delivers heat pulses with adjustable peak temperatures. This stimulus was applied using an MR-compatible, commercially available computer controlled peltier-type thermode (Medoc Pathway System, Medoc Ltd, Ramat Yishai, Israel). The foot was chosen as the primary stimulation area as this is the anatomical location where patients with CIPN develop pain-related symptomatology. The thigh was the secondary stimulation site. As this site was not being associated with neuropathic pain, this area was chosen as a potential control site.

Prior to each subject entering the MR scanner, pain thresholds were ascertained at both thermode placement sites. At these and subsequent temperature iterations, each subject reported their subjective pain status with reference to a 0-10 point numeric rating (Likert) scale (where 0 represented no pain and 10 represented the most extreme pain). The temperature was increased or decreased by 0.5-2°C until a pain score of 7/10 was identified. The maximum temperature at either site was set at 47.9°C (Figure 2.15).

Start temperature 44.9°C (foot) & 42.9°C (thigh) for 30 seconds

NRS ≥7

NRS < 7

If can tolerate this, repeat to confirm NRS ≥ 7 and use this temperature

If cannot tolerate this, then not suitable to continue

↑ by 2°C max 47.9°C → If this is not tolerated, then ↓ by 1°C or by 0.5°C
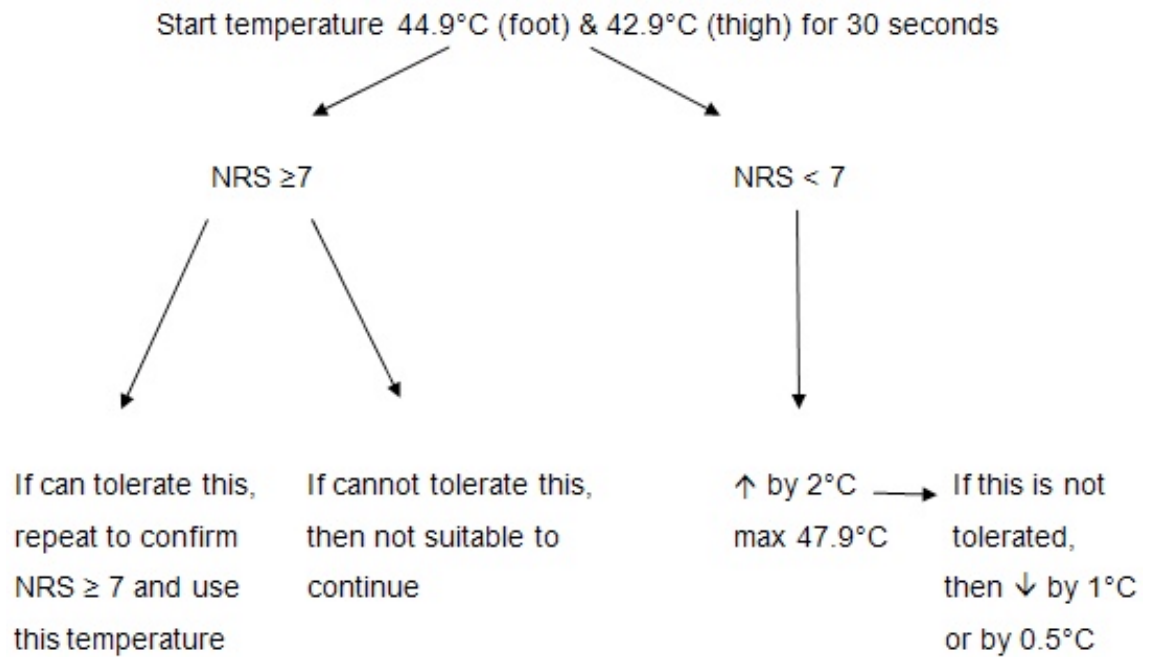
Figure 2.15: Heat pain rating score

All MR imaging was performed in the Academic Unit of Radiology, Royal Hallamshire Hospital. Data was acquired at 3 Tesla (Achieva 3.0T, Phillips Medical Systems, Holland). The scanning protocol included acquisition of standard T1-weighted, 3-dimesional and T2-weighted, 2-dimensional anatomical images to screen for overt pathology prior to two fMRI runs. Whole-brain fMRI datasets were acquired on each subject using standard technique. This comprised the use of a single-shot, T2*-weighted, gradient-recalled, echo-planar imaging sequence: time to echo (TE)=35ms; time to repeat (TR)=3000ms; SENSE-encoding factor=1.5 to acquire 35 contiguous, trans-axial slices of thickness 4mm, having an in-plane resolution of 1.8mm x 1.8mm at every functional imaging 'time-point' or dynamic. All the subjects were instructed to lie motionless throughout the scanning procedure, aside from the requested finger tapping task. Additional foam pads within the head coil helped secure head fixation and prevent motion of the head (Figure 2.16).
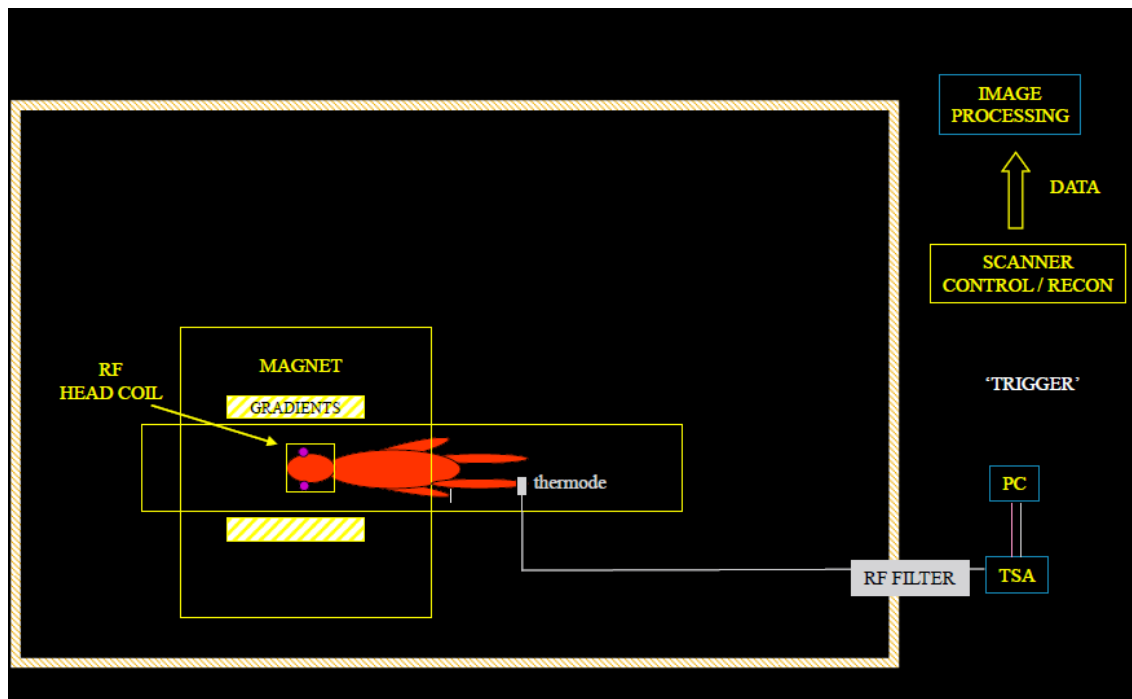
Figure 2.16: fMRI and pain stimulator setup

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Each subject underwent heat-pain stimulation and finger tapping task whilst in the fMRI.

2.6.1 Heat-pain stimulation

The fMRI response to heat-pain stimulation was determined independently at the 2 anatomical levels on the right side detailed above. One fMRI run was performed at each of these anatomical sites. Each run comprised the presentation of 7 blocks of heat-pain stimuli (duration 30 seconds) at the pre-determined 7/10 pain-inducing temperature, interspersed with 7 blocks of baseline temperature 32°C (each baseline block duration was pseudo-randomised to 55, 60 or 65 seconds). The pre-programmed thermode-device computer controlled the timing of the applied temperature protocol. For each anatomic site a 600 second (10 minute) long protocol was used (Figure 2.17). The onsets of the heat pain stimuli were at: 30, 125, 205, 330, 390, 475, 570 seconds whilst the onsets of the baseline temperature were at 60, 145, 235, 330, 420, 505 seconds. Each stimulus was applied via an MR-compatible thermode and the temperature used to provide a heat-pain stimulus was determined prior to imaging, as detailed above. The radiographer and a member of

the team operating the CHEPS thermode verified synchronisation between the start of the heat-pain stimulation and fMRI acquisition.
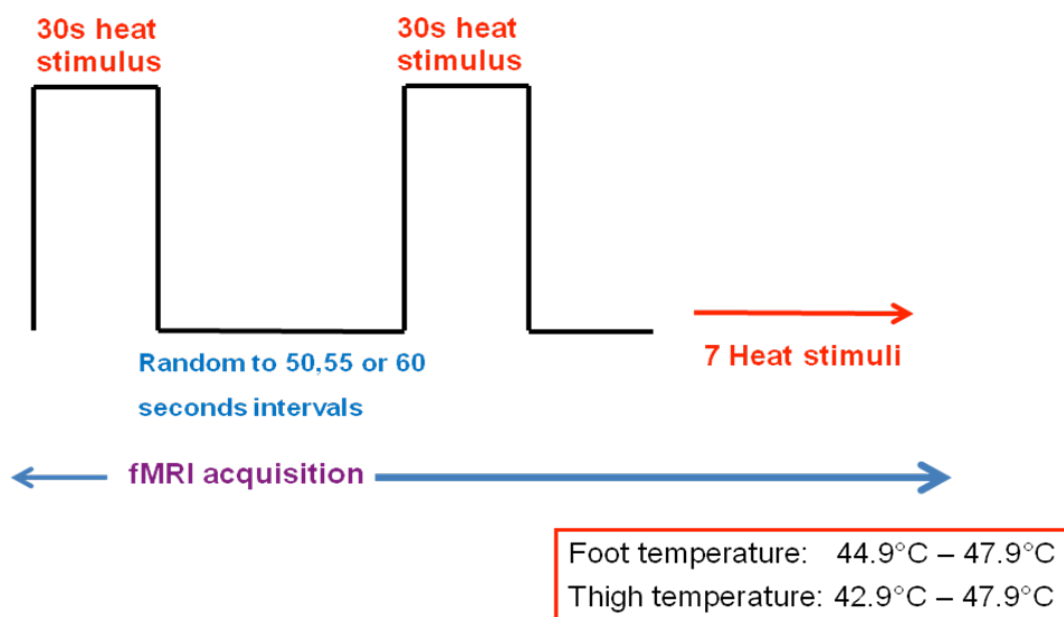
Figure 2.17: Experimental setup for heat-pain stimulation

A 30-second thermal stimulation followed by a random pain free interval (32$^{°}$C) of 50, 55 or 60 seconds, this was performed 7 times first on the right foot and repeated on the right thigh

At the end of the imaging session, all participants rated their overall pain when the heat-pain stimulus was delivered to the foot and to the thigh.

2.6.2 Auditory-motor finger tapping task

Inter-individual variation in non-pain related BOLD response was assessed and controlled for by the addition of a functional run assessing the brain's response to an auditory-motor finger tapping task. A 180 second/60 dynamic scanning sequence was used. Patients were asked to touch their thumbs against the tips of the other four fingers consecutively on both hands in time with an audio cue which sounded every 2 seconds. This single-finger opposition task was explained to the subjects before the scanning, and subjects were allowed a brief practice session. A steady rate was chosen to eliminate the effects of varying motor rate. The subjects were instructed to maintain the pace during the activation periods. The task was monitored visually by the researcher (myself) during these periods. During the rest period, subjects were asked to relax their hands at their sides. The task began with

a rest period of 30 seconds (10 dynamics) then finger tapping for 30 seconds and this was repeated three times.

2.6.3 Scanning Protocol

A fixed scanning protocol (Table 2.5) was used on every subject for consistency.

Table 2.5: fMRI scanning protocol

TASK	Description
1) Survey Scan	Provides anatomical survey images and sets up MRI for all subsequent sequences.
2) SENSE Ref Scan	This is a set up scan, which prepares the coil.
3) T2 Weighted/Turbo Spin Echo Scan	An axial anatomical scan using T2 weighted imaging which is good for detecting pathologies in the brain and used for screening patients.
4) Functional-Quality Analysis (F-QA)	A short 1 dynamic long functional sequence which checks the quality of functional images before proceeding.
5) Functional-Baseline (F-BASE)	A functional scan which images brain activity at baseline (no stimulus/task). Patients are instructed to look at red cross (this is to ensure fixation of attention) for a total of 100 dynamics.
6) Functional imaging with audio cue initiated finger tapping	A functional scan to collect information about inter-individual non-pain related BOLD variation. Patients are instructed to finger tap to an auditory cue for a total of 60 dynamics (toggling between 10 dynamics at rest followed by 10 dynamics of finger tapping).
7) Functional imaging with heat pain stimulation to foot	Functional scan. During this sequence heat pain stimulus is applied to the dorsum of right foot (see heat pain protocol below).
8) Functional imaging with heat pain stimulation to thigh.	Functional scan. During this sequence heat pain stimulus is applied to the thigh as in Task 7.
9) T1 MPRAGE	A sagittal anatomical scan used to produce high quality anatomical images of the brain.

2.6.4 Data Storage

Data collected from assessments for each subject was stored in a secure room. A unique scan number and an MRI screening form were stored in the MRI department. Imaging data for each subject was transferred and stored on a secure analysing computer in the imaging laboratory.

2.6.5 Data analysis & statistical assessments

Non-imaging data collected were converted into numerical scores and entered into Microsoft Excel for subsequent analysis. This was then entered into the Statistical Package PASW 18.0 for Windows and statistical analyses were performed (SPSS 2009).

The analysis of imaging data was carried out using statistical parametric mapping software (SPM5 - www.fil.ion.ucl.ac.uk/spm). Following spatial pre-processing (as described above in section 1.4.6), images were analysed. Blood Oxygen Level Dependent response was modelled using a box-car waveform convolved with a canonical haemodynamic response function.

2.6.5.1   Individual images analysis

Blood oxygen level dependent response was modelled using a box-car waveform convolved with a canonical haemodynamic response function. Following spatial pre-processing, which included realignment, spatial normalisation and spatial smoothing, images were analysed using the General Linear Model. All activation results are displayed in the anatomical space as defined by the Montreal neurological institute (MNI); however the convention in the neuroscience community is to report brain locations in the standard space of the Talairach and Tournoux atlas (Talairach and Tournoux 1988). The location of regions with BOLD activity was determined by converting MNI (x,y,z) co-ordinates as provided by SPM5 into Talairach co-ordinates, which were then interpreted using the Talairach and Tournoux atlas (Talairach and Tournoux 1988).

First-level functional images were produced comparing the BOLD response under the heat pain condition with the baseline BOLD signal. Images were produced for every functional run at each of the two anatomical stimulation sites (foot and thigh)

for each subject. Therefore, the first-level analysis yielded 2 contrast images per subject, comparing BOLD response under the heat-pain condition with baseline at the foot and thigh stimulation sites.

2.6.5.2   Comparing groups analysis

These resultant first-level contrast images were combined at the group level in a flexible factorial model with factors of subject, group (CIPN-myeloma and healthy volunteers) and site (foot and thigh). This appropriately modelled the independence of observations between subjects and groups, and the inequality of variance across all factors. The exception was the finger tapping analysis where 2-sample t-test was used.

Within-group images of all pain (foot and thigh) response versus baseline were produced for each of the CIPN-myeloma and healthy volunteer groups. The statistical threshold for reporting within-group pain contrast activation was $p<0.05$, family-wise error (FWE) corrected across the whole brain volume.

A functional anatomical mask of voxels activated in response to all heat-pain stimuli (compared with baseline) in both groups of subjects (CIPN-myeloma and healthy volunteers) was created at voxel-level statistical threshold $p<0.001$, uncorrected. This mask was used as a volume-of-interest for correction for multiple comparisons in subsequent between-group contrasts.

The results were evaluated using contrast analysis where a contrast was specified in terms of weights for each condition. As an example, in the experiment of finger tapping comparing healthy volunteers to CIPN-myeloma patients; the design matrix had two columns and a contrast vector (e.g: 1 0, 0 1, 1 -1, -1,1) was added to observe what happened at the voxel level and whether there was any significant difference between the two groups (Figure 2.18).
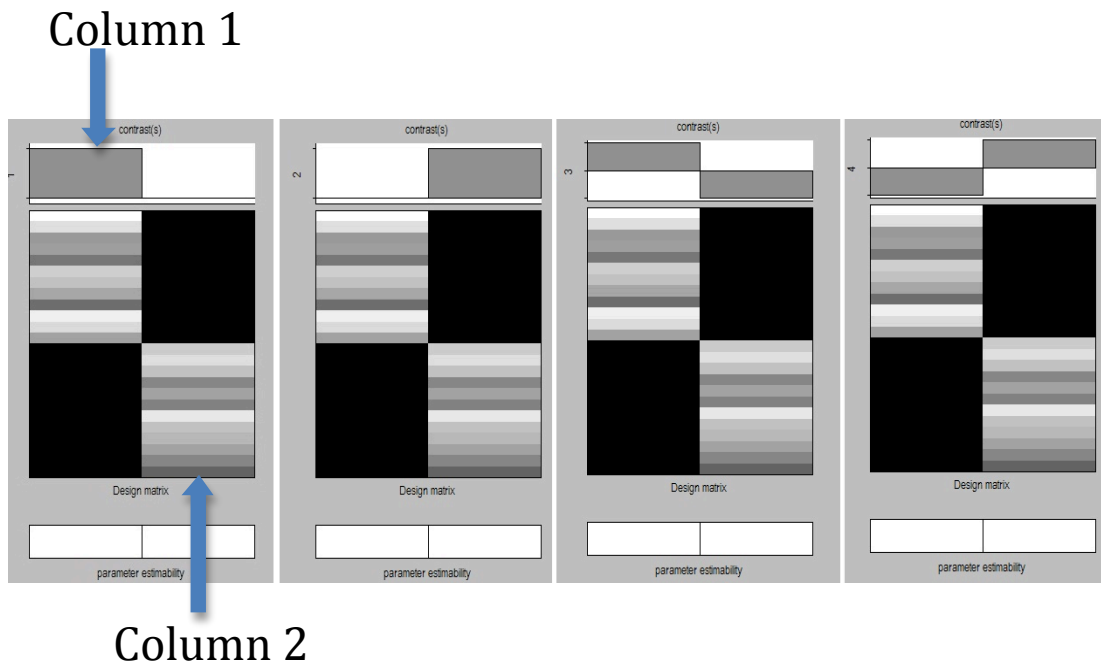
Column 1

Column 2

Contrast: 1 0   0 1   1 -1   -1 1

Figure 2.18: Design matrix for finger tapping showing contrast analysis

There are 2 groups in each figure: Columns 1 are collated images from HV and columns 2 are collated images from CIPN-myeloma patients. The contrast 1 0 means that the analysis is only looking at brain activation in HV whilst tapping their fingers; 0 1 looking at brain activation only in CIPN-myeloma patients whilst tapping their fingers; 1 -1 is looking whether there was significant different activation of voxels (brain activity) in HV compared to CIPN-myeloma patients whilst tapping their fingers and -1 1 is vice versa of 1 -1.

The statistical threshold for reporting between-group differences in pain-evoked activation was p<0.05 family-wise error corrected (voxel-level) or p<0.05 corrected for extent of activation (cluster level) in either the whole brain volume or functionally defined region-of-interest (above). All activation results were displayed in the anatomical space as defined by the Montreal Neurological Institute (MNI) (Ashburner and Friston 2005), with stereotactic co-ordinates converted to the standard space of Talairach and Tournoux (Talairach and Tournoux 1988) for the purposes of neuroanatomical labelling. BOLD response maps for painful stimulation versus baseline were correlated with single covariates of interest: TNSr, CPAQ and PCS in multiple regression analysis. In a GLM using SPM, the model assumes a linear relationship between regional cerebral blood flow and the covariate.

2.6.5.3　Test for habituation

In the first level analysis, the first 80 images and the last 80 images (out of 203 images) for each subject were analysed individually as this included the first 3 heat-pain stimuli and the last 3 heat-pain stimuli both for the foot and for the thigh. In the second level analysis, habituation was looked for comparing the 1st 3 stimuli vs the last 3 stimuli in:

- healthy volunteers when the heat-pain stimuli was delivered to the foot and secondly to the thigh;

- CIPN-myeloma patients when the heat-pain stimuli was delivered to the foot and secondly to the thigh;

- comparing healthy volunteers and CIPN-myeloma patients when the heat-pain stimuli was delivered to the foot and secondly to the thigh

The conditions specified in the second-level analysis were:

The onset (seconds) of timing for the first three heat-pain stimuli: 30 115 145 The duration of each heat-pain stimuli was 30 seconds.

The onset (seconds) of the timing for the last three heat-pain stimuli: 21 106 201 The duration of each heat-pain stimuli was 30 seconds.

2.6.6 Power calculation

In fMRI experiments, it is estimated that the inclusion of 12 subjects per study cell allows for between-groups comparisons with inter-subject variability properly modelled as a random effect in the statistical analysis (Friston et al. 1999). A pilot study carried out in Sheffield looking at diabetic neuropathy has shown significant differences when comparing painless and painful diabetic neuropathy in a limited sample size (n=6 in each group).

2.6.7 Statistical analysis

Standardised measures were scored as directed in manuals and published reports. Descriptive statistics were calculated. Quantitative data were analysed using PASW version 18 (SPSS 2009). Cronbach's alpha is a psychometric statistic measuring the coefficient of reliability and internal consistency of a multi-item scale and a score >0.8 is regarded as good. This was used for the questionnaires used.

To check if the data was normally distributed or not, the skewness and the histogram plot with normal curve overlay were performed. The age of the CIPN-myeloma patients and the healthy volunteers' age were tested for normal distribution (Table 2.6, Figure 2.19, 2.20) and results show that the data was not normally distributed. Skewness is a measure of the degree of asymmetry of a distribution. If the left tail (tail at small end of the distribution) is more pronounced than the right tail (tail at the large end of the distribution), the function is said to have negative skewness. If the reverse is true, it has a positive skewness (Weisstein 2012). If the two are equal, it has zero skewness. The histogram is a representation of a frequency distribution. Therefore non-parametric tests were used for descriptive statistics.

Table 2.6: The mean, median, skewness of the data

Statistics	CIPN-MM Age	Healthy volunteers Age
Mean	61.25	47.25
Median	63.00	50.50
Skewness	-.421	-0.02
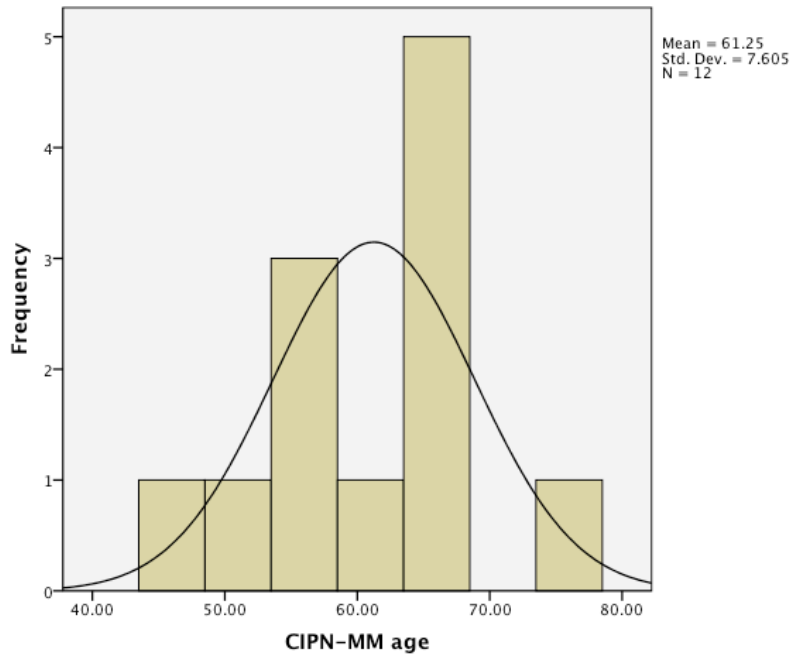
Figure 2.19: Histogram plot with overlay for CIPN-MM age

The data is moderately skewed to the left: the left tail is slightly longer and more data of the distribution is at the right.
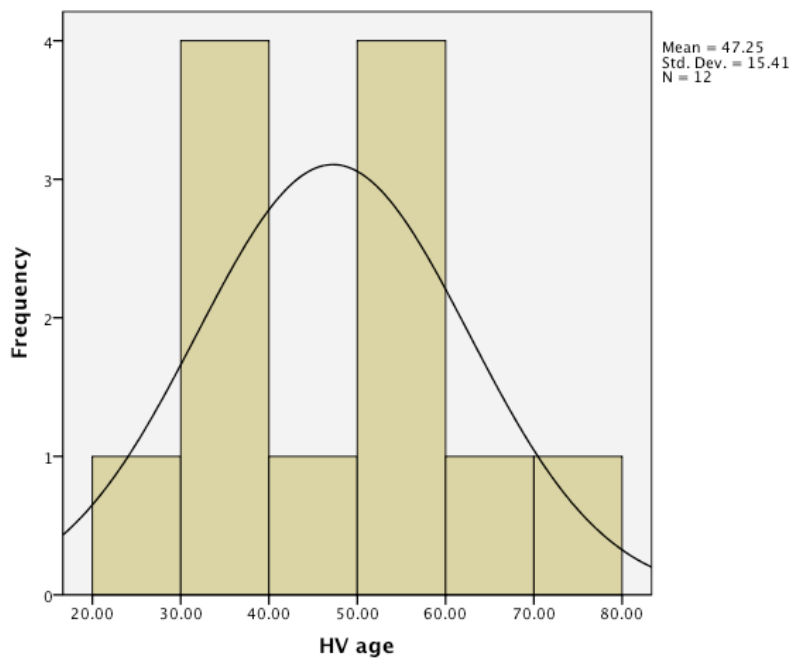
Figure 2.20: Histogram plot with overlay for healthy volunteers age

The data is very slightly skewed to the left: the left tail is very slightly longer.

2.7 Results

2.7.1 Baseline characteristics

The demographic details and disease characteristics of the MM patients and healthy volunteers are shown in Table 2.7. Ten MM patients (83%) had undergone autologous haemopoietic stem cell transplantation (HSCT) as part of their treatment and 4 (33%) received a second more recent autologous HSCT. Other anti-myeloma therapies that these patients received which are known to cause peripheral neuropathy varied between patients:

5 (42%) vincristine, 9 (75%) thalidomide and 8 (67%) bortezomib.

Table 2.7: Baseline characteristics (median [IQR]) of the subjects recruited

	Multiple Myeloma patients	Healthy Volunteers
Group Size	12	12
Age (Years)	63 [56-67]	53 [35-58]
Sex	8 Males; 4 Females	6 Males; 6 Females
Duration of MM (Years)	4.1 [2.0-7.7]	Not relevant
Duration of neuropathic pain (Years)	2 [0.9-3.2]	Not relevant

A Mann-Whitney's U test was performed to evaluate the differences in the age between the two groups and a statistically significant difference was found (p=0.04).

These CIPN-myeloma patients have had neuropathic pain for a median of 2 years and all patients were on a variety of analgesics. Seven patients (58%) were on a stable dose of opioids ranging from one (14.25%) being on tramadol and codeine, one (14.25%) being on fentanyl patch, three (43%) being on buprenorphine patches, one being on morphine slow release tablets (14.25%) and one (14.25%) being on OxyContin slow release tablets. Out of the 12 patients, 10 (83%) were on calcium channel blockers as a form of analgesia namely, three being on gabapentin (30%) and seven being on pregabalin (70%). The opioid doses had been stable for at least

one month and were not stopped, whilst the anticonvulsants were stopped 48hours prior to scanning.

2.7.2 Quantitative results for multiple myeloma patients

2.7.2.1 Quantitative Sensory Testing

Quantitative sensory testing was conducted for cooling detection threshold (CDT) and vibration detection thresholds (VDT). The median [IQR] CDT was 19.2 [4.73] and VDT was 23.1 [15.3-21.0] as shown Table 2.8.

Table 2.8: Cooling detection threshold and Vibration detection thresholds

	Median	IQR	Minimum	Maximum
Cooling Detection Threshold JND	19.20	15.3-21.0	5.44	22.20
Vibration Detection Threshold JND	23.10	21.2-24.2	14.83	25.00

JND – Just noticeable differences

2.7.2.2 Nerve Conduction Studies

The nerve conduction studies were not elicited in all MM patients; in those patients where the nerve could not be stimulated and no recording measured, it was assumed that the nerve conduction was abnormal (Table 2.9). In those elicited the results below show that they varied from the norm. Normal ranges adjusted for age were derived from a nerve conduction study reference text (Liveson 1992).

Table 2.9: Nerve Conduction Study Results

Nerves studied	Mean	Standard Deviation	Minimum	Median	IQR	Maximum
Sural Amplitude (μV)	5.49	9.39	0.00	0.00	0 -11.70	30.00
Common Peroneal Nerve Velocity (m/secs)	42.20	5.45	37.30	39.40	37.80 - 48.05	49.30
Common Peroneal Nerve Amplitude (μV)	1.5	2.09	0.00	0.95	0 - 2.52	5.60
Common Peroneal Nerve Latency (msecs)	3.12	2.46	0.00	3.6	0 - 5.30	5.80
Tibial Nerve Latency (msecs)	4.06	3.86	0.00	4.12	0 - 8.09	9.25

2.7.2.3   Autonomic testing

From the heart rate variability values derived from the cardiac autonomic tests, patients' age related reference ranges were calculated and displayed on graphs showing O'Brien normal ranges for their age. The median [IQR] for the R-R variability during deep inspiration was 1.24 [1.15 – 1.29]. In these patients, the autonomic cardiac studies were normal (three patients did not have this done: two patients had atrial fibrillation; one patient died prior to attending this test).

2.7.2.4   Assessment tools

2.7.2.4.1 Total Neuropathy Score (reduced version)

Motor and sensory symptoms and signs, pinprick and vibration perception threshold, and neurophysiological examination of the sural nerve amplitude and common peroneal nerve amplitude were used to obtain the TNSr. A higher score indicates worse peripheral neuropathy. Table 2.10 shows the score for the MM patients.

Table 2.10: Total Neuropathy Score, reduced version

Parameter	Mean	SD	Median	IQR
	TNSr (0-32)			
Sensory symptoms	2.1	0.8	2	2-3
Motor symptoms	0	0	0	0
Pin sensibility	2.2	0.4	2	2-3
Vibration sensibility	2.2	0.4	2	2-3
Strength	0	0	0	0
Tendon reflexes	0.3	1.1	0	0
Sural nerve amplitude	3.1	1.6	4	3-4
Peroneal nerve amplitude	3.4	0.69	3	3-4

Median	IQR	Mean	SD	Minimum	Maximum
		TNSr			
14.00	11-17	13.6	3.6	9.00	21.00

(1 missing data, patient passed away acutely prior to returning for nerve conduction studies).

The peripheral neuropathy was graded on the basis of the TNSr the subjects were grouped according to:

TNSr 2-8 = Grade 1; TNSr 9-16 = Grade 2; TNSr 17-24 = Grade 3; TNSr 25-32 = Grade 4 (Ware 1996).

For the sample of patients, according to this grading system, the group comparison was:

Grade 1 = 0

Grade 2 = 67% (8 patients)

Grade 3 = 25% (3 patients)

Grade 4 = 0

2.7.2.4.2 Dyck's Score

Assessment of neuropathy was also calculated using the Dyck's composite score. The median [IQR] for Dyck's Score (NIS - Lower limb) + 7) was found to be 12.3 [8-20] (Table 2.11).

Table 2.11: Dyck's score

Dyck's score					
Mean	**SD**	**Median**	**IQR**	**Minimum**	**Maximum**
13.7	6.5	12.3	8-20	5	24

(1 missing data entry as the patient passed away acutely prior to returning for nerve conduction studies).

According to the classification of Dyck's, this cohort of patients would be classified as all having clinical neuropathy since the scores were ≥4.5.

2.7.3 Qualitative results for multiple myeloma patients

2.7.3.1   EORTC QLQ-C30 and the myeloma module QLQ-MY20

From the EORTC QLQ-C30, the commonest symptoms were pain and fatigue; whilst in terms of the functioning scales the lowest mean score, indicating the most severe impairment was found in social functioning (Table 2.12).

The individual item of the EORTC QOL scale that were reported as most bothersome by patients (>25% of patients reporting 'very much' or 'quite a bit' of bother) included the inability to do strenuous activities (58.4%) or take long walks (75%); patients felt limited in doing either their work or other daily activities (41.6%) and in pursuing their hobbies or other leisure time activities (41.6%) and they felt this interfered with their family life (50%) and with their social activities (41.7%). Over three-quarters of the patients had pain (83.4%), and felt fatigued (41.7%).

Table 2.12: EORTC-QLQ-C30

EORTC QLQ-C30	Median [IQR]	Mean [SD]
Functional Scales		
Physical Functioning	63.5 [28.5-85.25]	59.5 [27.3]
Role Functioning	67.0 [33.0-83.0]	61.0 [31.3]
Emotional Functioning	83.5 [69.0-100]	78.6 [28.1]
Cognitive Functioning	67.0 [21.0-79.0]	57.1 [32.9]
Social Functioning	58.5 [33.0-67.0]	51.4 [28.9]
Global Health Status/QoL		
Global Health Status/QoL	67.0 [50.0-73.0]	60.6 [18.9]
Symptom Scales/Items		
Pain	50.0 [33.0-79.0]	54.1 [24.7]
Fatigue	44.0 [24.75-75.35]	51.7 [28.7]
Dyspnoea	0.0 [0.0-33.0]	19.4 [26.5]
Insomnia	33.0 [33.0-58.5]	36.0 [22.4]
Appetite loss	33.0 [0.0-33.0]	27.7 [31.3]
Nausea & vomiting	0.0 [0.0-17.0]	7.0 [11.1]
Constipation	0.0 [0.0-58.5]	19.5 [30.1]
Diarrhoea	0.0 [0.0-33.0]	19.4 [26.5]

A high score (maximum =100) in the functional scales indicates better health-related quality of life but a high score (maximum = 100) in the symptom scales indicate a high level of symptomatology / problems.

From the EORTC QLQ-MY20, patients reported tingling in hands or feet as the most troublesome symptom (66.7%) followed by feeling drowsy (50%). From the subscales of the EORTC QLQ-MY20, patients were mainly worried about future perspectives (such as worrying about their illness, about their health in the future). Interestingly, 58% were not worried at all about dying (Table 2.13).

Table 2.13: EORTC QLQ-C20 myeloma module

EORTC QLQ-C20	Median [IQR]	Mean [SD]	Range
Subscales			
Disease specific [a]	43.0 [24.0-51.0]	38.6 [22.1]	0-100
Have you had bone aches or pain?	2.0 [2.0-4.0]	2.58 [1.16]	1-4
Have you had pain in your back?	2.0 [1.0-2.0]	1.92 [1.08]	1-4
Have you had pain in your hip?	1.0 [1.0-3.0]	1.92 [1.24]	1-4
Have you had pain in your arm or shoulder?	1.0 [1.0-2.75]	1.67 [1.07]	1-4
Have you had pain in your chest?	1.0 [1.0-1.0]	1.17 [0.39]	1-4
If you had pain did it increase with activity?	2.0 [1.0 – 3.0]	2.17 [1.19]	1-4
Side effects of treatment [a]	26.0 [12.0-36.0]	25.3 [13.6]	0-100
Do you feel drowsy?	2.5 [2.0-4.0]	2.75 [1.05]	1-4
Do you feel thirsty?	1.5 [1.0-2.0]	1.67 [.078]	1-4
Have you felt ill?	1.0 [1.0-2.0]	1.42 [0.67]	1-4
Have you had a dry mouth?	2.0 [1.0-2.75]	1.92 [0.99]	1-4
Have you lost any hair?	1.0 [1.0-1.0]	1.25 [0.87]	1-4
Did you have any tingling hands or feet?	3.0 [2.0-4.0]	3.0 [1.04]	1-4
Did you feel restless or agitated?	2.0 [1.0-2.75]	2.0 [1.13]	1-4
Have you had acid indigestion or heartburn?	1.0 [1.0-2.75]	1.67 [1.07]	1-4
Have you had burning or sore eyes?	1.0 [1.0-1.0]	1.08 [0.29]	1-4
Body Image [b]	33.0 [0-67.0]	33.4 [33.5]	0-100
Have you felt physically less attractive as a result of your disease or treatment?	2.0 [1.0-3.0]	2.0 [1.0]	1-4
Future Perspective [b]	67 [47.0-78.0]	62.2 [22.6]	0-100
Have you been thinking about your illness?	2.0 [2.0-3.0]	2.17 [0.72]	1-4
Have you been worried about dying?	1.0 [1.0-2.0]	1.67 [0.98]	1-4
Have you worried about your health in the future?	2.5 [2.0-3.0]	2.58 [0.67]	1-4

a) High scores indicate worse symptoms, b) High scores indicate better support/functioning.
Individual items score: 1 = not at all, 2 = a little bit, 3 = quite a bit, 4 = very much

2.7.3.2   Self-report Leeds Assessment of Neuropathic Symptoms and Signs (s-LANSS)

75 % (nine patients) scored >12 of the s-LANSS which suggests that their pain is predominantly of neuropathic origin. This was supported by the neurological examination, however examination showed all (n=12) patients had signs of sensory neuropathy in their feet.

2.7.3.3   Neuropathic Pain Scale (NPS)

From the NPS (range 0 – 10), MM patients scored itchy as the most troublesome and allodynia as the least symptom present. The different aspects of pain are reported in table 2.14.

Table 2.14: Neuropathic Pain Score values for CIPN-myeloma group

Neuropathic pain	Mean [SD]	Median [IQR]
Itchy	6.0 [1.5]	6.0 [5.25-7.0]
Intense	5.9 [1.7]	6.0 [4.5-7.0]
Deep	5.8 [2.0]	6.0 [4.25-7.75]
Dull	4.9 [2.5]	5.0 [3.25-7.5]
Surface	4.8 [2.2]	5.5 [3.0-6.0]
Hot	4.2 [3.0]	4.5 [0.75-6.75]
Sensitive	4.2 [3.0]	5.0 [1.25-7.0]
Cold	3.9 [2.8]	4.0 [1.25-6.5]
Sharp	3.9 [2.9]	3.5 [1.25-6.75]
Unpleasant	1.9 [2.5]	1.0 [0.0-3.0]

Spearman's rho correlation between each of the ten NPS descriptors for the twelve MM patients was calculated. The strongest positive correlation was between neuropathic pain that was described as cold and unpleasantness (r=0.801; p=0.002) followed by intensity and deep pain (r=0.765; p=0.004) and intensity and itchy (r=0.760; p=0.004). Another strong correlation was between sharp and cold feeling (r=0.715; p=0.009).

2.7.3.4 Neuropathy Total Symptom Score (NTSS-6)

NTSS-6 total median [IQR] score was 7.8 [5.6-10.5]. Seventy-five percent of patients scored >6 points indicating clinically significant symptoms of neuropathy. The most frequently reported symptom (a symptom score of >1 on NTSS-6) was numbness (83%) followed by prickling (75%). Forty-one percent of patients reported lancinating and burning pain whilst 33% had aching pain. The least reported symptom was allodynia (16%). Eight-three percent of patients reported ≥3 symptoms.

2.7.3.5 Chronic Pain Assessment Questionnaire (CPAQ)

In the CPAQ, a higher score indicates a higher acceptance of pain (range 0 – 120). Two components were derived for scoring: activities engagement and pain willingness as described in section 2.5.2.5. The median sum score for activities engagement was 45; pain willingness was 31. The total score for CPAQ was 75. The items pertained to activities engagements were correlated (alpha coefficient = 0.775) as were items pertained to pain willingness (alpha coefficient =0.948).

The more patients accepted their pain, the better their social functioning was (r=0.747; p=0.005) and the better their QoL (r=0.591; p=0.04)

2.7.3.6 Pain catastrophizing scale (PCS)

Higher scores indicate more pain catastrophizing. Table 2.15 contains the scoring from the MM patients. Alpha coefficients in this study were .80, .95, and .90 for the subscales magnification, rumination and helplessness respectively. For the total PCS, the alpha coefficient was .95.

Table 2.15: PCS scores for CIPN-myeloma patients

PCS	Mean [SD]	Median [IQR]	Range
Total score	15.7 [13.1]	11.9 [4.75-27.5]	0-52
Magnification	3.25 [3.1]	2.5 [1.0-6.25]	0-12
Rumination	6.30 [4.9]	5.0 [1.5-11.75]	0-16
Helplessness	6.08 [5.9]	4.5 [1.25-10.5]	0-24

2.7.3.7   Hospital Anxiety and Depression Scale (HADS)

Fifty-eight percent of MM patients reported signs of anxiety, depression or a combination of both (Table 2.16). Those patients that reported anxiety were also depressed.

Table 2.16: HADS results

	CIPN- Myeloma patients
No anxiety & depression	42% (5)
Borderline for anxiety & depression	17% (2)
Anxiety	25% (3)
Depression	8% (1)

2.7.4 Correlations between clinical demographics, questionnaires and quantitative data

The EORTC MY20:

Side effects of treatment were strongly correlated with cognitive functioning (r=-0.0856; p<0.0001), social functioning (r=-0.784; p=003), fatigue (r=0.593; p=0.04) and insomnia (r=0.632; p=0.02) from the EORTC QLQ-c30.

If the patient felt *physically less attractive* as a result of their disease or treatment, they were more likely to feel anxious (r=0.651; p=0.03); depressed (r=0.778; p=0.005); there was a negative significant correlation with emotional functioning from the EORTC QLQ-C30 (r=-0.765; p=0.006) and also this was strongly correlated with appetite loss (r=0.811; p=0.002).

If the patient was *worried about their future perspectives* (such as thinking about their illness, been worried about dying and worried about their health in the future) then these patients scored higher on the pain catastrophizing questionnaire in the three domains of rumination, magnification and helplessness (r=-0.734; p=0.007).

The Chronic Pain assessment Questionnaire:

The more the patients accepted their *pain* (total pain acceptance score) the better their QoL was (r=0.591; p=0.04) and the better their social functioning (r=0.747; p=0.005).

The s-LANSS:

Patients with neuropathy as indicated from the *s-LANSS* had lower levels of physical functioning (r=-0.591, p=0.04) and role functioning (r=-0.593; p=0.04).

The Total Neuropathy Score, reduced version and Dyck's score:

There was a strong negative correlation with *TNSr* and role functioning (r=-0.801; p=0.003) from the EORTC QLQ-C30 and symptoms related to the disease from the EORTC myeloma module (r=-0.670; p=0.02). There was a strong correlation between *TNSr* and *Dyck's score* (r=0.841; p=0.001). *Dyck's score* was negatively correlated with physical functioning (r=-0.816; p=0.002) and role functioning (r=-0.716; p=0.013) from the EORTC QLQ-c30. There was a strong correlation between NTSS-6 and Dyck's score (r=0.719; p=0.013) but a weak correlation between NTSS-6 and TNSr (r=0.605; p=0.049).

There was no correlation between S-LANSS and TNSr, Dyck's score and NTSS-6.

There were no statistically significant correlations between age, sex, and years since diagnosis and years since their neuropathic pain started.

2.7.5 fMRI analysis results

2.7.5.1 Finger tapping

All healthy volunteers and CIPN-myeloma patients underwent finger tapping fMRI assessment whilst in the scanner. Average group activation results for each cohort were calculated. In both cohorts the areas of significant activation included the primary motor cortex, the supplementary motor area and the cerebellum ($P_{FWE-corr}$ at voxel level $p<0.0001$) (Table 2.17 and Figure 2.21).

Table 2.17: Areas of BOLD fMRI activation with finger tapping

Neuroimaging Data *HV + CIPN-myeloma finger tapping*	Talairach co-ordinates (x,y,z)	t Statistic
Rt primary motor cortex	42 -18 60	11.89
Lt cerebellum	-18 -52 -24	10.85
Rt superior frontal gyrus	40 36 32	7.24

a)

b)
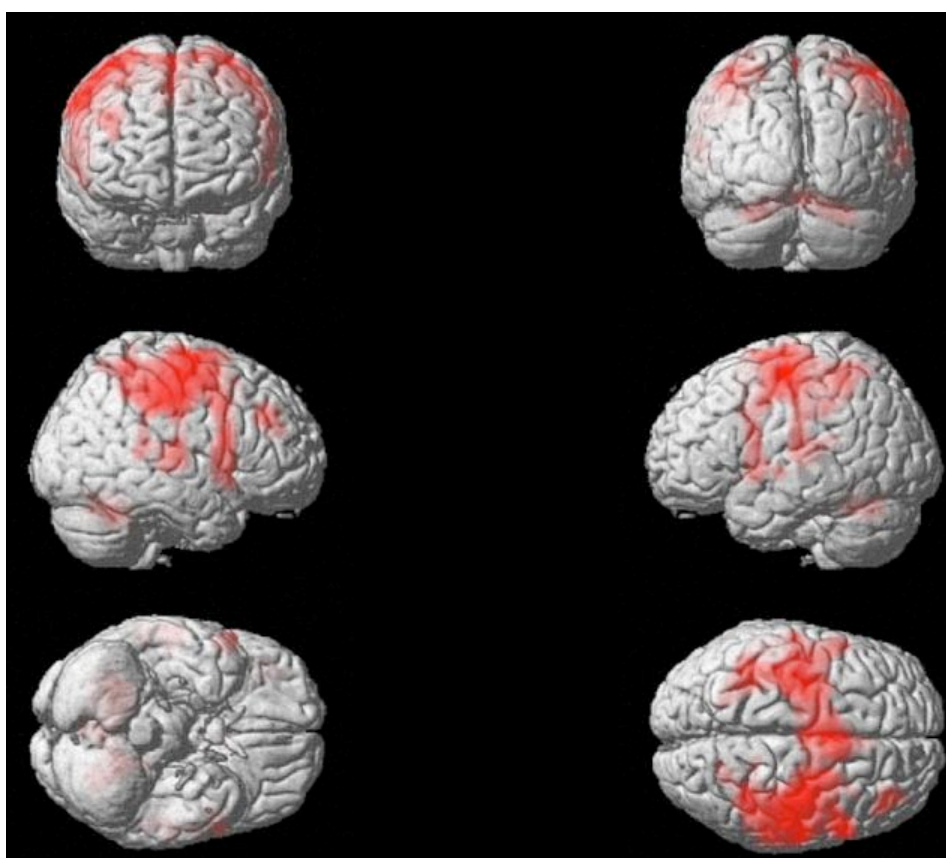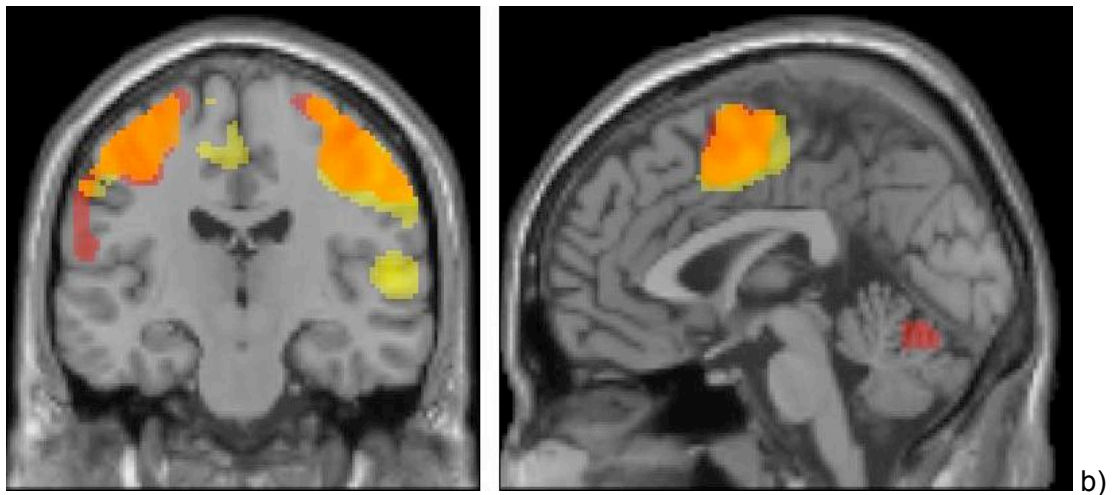
Figure 2.21: SPM generated brain images showing activation due to finger tapping

a) SPM rendered to a generic 3D brain shown from six angles demonstrating areas of activation on finger tapping.

b) Coronal and sagittal views of the brain superimposed on a normalized anatomical T1 image of subjects with activated regions shown in red for healthy volunteers, yellow for CIPN-myeloma patients and orange for overlap. The activation is concentrated in the primary motor area (precentral gyrus), motor associated areas (frontal gyrus) and cerebellum. (Activation maps used threshold $p < 0.01$ and extent threshold adjusted for illustrative purposes.)

There were no significant differences in the brain activation between the healthy volunteers and the CIPN-myeloma patients during finger tapping.

2.7.5.2   Pain stimulation

All 24 subjects completed this part of the study.

Pain rating and temperature used for pain stimulation

A rating for pain intensity was taken before and after MRI scans (Table 2.18).

Table 2.18: Group median [IQR] temperature for pain stimulation and pain rating

	Healthy Volunteers	MM patients	p-value
Foot stimulation			
Temperature °C	46.7 [45.5-47.8]	47.2 [46.6-47.9]	0.23
Pre-scan pain rating (0-10)	7.5 [6.6-8.0]	7.0 [7.0-8.0]	0.74
Post-scan pain rating (0-10)	7.7 [6.6-8.7]	7.7 [7.0-8.0]	0.95
Thigh stimulation			
Temperature °C	45.9 [45.6-47.9]	46.7 [45.5-47.9]	0.88
Pre-scan pain rating (0-10)	7.7 [5.9-8.0]	8.0 [7.6-8.0]	0.28
Post-scan pain rating (0-10)	8.0 [6.2-8.9]	8.0 [8.0-9.0]	0.29

The median pain ratings are shown in Figure 2.22.
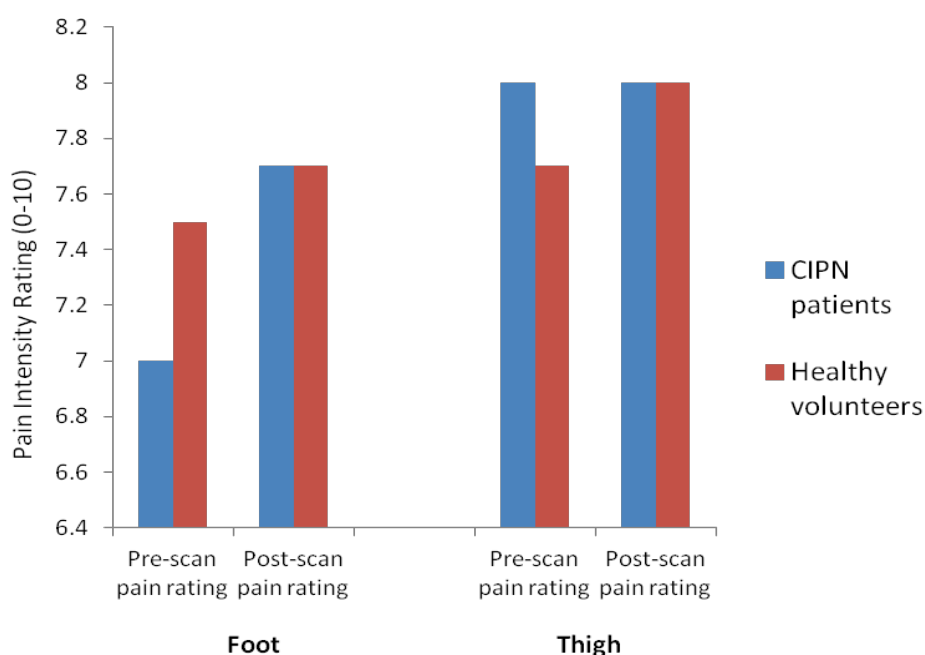
Figure 2.22: Median pain rating pre and post scanning for foot and thigh stimulation.

There were no significant differences (p>0.05) between the two groups.

A Wilcoxon Signed Ranks Test showed that the pain ratings and the temperatures used as noxious stimulus for the foot and the thigh in both the healthy volunteers and MM patients were not statistically significant.

2.7.5.3 Brain activation in response to heat-pain stimulation in both healthy volunteers and CIPN-myeloma patients

2.7.5.3.1 Individual group analysis

Statistical parametric activation maps were constructed for the CIPN-myeloma group and the healthy volunteers control group individually. Firstly, compared with baseline, thermal stimulation on the foot evoked brain activation in both healthy volunteers and CIPN-myeloma patients and the areas of significant brain activation are shown in figures 2.23 and 2.24. Tables 2.19, 2.20 show the areas of activation on painful stimulation on the foot in healthy volunteers and CIPN-myeloma patients.
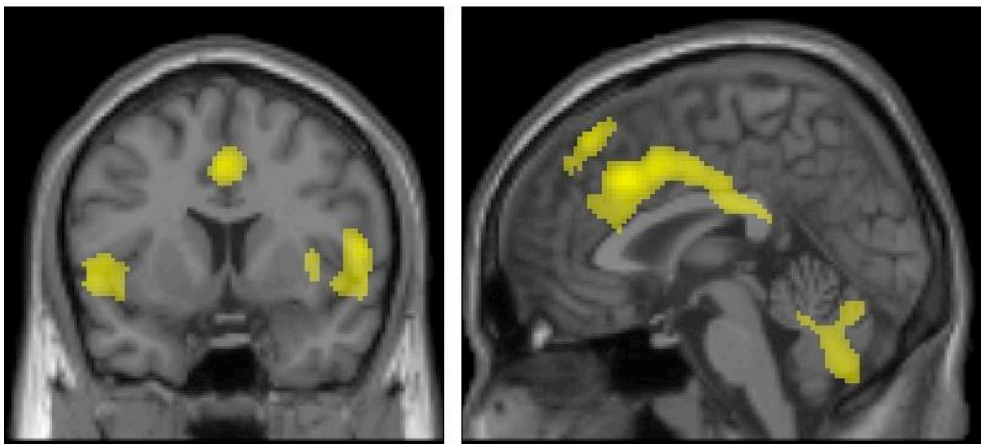
Figure 2.23: Regions of significant activation (yellow) following application of the thermal stimuli on the foot (painful versus baseline) for healthy volunteers
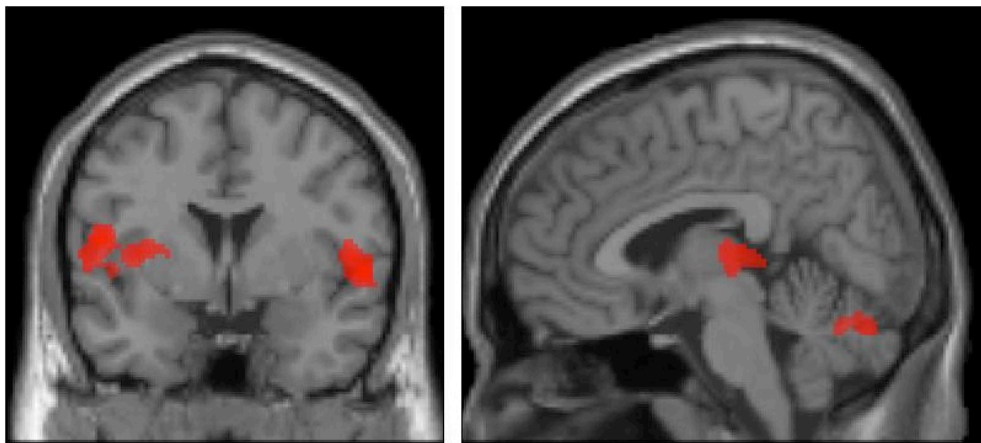
Figure 2.24: Regions of significant activation (red) following application of the thermal stimuli on the foot (painful versus baseline) for CIPN-myeloma patients

For both figures: Foci with a maximum exceeding statistical threshold $p<0.05$, FWE-corrected in the whole brain, are shown at visualisation threshold $p<0.001$ uncorrected

Table 2.19: Areas of significant BOLD response in the Healthy volunteers: where Foot Pain response > Baseline signal

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels*
Rt medial prefrontal lobe	32	4 23 32	7.63	<0.001	1414
Rt supramarginal gyrus	40	63 -41 33	6.92	0.001	617
Rt inferior frontal gyrus	47	44 20 -9	6.72	0.001	1234
Lt posterior cerebellar lobe	-	-30 -66 -16	6.37	0.003	2391
Rt medial frontal gyrus	8	5 39 42	6.32	0.004	325
Lt anterior lobe of temporal gyrus	38	-50 13 -8	6.16	0.006	679

Table 2.20: Areas of significant BOLD response in the CIPN-myeloma patients: where Foot Pain response > Baseline signal

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels*
Rt superior temporal gyrus	22	55 4 0	6.04	0.009	490
Lt precentral gyrus	6	-52 2 12	5.48	0.044	648
Lt middle frontal gyrus	10	-34 40 20	5.37	0.05	163

.

Next, looking at brain activation by the application of the thermal stimuli on the thigh and compared with baseline, evoked significant brain activation in both healthy volunteers and CIPN-myeloma patients are shown in Figure 2.25 and 2.26 respectively. Table 2.21 and 2.22 show the areas of activation on painful stimulation on the thigh in healthy volunteers and CIPN-myeloma patients.
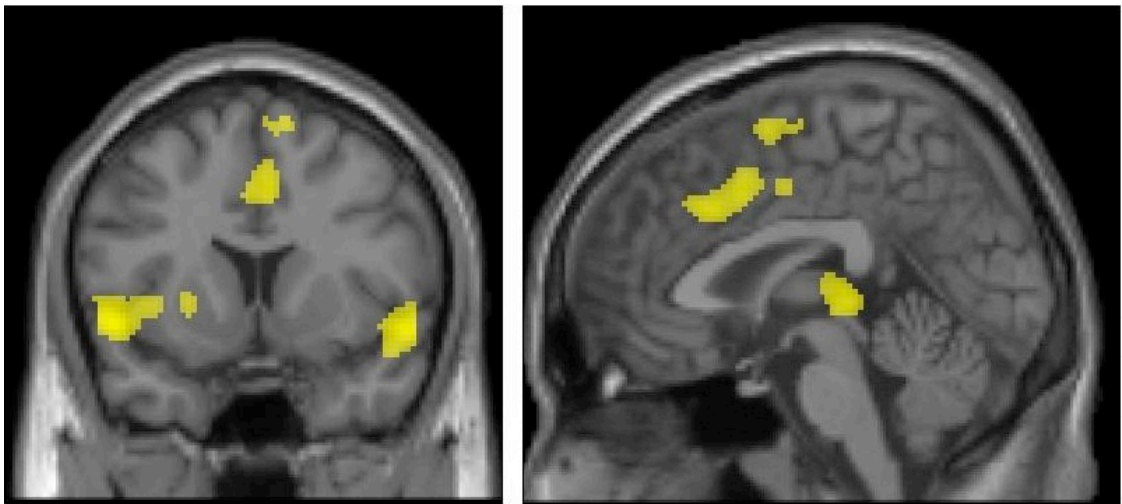
Figure 2.25: Regions of significant activation following application of the thermal stimuli on the thigh (painful versus baseline) for healthy volunteers
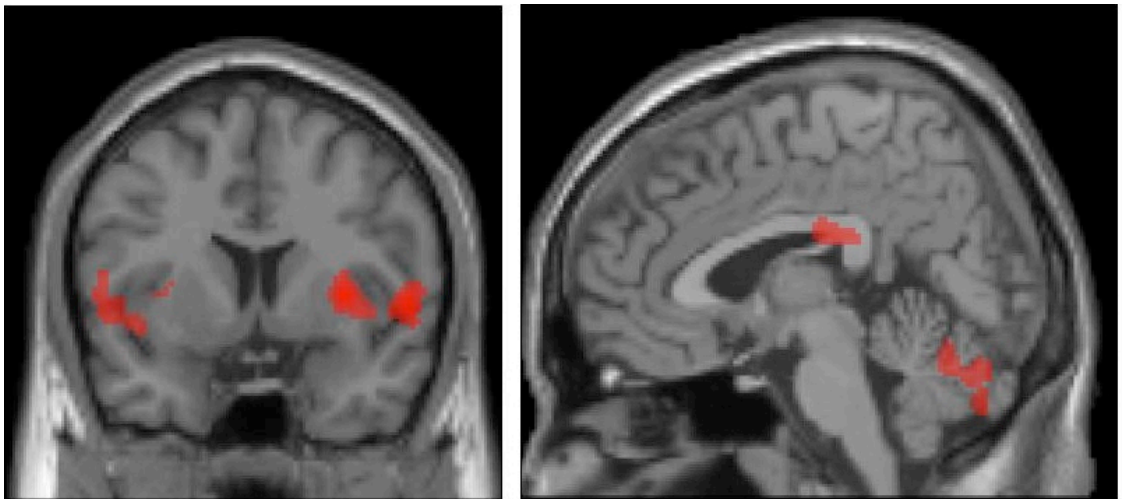
Figure 2.26: Regions of significant activation following application of the thermal stimuli on the thigh (painful versus baseline) for CIPN-myeloma patients

For both figures: Foci with a maximum exceeding statistical threshold $p<0.05$, FWE-corrected in the whole brain, are shown at visualisation threshold $p<0.001$ uncorrected

Table 2.21: Areas of significant BOLD response in the Healthy volunteers: where Thigh Pain response > Baseline signal

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels*
Lt insula in lateral fissure	-	-40 6 2	6.16	0.006	1257
Rt anterior lobe of temporal gyrus	38	-55 13 -9	5.87	0.01	539
Lt superior temporal gyrus	42	-60 -32 20	5.43	0.05	432
Rt middle frontal gyrus	10	-32 48 20	5.42	0.05	260

Table 2.22: Areas of significant BOLD response in the CIPN-myeloma patients: where Thigh Pain response > Baseline signal

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels*
Rt supramarginal gyrus	40	61 -41 35	7.23	<0.001	528
Rt claustrum	-	34 5 4	6.68	<0.001	936
Lt posterior cerebellar lobe	-	-34 -58 -24	5.61	0.031	491

Afterwards, statistical parametric activation maps were constructed for the healthy volunteers control group (foot + thigh) and the CIPN-myeloma group (foot + thigh) and shown in figure 2.27.
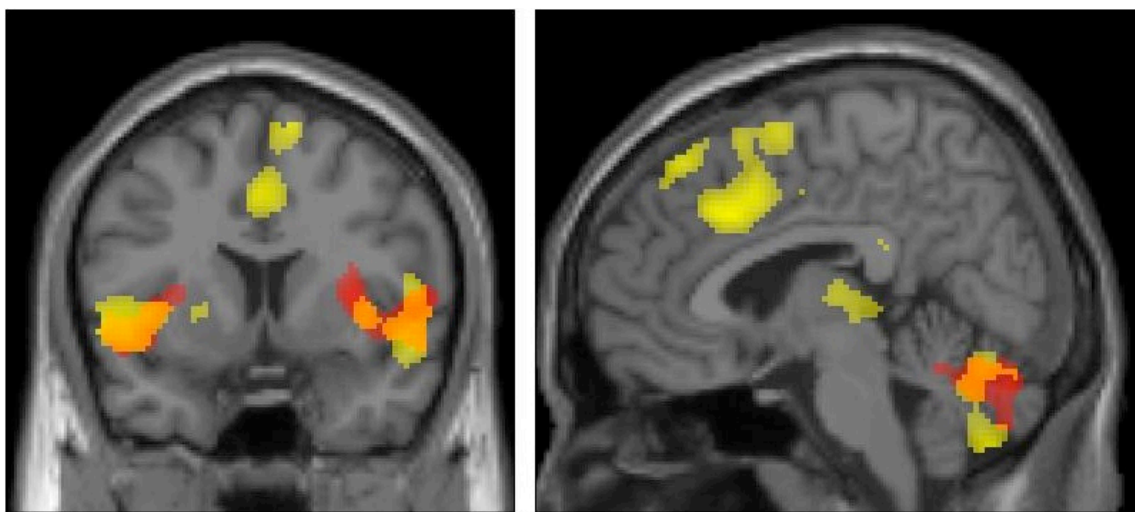
Figure 2.27: Regions of activation following application of the thermal stimuli (painful versus baseline) for CIPN-myeloma patients (red), healthy volunteers (yellow), and overlapping common regions of activation (orange).

Foci with a maximum exceeding statistical threshold $p<0.05$, FWE-corrected in the whole brain, are shown at visualisation threshold $p<0.001$ uncorrected

Table 2.23 and 2.24 show the areas of activation when the application of the thermal stimuli on the foot and thigh were applied in healthy volunteers and CIPN-myeloma patients respectively.

Table 2.23: Areas of significant BOLD response in the Healthy volunteers: where Foot + Thigh Pain response > Baseline signal

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt anterior cingulate gyrus	32	4 24 23	7.47	<0.001	1643
Lt superior temporal gyrus	38	-50 13 -8	7.19	<0.001	1491
Lt posterior cerebellar lobe	-	-30 -67 -20	6.56	0.002	2917
Rt inferior frontal gyrus	47	44 20 -9	6.52	0.002	1151
Rt supramarginal gyrus	40	64 -41 34	6.36	0.004	687
Lt superior temporal gyrus	42	-60 -30 18	6.13	0.007	709
Lt superior frontal gyrus	10	-32 48 20	5.80	0.018	469
Rt middle frontal gyrus	46	42 44 24	5.73	0.02	390
Rt superior frontal gyrus	8	6 39 48	5.68	0.025	216
Lt postcentral gyrus	7	-20 -41 68	5.66	0.026	212

Table 2.24: Areas of significant BOLD response in the CIPN-myeloma patients: where Foot + Thigh Pain response > Baseline

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt superior temporal gyrus	22	56 4 0	8.10	<0.001	1356
Rt supramarginal gyrus	40	62 -41 35	7.65	<0.001	735
Lt middle frontal gyrus	46	-34 40 22	6.25	0.005	544
Lt area opercularis	44	-54 2 12	6.07	0.008	2228
Lt posterior cerebellar lobe	-	-34 -58 -24	6.00	0.01	2535

2.7.5.3.2 Comparison between groups

The mean BOLD activation during the painful stimulus in healthy volunteers and CIPN-myeloma patients was compared and the differential cortical activation between the 2 groups during application of the thermal stimuli to the foot, to the thigh and to the foot + thigh together were observed.

During application of the thermal stimuli to the foot, there was a significant activation in the right superior frontal gyrus (Talairach co-ordinates 5 41 47) in healthy volunteers compared to CIPN-myeloma patients (t=4.77; p=0.04 FWE corrected within the VOI (Figure 2.28).
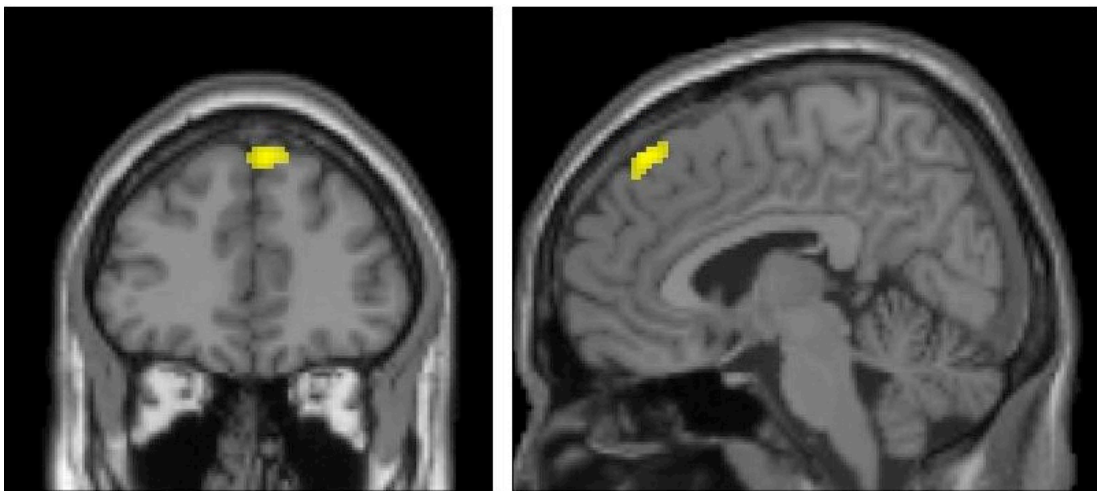
Figure 2.28: Functional image data (coronal and sagittal views) of the right superior frontal gyrus activation during application of the thermal stimuli to foot in healthy volunteers compared to CIPN-myeloma patients.

Images are displayed against sections through a canonical single subject T1-weighted image. For display purposes, the voxel-level threshold is p<0.001, uncorrected at threshold level (k_E) of 170.

From the contrast estimates and 90% confidence interval (CI), it is suggested that CIPN-myeloma patients demonstrated hypo-activation at the superior frontal gyrus, brodmann area 8, whilst healthy volunteers showed activation (Figure 2.29).
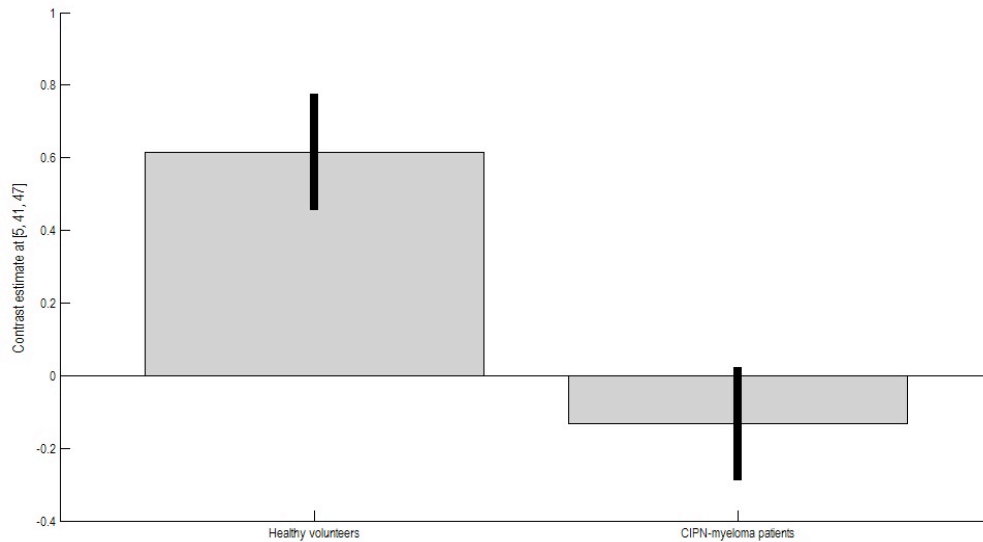
Figure 2.29: Contrast estimates and 90% CI at superior frontal gyrus (co-ordinate 5, 41, 47) depicted in Figure 2.28.

Comparing activation at the superior frontal gyrus when applying thermal stimuli to the foot: in healthy volunteers (activation) and CIPN-myeloma patients (hypo-activation).

An independent-sample t-test was conducted to compare the BOLD activation during thermal pain stimulation of the foot in CIPN-myeloma patients and healthy volunteers. There was a significant difference in the BOLD response for CIPN-myeloma patients (mean = -0.12, SD = 0.38) and healthy volunteers (mean = 0.46, SD = 0.53) conditions; $t(22) = -3.08$, $p = 0.005$ as seen from the box plot in figure 2.30.
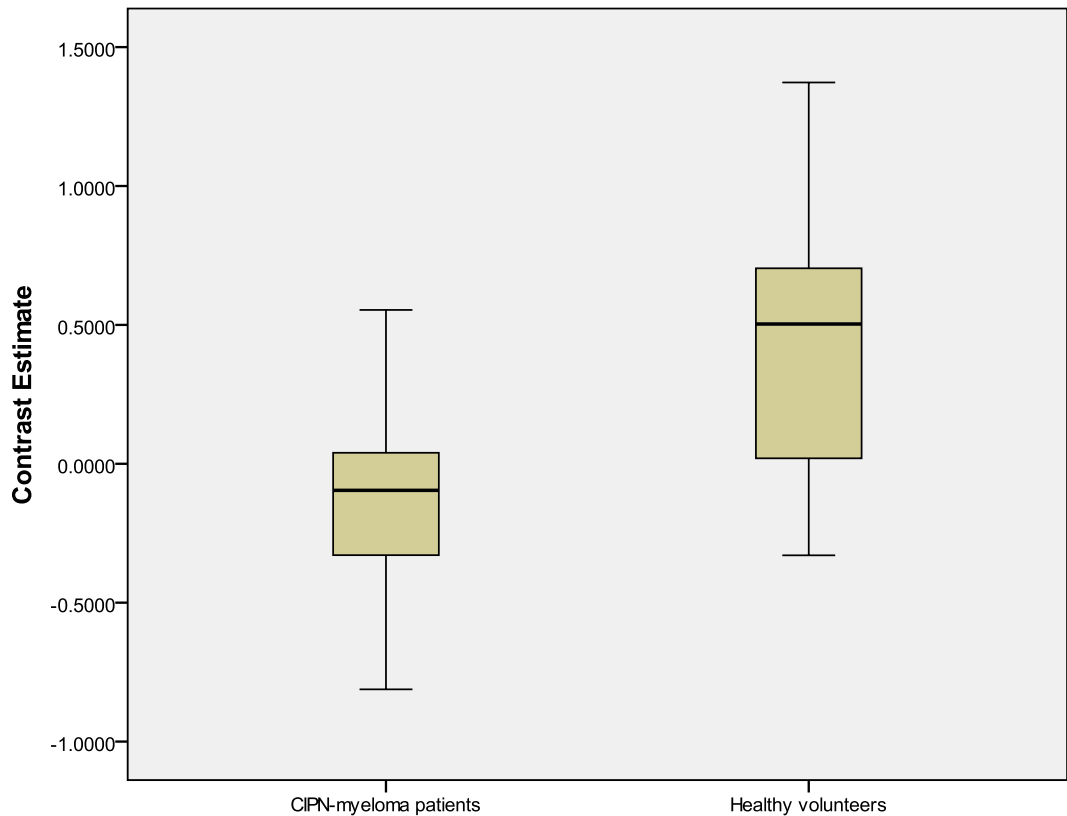
Figure 2.30: Boxplot of contrast estimates of the superior frontal gyrus for CIPN-myeloma patients and healthy volunteers for the comparison results depicted in figure 2.29.

Talairach coordinates (5 41 47) shown for this activation of increased BOLD response during application of thermal stimuli to the foot in CIPN-myeloma patients compared to healthy volunteers.

During application of the thermal stimuli to the thigh, there was no significant brain activation when comparing healthy volunteers to CIPN-myeloma patients.

When looking at the combined brain activation when applying the thermal stimuli to the foot + thigh, compared with CIPN-myeloma patients, healthy volunteers demonstrated significantly greater activation in the right superior frontal gyrus, close to the midline (Figure 2.31; Brodmann area 8; Talairach co-ordinates: 6, 39, 48; peak t = 4.87; $p = 0.03$, FWE-corrected within the volume-of-interest; 97 voxels exceeded height threshold $p < 0.001$, uncorrected).
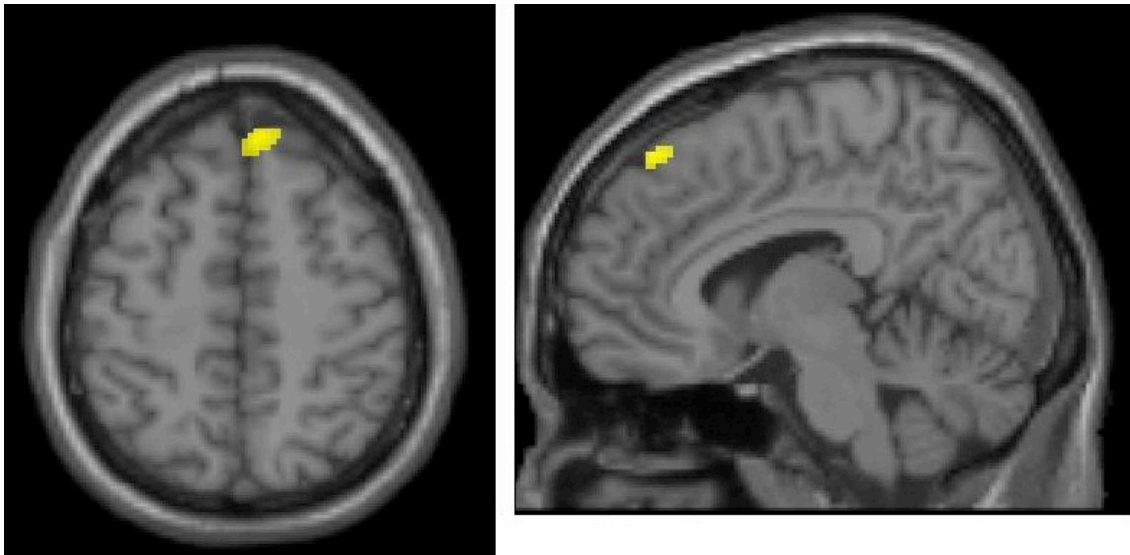
Figure 2.31: CIPN-myeloma patients demonstrated hypoactivation of superior frontal gyrus during application of the thermal stimuli, compared with healthy controls.

Functional imaging data are shown against axial (z=48mm) and sagittal (x=6mm) slices through a canonical single-subject T1-weighted image. For display purposes, the statistical threshold is $p < 0.001$, uncorrected, at the voxel-level.

From the contrast estimates, it is suggested that CIPN-myeloma patients demonstrated hypo-activation at the superior frontal gyrus, whilst healthy volunteers (HV) showed activation (Figure 2.32).
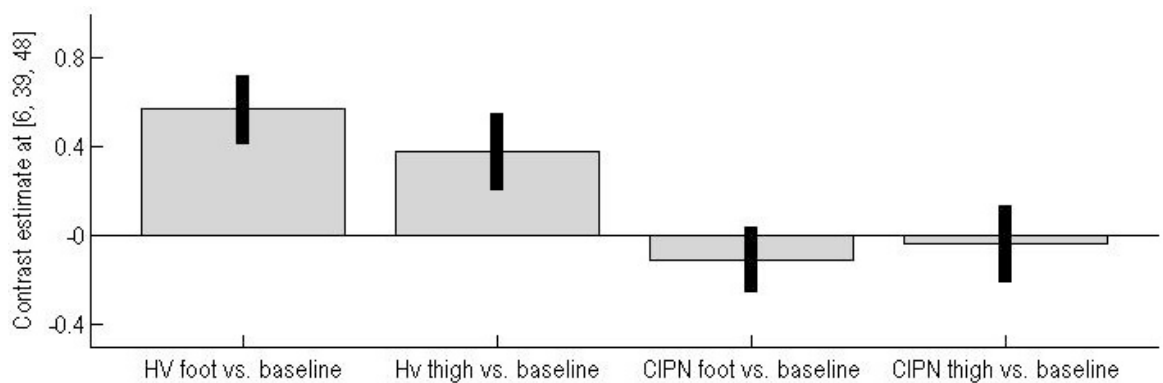
Figure 2.32: Contrast estimates and 90% CI at the superior frontal gyrus (co-ordinate 6, 39, 48) when comparing healthy volunteers to CIPN-myeloma patients depicted in figure 2.31.

Comparing activation at the superior frontal gyrus when applying thermal stimuli to the foot and to the thigh: in healthy volunteers (activation) and CIPN-myeloma patients (hypo-activation).

When looking at the combined brain activation when applying the thermal stimuli to the foot + thigh, CIPN-myeloma patients demonstrated significantly greater activation in left precuneus when compared with healthy volunteers (Figure 2.33; Brodmann area 31; Talairach co-ordinates: -18, -61, 27; peak t = 4.82; 244 voxels exceeded height threshold p < 0.001, uncorrected; p = 0.01, corrected with respect to extent of activation).
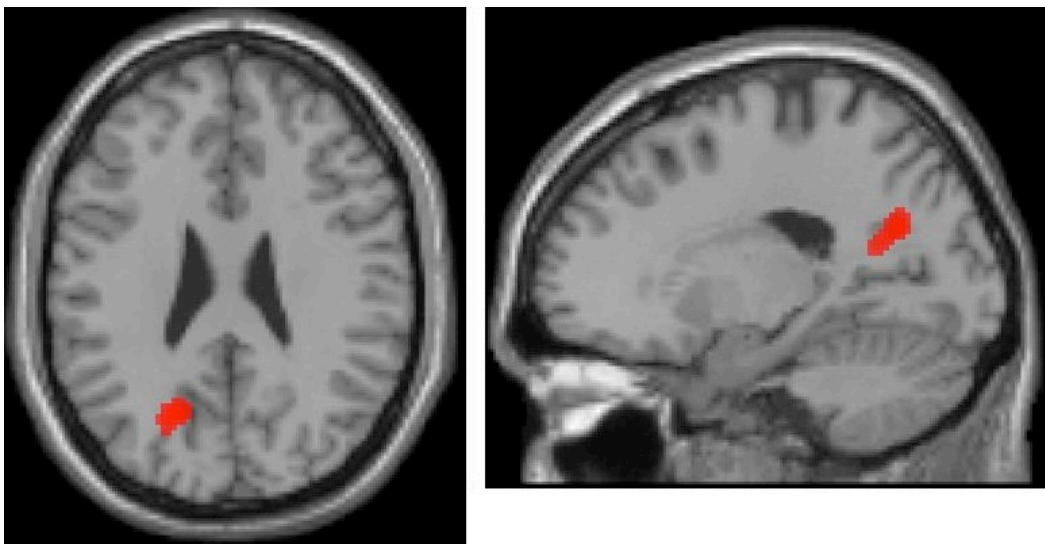
Figure 2.33: CIPN-myeloma patients demonstrated hyperactivation of precuneus during application of the thermal stimuli, compared with healthy controls.

Functional imaging data are shown against axial (z=27mm) and sagittal (x=-18mm) slices through a canonical single-subject T1-weighted image. For display purposes, the statistical threshold is p < 0.001, uncorrected, at the voxel-level.

From the contrast estimates, it is suggested that CIPN-myeloma patients demonstrated hyper-activation at the precuneus, whilst healthy volunteers showed de-activation (Figure 2.34).
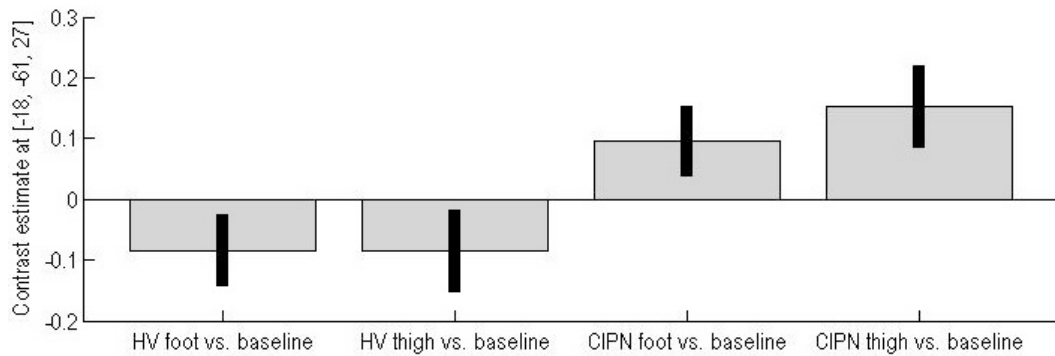
Figure 2.34: Contrast estimates and 90% CI at the precuneus (co-ordinate -18, -61, 27) when comparing healthy volunteers to CIPN-myeloma patients depicted in figure 2.33.

Comparing activation at the precuneus when applying thermal stimuli to the foot and to the thigh: in healthy volunteers (hypo-activation) and CIPN-myeloma patients (activation).

There was a significant group-by-site interaction in the right prefrontal cortex, with CIPN-myeloma patients demonstrating significantly greater activation in response to thigh than foot pain thermal stimulation whereas healthy volunteers exhibited greater activation in response to foot than thigh thermal stimulation at this focus (Brodmann area 9; Talairach co-ordinates: 38, 37, 37; peak t = 6.21; p=0.005, FWE-corrected in the whole brain; 236 voxels exceeded height threshold p < 0.001, uncorrected), Figure 2.35 and this is also seen from the contrast estimates (figure 2.36).
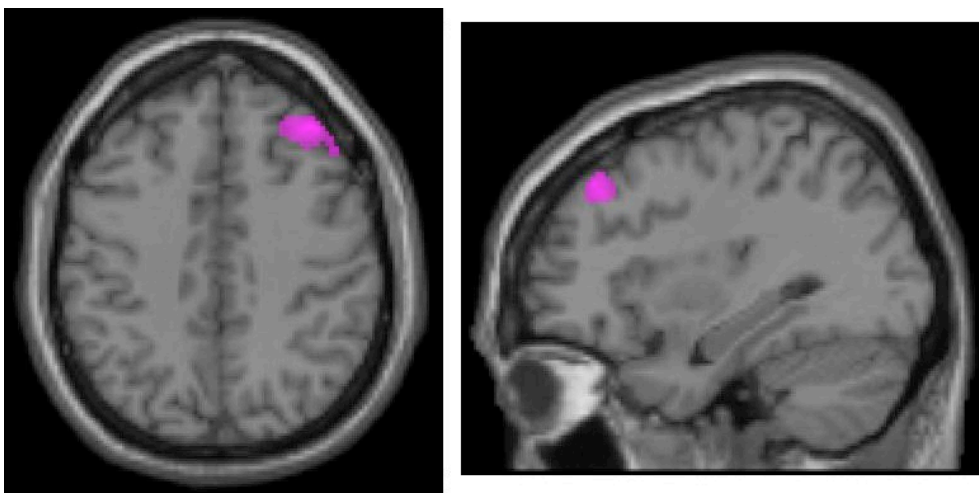
Figure 2.35: Right prefrontal gyrus activation in CIPN-myeloma group and in healthy volunteers.
There was a significantly greater BOLD response with the application of the thermal stimuli to the thigh than foot in CIPN-myeloma patients whereas healthy volunteers exhibited greater activation in response to foot than to thigh stimulation. Functional imaging data are shown against axial (z=37mm) and sagittal (x=38mm) slices through a canonical single-subject T1-weighted image. For display purposes, the statistical threshold is p < 0.001, 236 voxels exceeded, uncorrected, at the voxel-level.
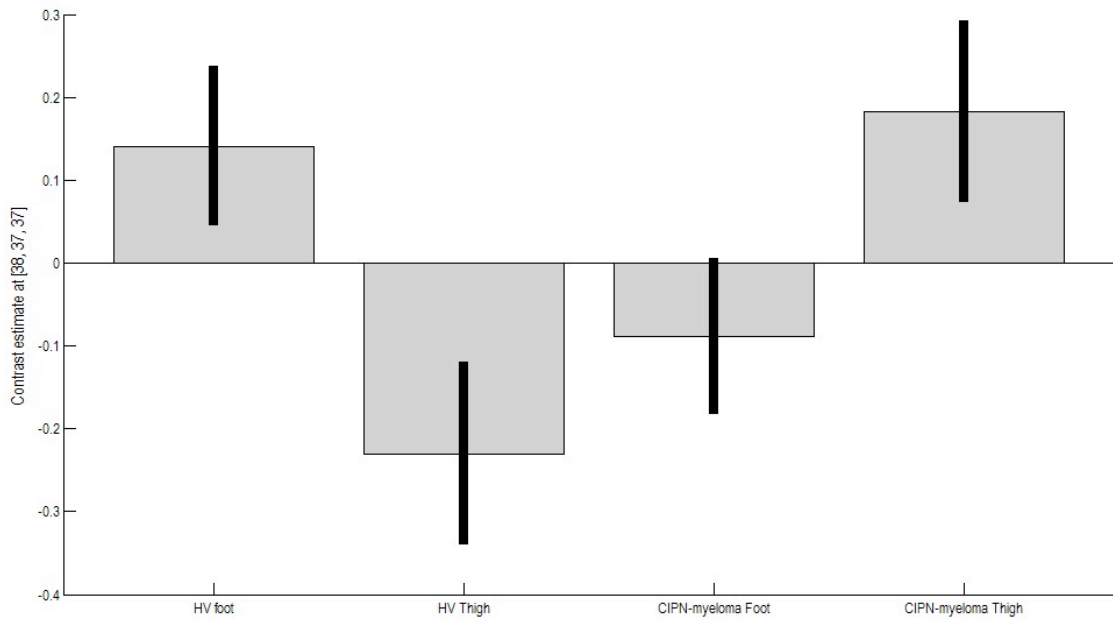
Figure 2.36: Contrast estimates and 90% CI at the right prefrontal gyrus (co-ordinate 38, 37, 37) when comparing healthy volunteers to CIPN-myeloma patients depicted in figure 2.35.

The reverse interaction effect was significant at the right cuneus, with CIPN-myeloma patients demonstrating significant greater activation in response to foot than thigh thermal stimulation whereas healthy volunteers exhibited greater activation in response to thigh than foot thermal stimulation at this focus (Brodmann area 17; Talairach co-ordinates: 2, -89, 8; peak t=4.78; p=0.04 FWE-corrected within the volume-of-interest), Figure 2.37. This is also seen from the contrast estimates, figure 2.38.
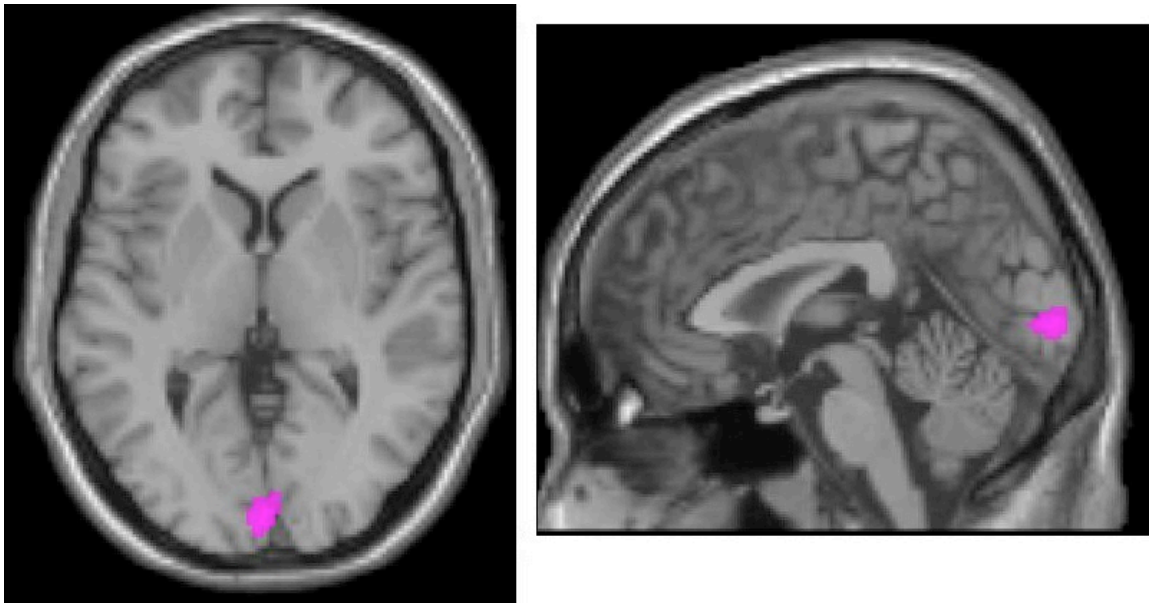
Figure 2.37: Right cuneus in CIPN-myeloma and healthy volunteers

There was a significant greater BOLD response with the application of the thermal stimuli to the foot than thigh in CIPN-myeloma patients whereas healthy volunteers exhibited greater activation in response to thigh than to foot stimulation. Functional imaging data are shown against axial (z=8mm) and sagittal (x=2mm) slices through a canonical single-subject T1-weighted image. For display purposes, the statistical threshold is p < 0.001, 62 voxels exceeded, uncorrected, at the voxel-level.
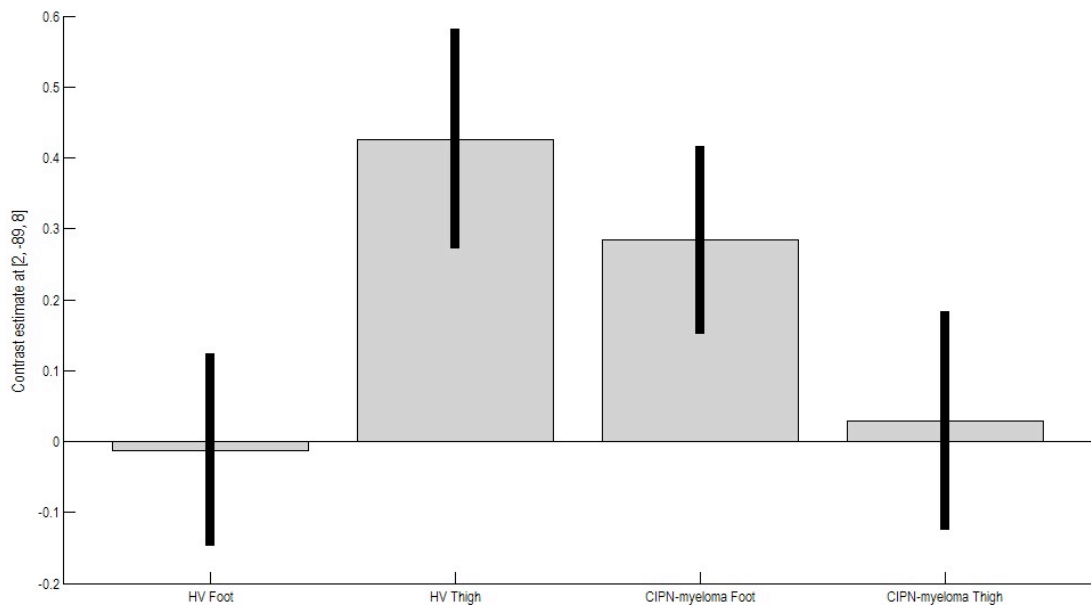
Figure 2.38: Contrast estimates and 90% CI at the right cuneus (co-ordinate 2, -89, 8) when comparing healthy volunteers to CIPN-myeloma patients depicted in figure 2.37.

2.7.6 Correlation of brain activation in CIPN-myeloma patients

Additionally, a multiple regression analysis of the brain activation (when the application of the thermal stimuli was applied to the foot) was performed with TNSr in CIPN-myeloma patients, which found significant activation in the left operculo-insular cortex (Talairach co-ordinates: -58, 2 4; peak t=8.02; p=0.03 FWE-corrected in the volume-of-interest for CIPN-myeloma foot activation) (Figure 2.39). There was a strong correlation between the functional contrast in this area of brain activation and the TNSr (Figure 2.40).

However, there was no correlation with Dyck's score and the brain activation when the application of the thermal stimuli was applied to the foot) in CIPN-myeloma patients.

There was also no significant correlation between brain activation levels in the CIPN-myeloma patients and the CPAQ and the PCS questionnaires when these were used as covariates.
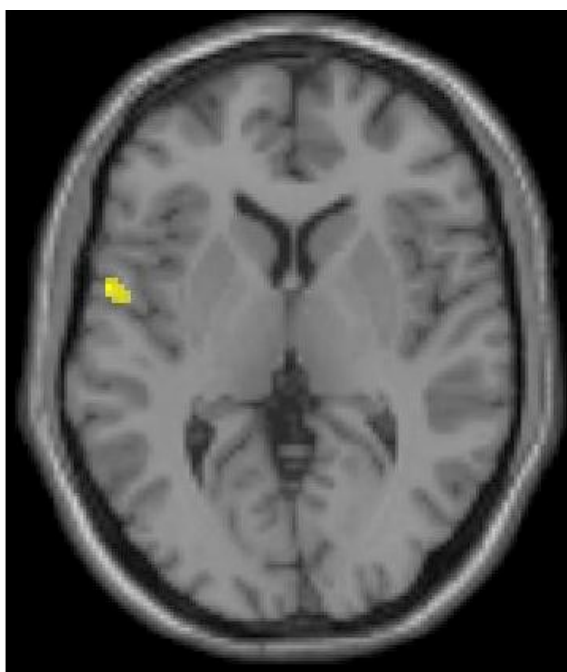
Figure 2.39: CIPN-myeloma patients demonstrated activation at operculo-insular cortex during the application of the thermal stimuli at the foot when correlated with TNSr

Functional imaging data is shown against axial (z=4mm) slice through a canonical single-subject T1-weighted image. For display purposes, the statistical threshold is p < 0.001, uncorrected, at the voxel-level
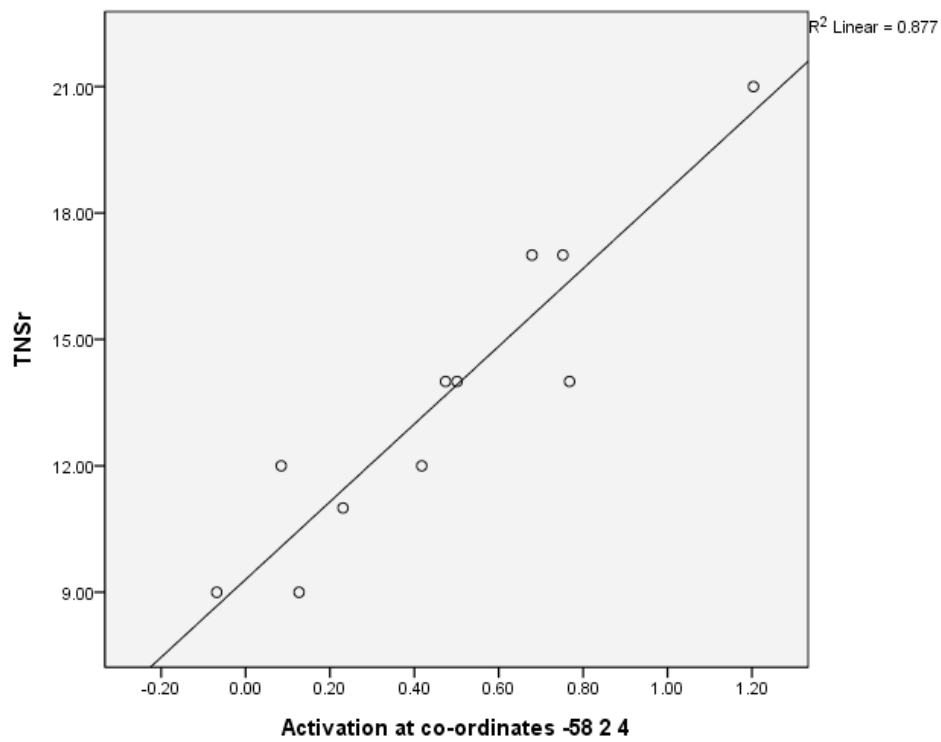
Figure 2.40: Scatter dot graph with a line to fit showing the r^2 linear regression

Significant positive correlation was present between activation in the operulo-insular cortex and scoring of the TNSr

2.7.7 fMRI measures in CIPN patients on opioids

There were seven CIPN patients (58%) who were on regular opioids for pain mainly neuropathic in origin, ranging from tramadol and codeine, morphine and oxycodone tablets and fentanyl and buprenophrine patches. These patients were asked to continue the opioids as the doses had been stable for more than one month.

2.7.7.1 Opioids: CIPN-myeloma group analysis

Statistical parametric activation maps were constructed for the CIPN-myeloma group who were on opioids and those who were not on opioids individually.

Firstly, compared with baseline, thermal stimulation to the foot and thigh (combined) evoked significant brain activation in both these groups as seen in Figure 2.41.
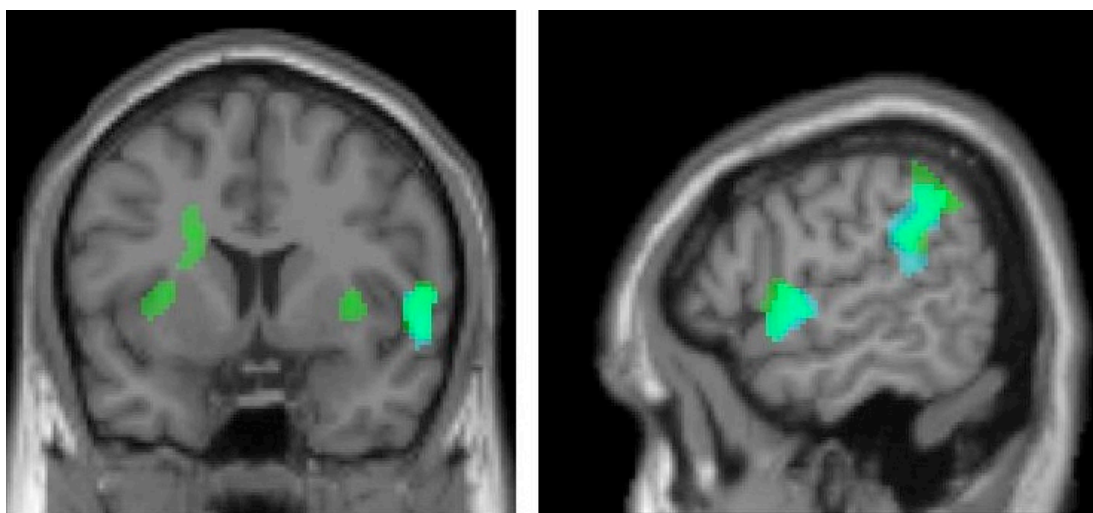
Figure 2.41: Regions of activation following application of the thermal stimuli (painful versus baseline) for CIPN-myeloma patients on opioids (green), CIPN-myeloma patients not on opioids (cyan), and overlapping common regions of activation

Foci with a maximum exceeding statistical threshold $p<0.05$, FWE-corrected in the whole brain, are shown at visualisation threshold $p<0.001$ uncorrected

Table 2.25 and 2.26 show the areas of activation on application of the thermal stimuli to the foot and thigh in CIPN-myeloma patients on opioids and those not on opioids.

Table 2.25: Significant brain activation on application of the thermal stimuli (foot + thigh) in CIPN-myeloma patients on opioids

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Lt posterior cerebellar lobe	-	-20 -64 -26	9.7	<0.001	3583
Rt superior temporal gyrus	22	56 4 2	8.37	0.005	521
Lt postcentral gyrus	43	-58 -20 21	7.08	0.039	4105
Rt inferior parietal lobe	40	61 -43 37	6.95	0.048	326

Table 2.26: Significant brain activation on application of the thermal stimuli (foot + thigh) CIPN-myeloma patients not on opioids

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt superior temporal gyrus	22	58 4 0	9.58	0.001	691
Lt middle frontal gyrus	46	-34 40 15	7.87	0.011	321
Lt postcentral gyrus	4	-20 -32 60	6.98	0.046	186

2.7.7.2 Opioids: comparison between CIPN-myeloma patients

The mean BOLD activation during the application of the thermal stimuli in CIPN-myeloma patients on opioids and those not on opioids was compared and the differential cortical activation between the 2 groups during foot + thigh stimulations was observed.

During the thermal stimulation, there was significant activation in the left posterior cerebellar lobe (Talairach co-ordinates -20 -64 -27) in CIPN-myeloma patients on opioids compared to those not on opioids (t=8.54; p=0.004 FWE-corrected (Figure 2.42).
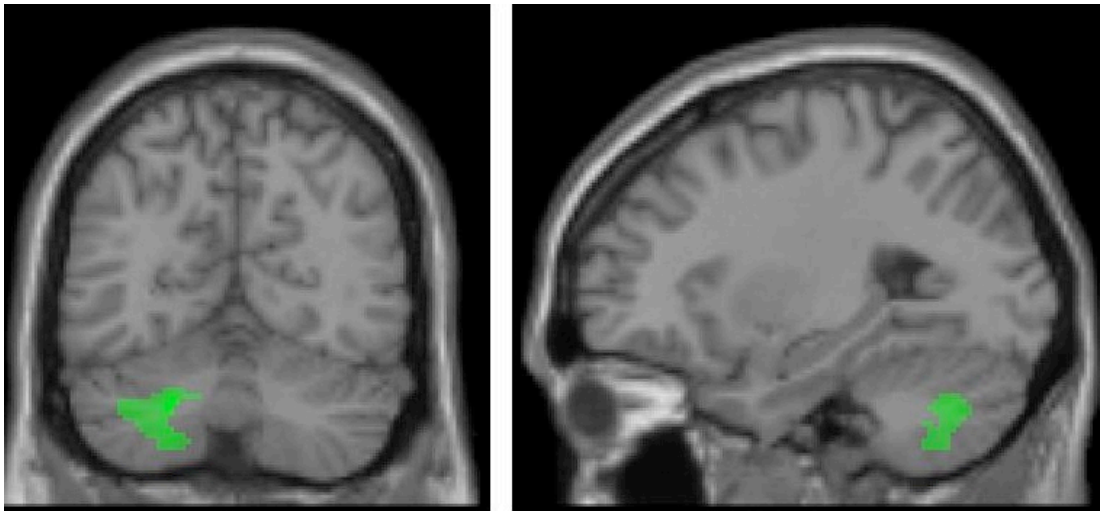
Figure 2.42: Activation of left posterior cerebellar lobe in CIPN-myeloma patients on opioids compared to those not on opioids

Functional imaging data are shown against coronal (y=-64mm) and sagittal (x=-20mm) slices through a canonical single-subject T1-weighted image. For display purposes, the statistical threshold is p < 0.001, 546 voxels exceeded, uncorrected, at the voxel-level

From the contrast estimates, it is suggested that CIPN-myeloma patients on opioids demonstrated significant activation at the cerebellum, whilst those not on opioids showed de-activation (Figure 2.43).
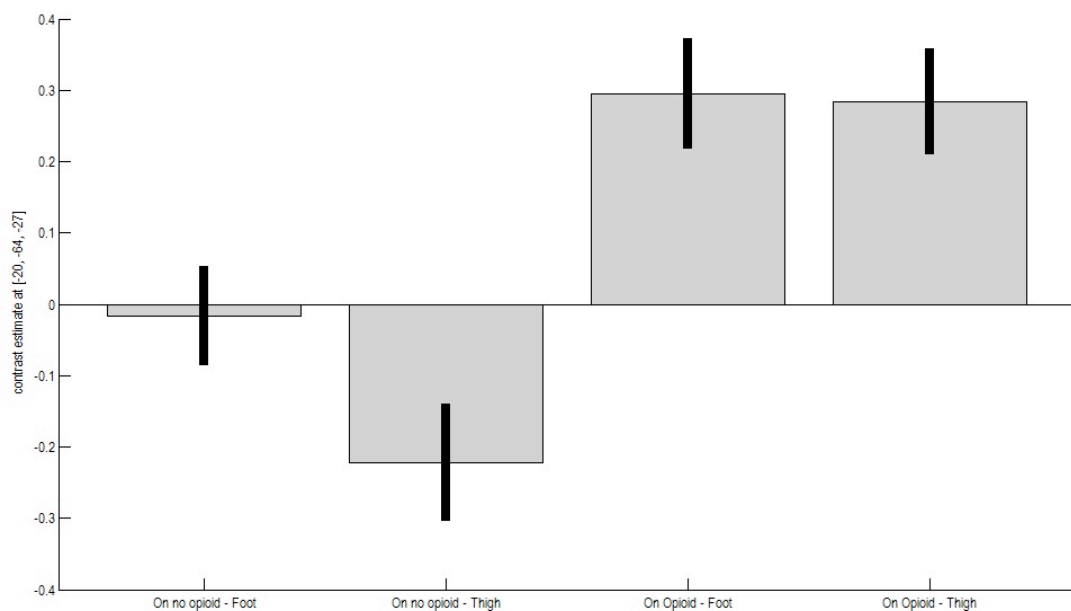
Figure 2.43: Contrast estimates and 90% CI at left posterior cerebellar lobe (co-ordinate -20 -64 -27) when comparing CIPN-myeloma patients on opioids to those not on opioids depicted in figure 2.42.

2.7.8 Attenuation of fMRI signal with noxious thermal heat stimulus

Statistical maps were constructed and analysed for the combination of the first three and the last three images when the thermal stimuli were applied in both healthy volunteers and CIPN-myeloma patients at the sites of the foot and the thigh.

2.7.8.1 Habituation: Individual group analysis

Firstly, compared with baseline, thermal stimulation of the foot for the first three stimuli evoked different brain activation in both healthy volunteers and CIPN-myeloma patients as seen in Figure 2.44 and 2.45 respectively.
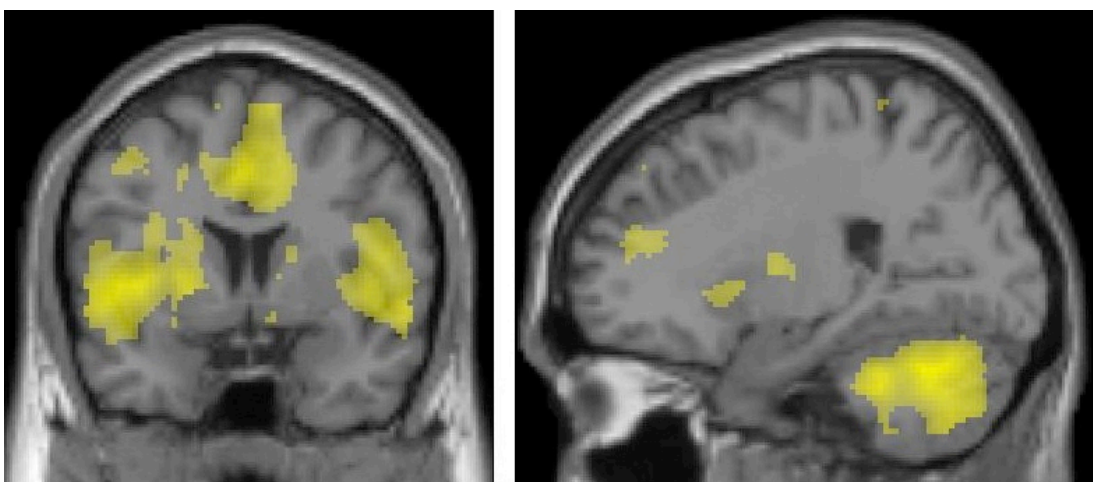
Figure 2.44: Regions of signification brain activation following the first three thermal stimulations on the foot (painful versus baseline) for healthy volunteers
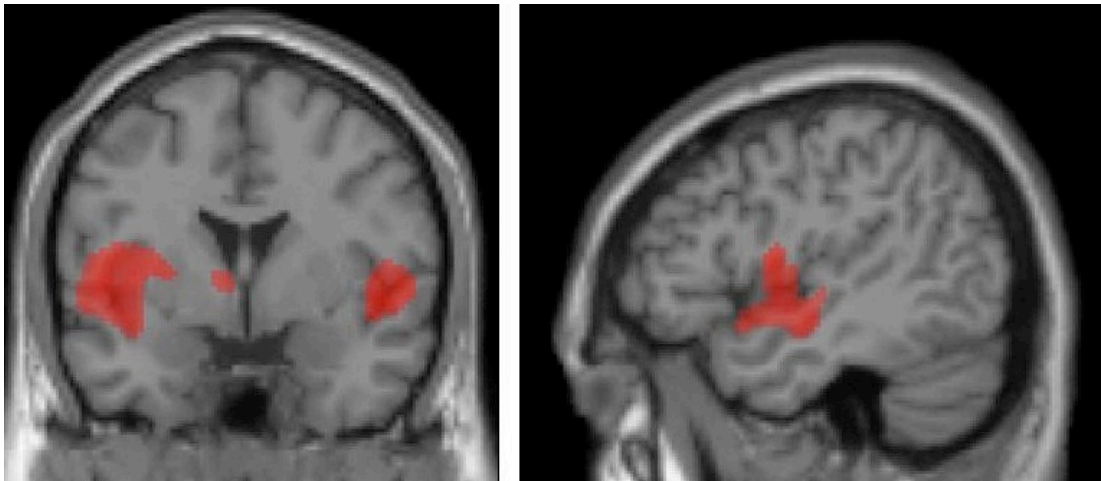
Figure 2.45: Regions of significant brain activation following the first three thermal stimulations on the foot (painful versus baseline) for CIPN-myeloma patients

For figures 2.44 and 2.45: Foci with a maximum exceeding statistical threshold p<0.05, FWE-corrected in the whole brain, are shown at visualisation threshold p<0.001 uncorrected

Table 2.27 and 2.28 show the areas of significant brain activation on first three painful stimulations on the foot in healthy volunteers and CIPN-myeloma patients.

Table 2.27: Significant brain activation on application of the thermal stimuli to the foot in healthy volunteers: first three thermal stimuli > Baseline

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Lt claustrum	-	-32 -21 8	9.64	<0.001	40897
Lt posterior cerebellum	-	-24 -59 -17	9.00	<0.001	40897
Rt inferior parietal lobule	40	62 -36 26	6.64	0.002	2302

Table 2.28: Significant brain activation on application of the thermal stimuli to the foot in CIPN-myeloma patients: first three painful stimuli > Baseline

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Lt insula	13	-40 -14 -5	8.55	<0.001	3033
Rt superior temporal gyrus	22	57 6 0	6.37	0.004	1891
Rt cuneus	17	4 -80 14	5.04	0.001 (cluster)	360

Next, looking at brain activation at the first three thermal stimulations on the thigh and compared with baseline, evoked different significant brain activations in both the healthy volunteers and CIPN-myeloma patients as shown in Figure 2.46 and 2.47 respectively.

Figure 2.46: Regions of significant brain activation following the first three thermal stimulations on the thigh (painful versus baseline) for healthy volunteers
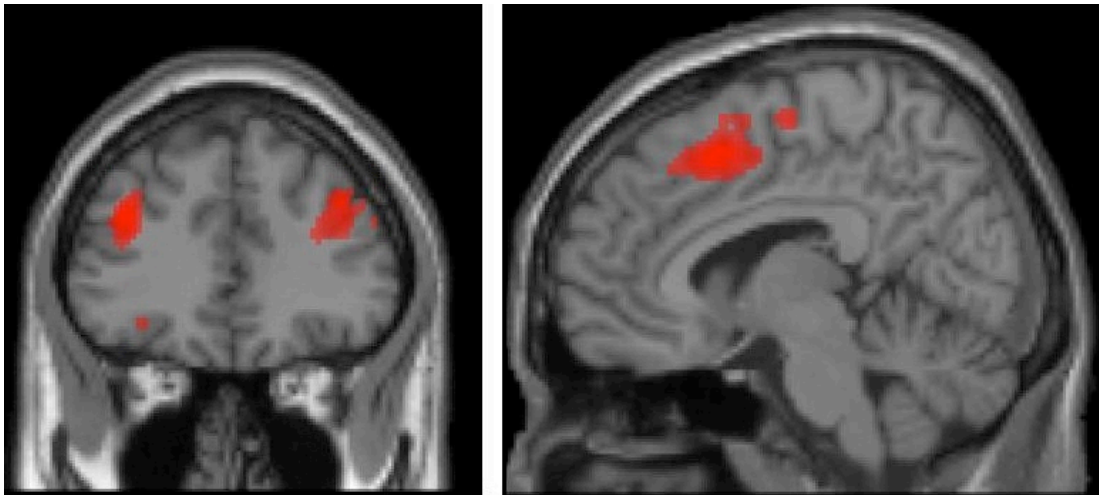
Figure 2.47: Regions of significant brain activation following the first three thermal stimulations on the thigh (painful versus baseline) for CIPN-myeloma patients

Table 2.29 and 2.30 show the areas of activation on first three painful stimulations on the thigh in healthy volunteers and CIPN-myeloma patients respectively.

Table 2.29: Significant brain activation on application of the thermal stimuli to the thigh in healthy volunteers: first three painful stimuli > Baseline

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Lt insula	-	-32 16 6	7.48	<0.001	2349
Rt middle frontal gyrus	10/47	44 43 -4	6.73	0.001	3249
Lt occipital lobe	-	-30 -69 18	6.41	0.003	3965
Lt superior parietal lobe (postcentral gyrus)	7	-20 -41 65	5.77	0.021	364
Rt inferior frontal gyrus	44	46 5 31	4.89	0.027	300

Table 2.30: Significant brain activation on application of the thermal stimuli to the thigh in CIPN-myeloma patients: first three painful stimuli > Baseline

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Lt middle frontal gyrus	46/9	-36 36 22	6.81	0.001	638
Rt medial frontal gyrus	6/8	8 18 45	6.77	0.001	929
Rt superior temporal gyrus	22	47 13 -2	6.49	0.003	1074
Rt inferior parietal lobe	40	60 -35 33	6.38	0.003	292
Rt precuneus	7	8 -68 38	6.10	0.008	116
Rt middle frontal gyrus	46/9	40 36 28	6.01	0.010	463
Lt middle temporal gyrus	21	-58 -12 -3	5.69	0.026	208
Rt posterior cerebellum	-	44 -60 -35	5.50	0.044	14

The next section will focus on the last three painful stimulations when compared with baseline at the foot and then at the thigh respectively. Figure 2.48 and 2.49 show the areas of significant brain activation for the last three thermal stimulations at the foot site and the thigh site respectively for healthy volunteers. There was no significant brain activation during the last three painful stimulations on the foot in CIPN-myeloma patients whilst there was an area of significant activation during the last three painful stimulations on the thigh when compared to the baseline in CIPN-myeloma group (Figure 2.50).
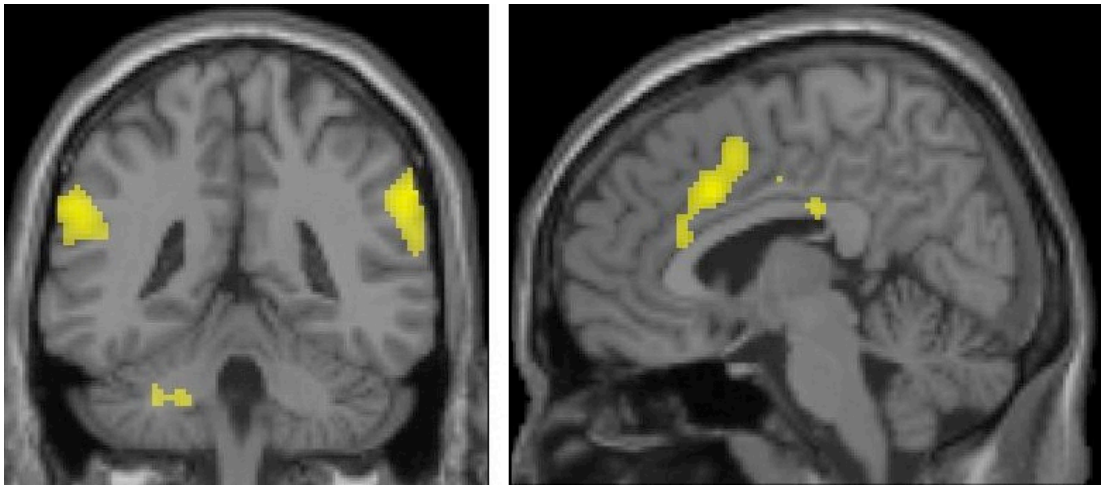
Figure 2.48: Regions of significant brain activation following the last three thermal stimuli to the foot (painful versus baseline) for healthy volunteers

Figure 2.49: Regions of significant brain activation following the last three thermal stimuli to the thigh (painful versus baseline) for healthy volunteers
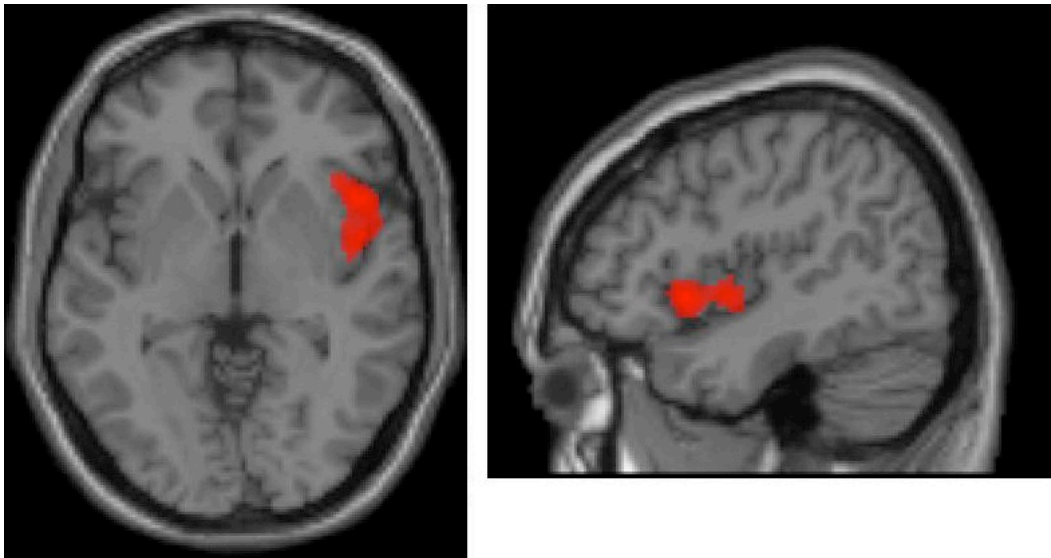
Figure 2.50: Regions of significant brain activation following the last three thermal stimuli to the thigh (painful versus baseline) for CIPN-myeloma patients

Table 2.31, 2.32 and 2.33 show the areas of activation on last three painful stimulations on the foot and thigh in healthy volunteers and CIPN-myeloma patients respectively.

Table 2.31: Significant brain activation on application of the thermal stimuli to the foot in healthy volunteers: last three painful stimuli > Baseline

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt supramarginal gyrus	40	59 -43 32	6.42	0.003	840
Lt supramarginal gyrus	40	-58 -43 28	6.10	0.009	461
Rt inferior frontal gyrus	44	53 10 11	5.77	0.023	1159
Rt cingulate gyrus	32	6 19 30	5.70	0.027	978
Lt posterior cerebellum	-	-30 -59 -19	5.61	0.035	1331

Table 2.32: Significant brain activation on application of the thermal stimuli to the thigh in healthy volunteers: last three painful stimuli > Baseline

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt superior temproral gyrus	22	63 -40 22	7.37	<0.001	698
Lt lentiform nucleus	-	-26 -8 8	7.09	<0.001	3481
Lt sub-lobar insula	13	-55 -32 20	6.44	0.003	697
Rt cingulate gyrus	24	8 -16 34	5.57	0.036	165
Rt inferior frontal gyrus	47	46 19 -8	5.40	0.050	435

Table 2.33: Significant brain activation on application of the thermal stimuli to the thigh in CIPN-myeloma: last three painful stimuli > Baseline

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt inferior frontal gyrus	47	46 15 -4	5.77	0.020	531

2.7.8.2   Habituation: comparison between first three thermal stimuli vs last three thermal stimuli

The mean BOLD activation during the first three thermal stimuli and the last three thermal stimuli delivered to the foot and then to the thigh in both the healthy volunteers and CIPN-myeloma patients were compared.

Healthy volunteers

In healthy volunteers, comparing the first three thermal pain stimulations delivered to the foot with the last three, showed significant activation in the left anterior lobe of the cerebellum and in the right claustrum. (Figure 2.51, Table 2.34) There was no significant brain activation when comparing the last three thermal pain stimulations delivered to the foot with the first three.
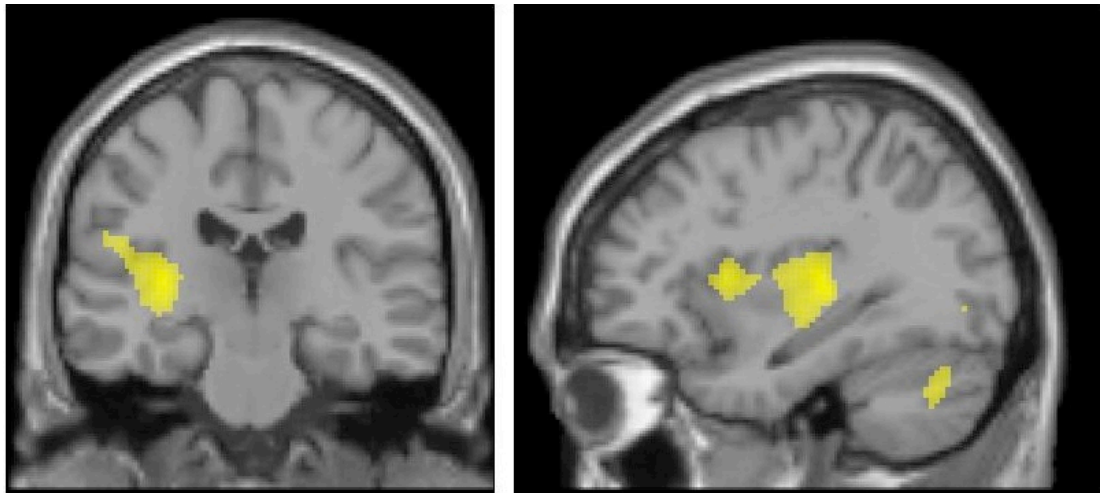
Figure 2.51: Functional image data (coronal and sagittal views) of the cerebellum and right claustrum activation during the application of the first three thermal stimuli to foot compared to the last three thermal stimuli in healthy volunteers

Images are displayed against sections through a canonical single subject T1-weighted image. For display purposes, the voxel-level threshold is p<0.001, uncorrected at threshold level (k_E) of 1683.

Table 2.34: Significant brain activation on application of the thermal stimuli to the foot in healthy volunteers: First three stimuli vs last three stimuli

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Lt anterior lobe of cerebellum	-	-18 -48 -14	6.56	0.002	4504
Rt claustrum	-	-32 -23 7	6.17	0.007	1683

From the contrast estimates, it is suggested that healthy volunteers demonstrated significant signal changes at these regions of activations (left anterior lobe of cerebellum and right claustrum) when comparing the first three thermal stimulations to the last three stimuli delivered to the foot (Figure 2.52 and 2.53).
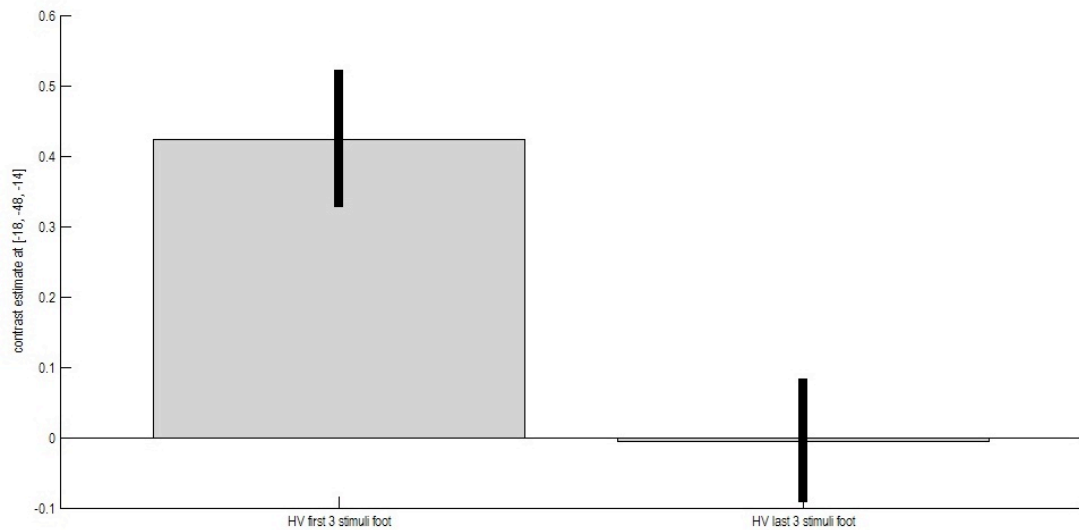
Figure 2.52: Contrast estimates and 90% CI at the cerebellum (co-ordinate -18 -48 -14) when comparing the first three thermal stimuli to the last three stimuli deliverd to the foot in healthy volunteers
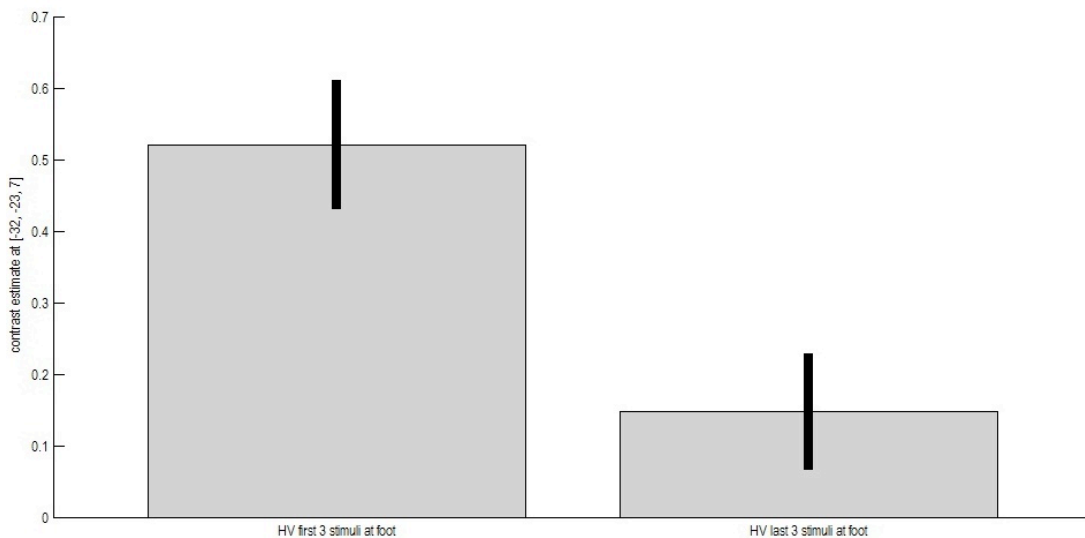
Figure 2.53: Contrast estimates and 90% CI at the claustrum (co-ordinate -32 -23 7) when comparing the first three thermal stimuli to the last three stimuli deliverd to the foot in healthy volunteers

In healthy volunteers, comparing the first three thermal pain stimulations delivered to the thigh with the last three stimuli, showed no significant activation in the brain; again no significant activation was demonstrated when comparing the last three thermal pain stimulations delivered to the thigh with the first three stimuli.

CIPN-myeloma patients

In CIPN-myeloma patients, comparing the first three thermal pain stimulations delivered to the foot with the last three, showed different significant areas of brain activations (Table 2.35, Figure 2.54). There was no significant brain activation when comparing the last three thermal pain stimulations delivered to the foot with the first three painful stimulations.
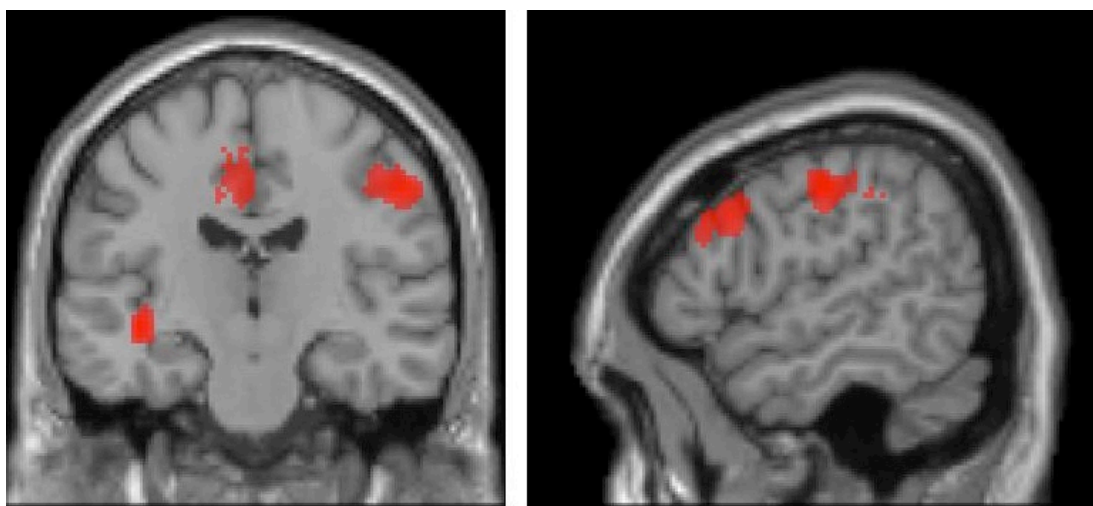
Figure 2.54: Functional image data (coronal and sagittal views) of the temporal gyrus and precentral gyrus activation during the first three thermal stimuli to foot compared to the last three thermal stimuli in CIPN-myeloma patients

Images are displayed against sections through a canonical single subject T1-weighted image. For display purposes, the voxel-level threshold is $p<0.001$, uncorrected at threshold level (k_E) of 144.

Table 2.35: Significant brain activation on application of the thermal stimuli to the foot in CIPN-myeloma patients: First three stimuli vs last three stimuli

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Lt medial temporal gyrus	21	-40 -15 -8	5.79	0.021	386
Rt precentral gyrus	4	53 -12 41	5.63	0.033	740
Rt precentral gyrus	6	36 -4 37	5.60	0.036	843
Rt sub-lobar insula	13	30 15 -6	5.59	0.038	144

From the contrast estimates, it is suggested that CIPN-myeloma patients demonstrated significant signal changes at the regions of activations comparing the first three thermal pain stimulations to the last three stimuli delivered to the foot (Figure 2.55 a,b,c,d).

a)

a) at co-ordinates -40 -15 -8 (medial temporal gyrus)

b)
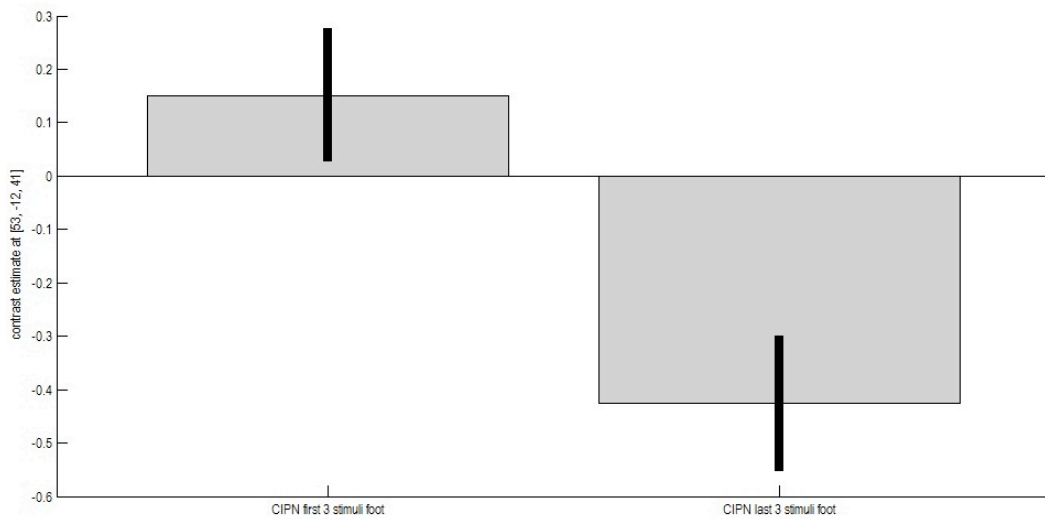
b) at co-ordinates 53 -12 41 (precentral gyrus, Brodmann 4)

c)
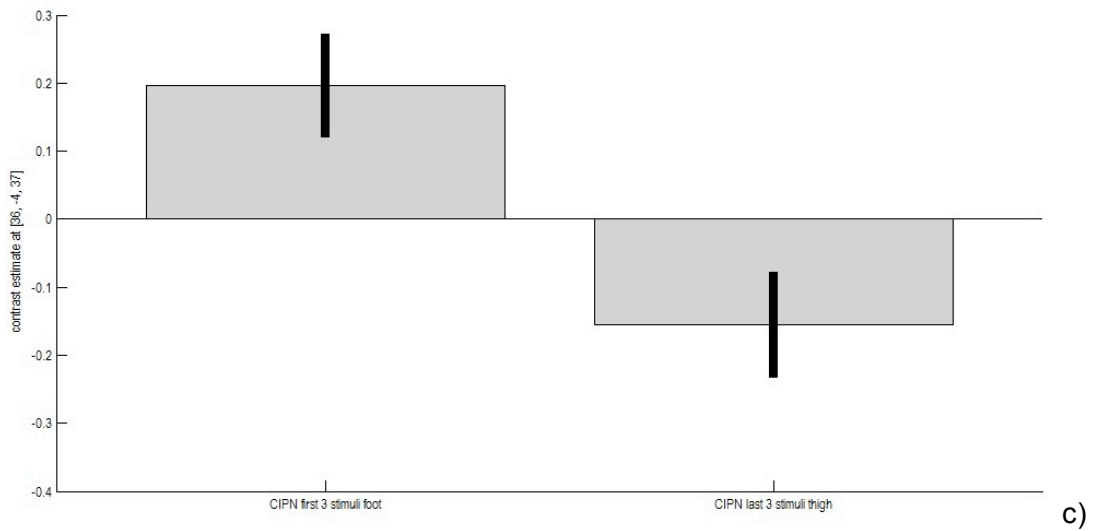
c) at co-ordinates 36 -4 37 (precentral gyrus, Brodmann 6)

d)
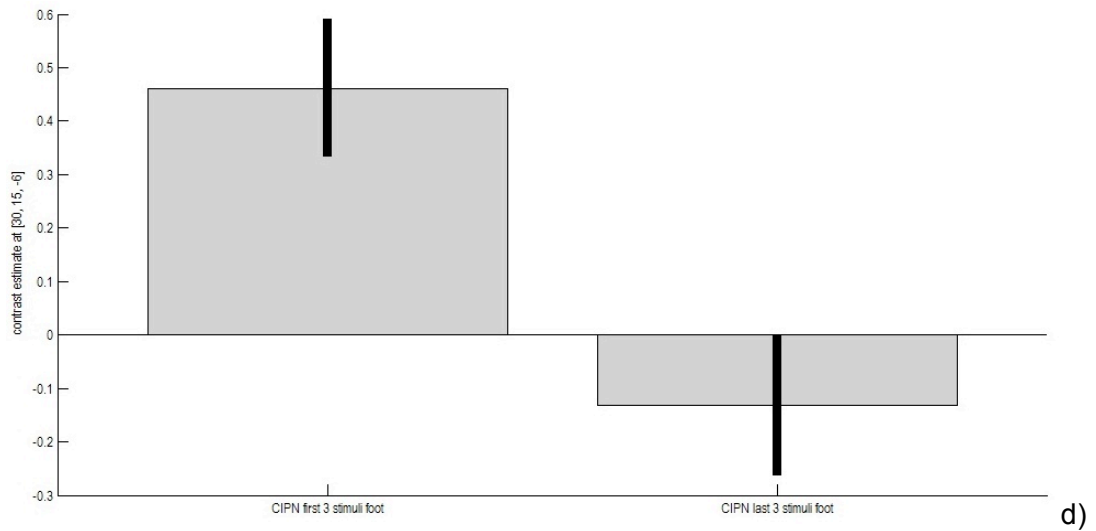
d) at co-ordinates 30 15 -6 (sub-lobar insula)

Figure 2.55: Contrast estimates and 90% CI when comparing the first three thermal stimuli to the last three stimuli deliverd to the foot in CIPN-myeloma patients

In CIPN-myeloma patients, comparing the first three thermal pain stimulations delivered to the thigh with the last three, showed no significant activation and vice versa.

2.7.8.3 Habituation: Comparison between healthy volunteers and CIPN-myeloma patients

The mean BOLD activation during the first three painful stimuli in healthy volunteers and CIPN-myeloma patients was compared and the differential cortical activation shown between the 2 groups during foot thermal stimulation and the thigh stimulation.

During first three thermal stimuli delivered to the foot, there was a significant activation in the right cingulate gyrus and right anterior cerebellar lobe in healthy volunteers compared to CIPN-myeloma patients (Figure 2.56 and Table 2.36).
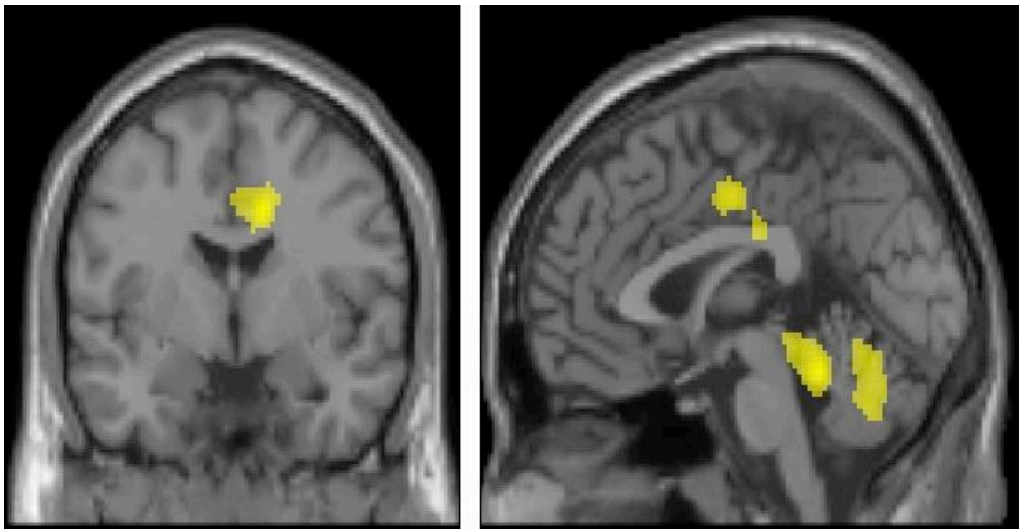
Figure 2.56: Healthy volunteers demonstrated significant activation of the cingulate gyrus and cerebellum during the first three thermal stimuli to the foot when compared with CIPN-myeloma patients.

Images are displayed against sections through a canonical single subject T1-weighted image. For display purposes, the voxel-level threshold is p<0.001, uncorrected at threshold level (k_E) of 689.
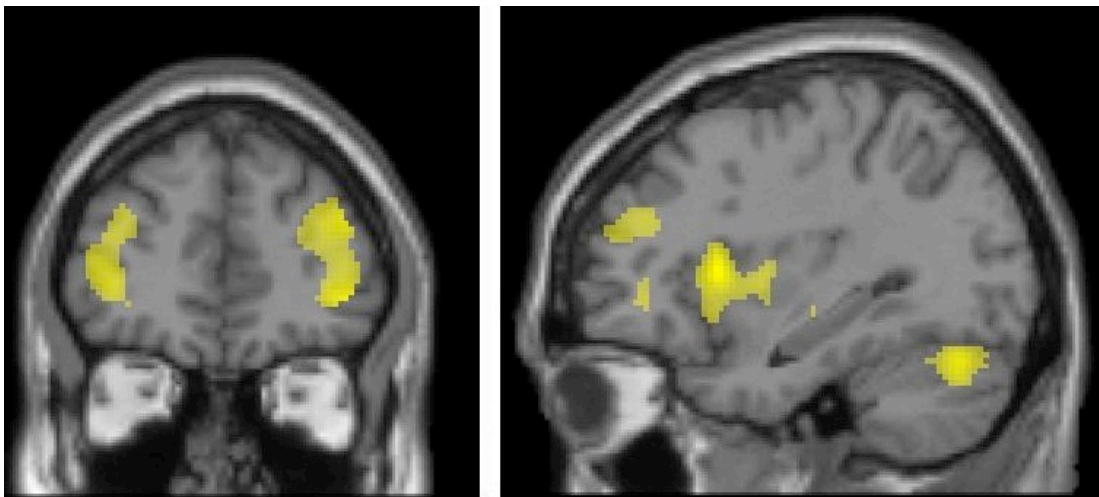
Table 2.36: Significant brain activation on application of the thermal stimuli to the foot in healthy volunteers vs CIPN-myeloma patients: First three stimuli

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt anterior cerebellar lobe	-	4 -43 -13	6.14	0.008	3569
Rt cingulate gyrus	24	14 2 35	5.84	0.018	689

From the contrast estimates, it is suggested that CIPN-myeloma patients demonstrated hypo-activation at the cingulate gyrus and the cerebellum, whilst healthy volunteers showed activation (Figure 2.57 a,b).

a)

a) at co-ordinates 4 -43 -13 (right anterior cerebellum)

b)

b) at co-ordinates 14 2 35 (right cingulate gyrus)

Figure 2.57: Contrast estimates and 90% CI when comparing the first three thermal stimuli deliverd to the foot in healthy volunteers and CIPN-myeloma patients.

During last three thermal stimuli to the foot, there was a significant activation in the right cingulate and middle frontal gyrus in healthy volunteers compared to CIPN-myeloma patients (Figure 2.58 and Table 2.37).
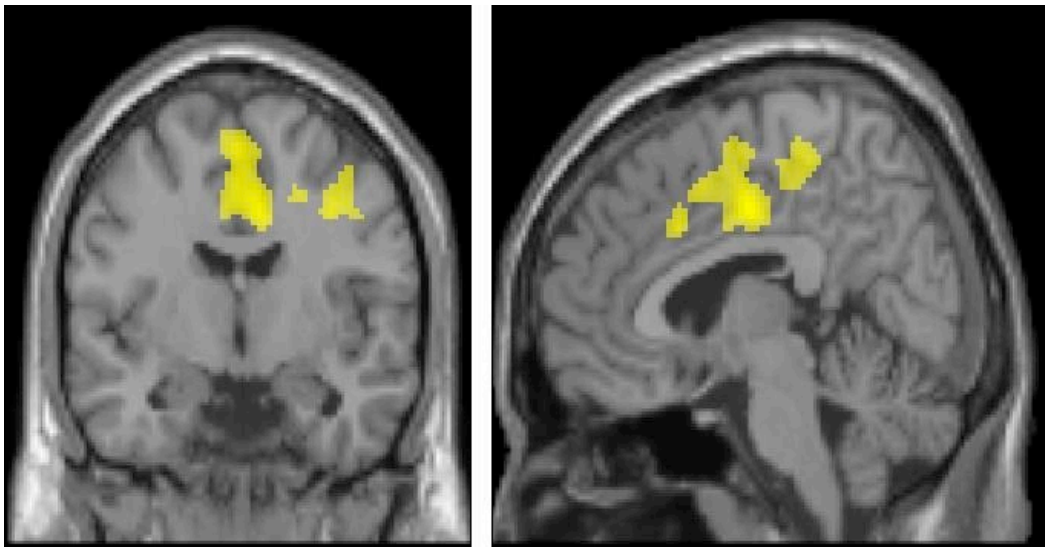
Figure 2.58: Healthy volunteers demonstrated significant activation of the anterior cingulate and middle frontal gyrus during the last three thermal stimuli to the foot when compared with CIPN-myeloma patients.

Images are displayed against sections through a canonical single subject T1-weighted image. For display purposes, the voxel-level threshold is p<0.001, uncorrected at threshold level (k_E) of 621.

Table 2.37: Significant brain activation on application of the thermal stimuli to the foot in healthy volunteers vs CIPN-myeloma patients: last three stimuli

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt cingulate gyrus	24	6 -6 39	5.73	0.025	2667
Rt middle frontal gyrus	6	50 4 42	5.57	0.039	621

From the contrast estimates, it is suggested that CIPN-myeloma patients demonstrated hypo-activation at the right anterior cingulate gyrus and the middle frontal gyrus, whilst healthy volunteers showed activation (Figure 2.59 a,b).

a)
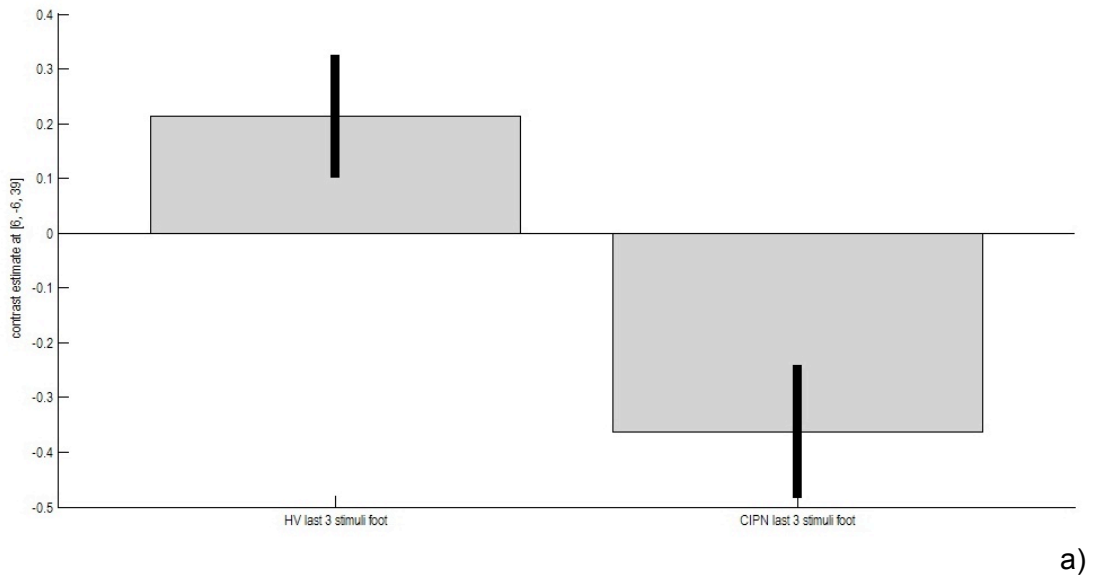
a) at co-ordinates 6 -6 39

b)

b) at co-ordinates 50 4 42

Figure 2.59: Contrast estimates and 90% CI when comparing the last three thermal stimuli deliverd to the foot in healthy volunteers and CIPN-myeloma patients

During first three thermal stimuli to the thigh, there was a significant activation in the temporal lobe in healthy volunteers compared to CIPN-myeloma patients (Figure 2.60 and Table 2.38).
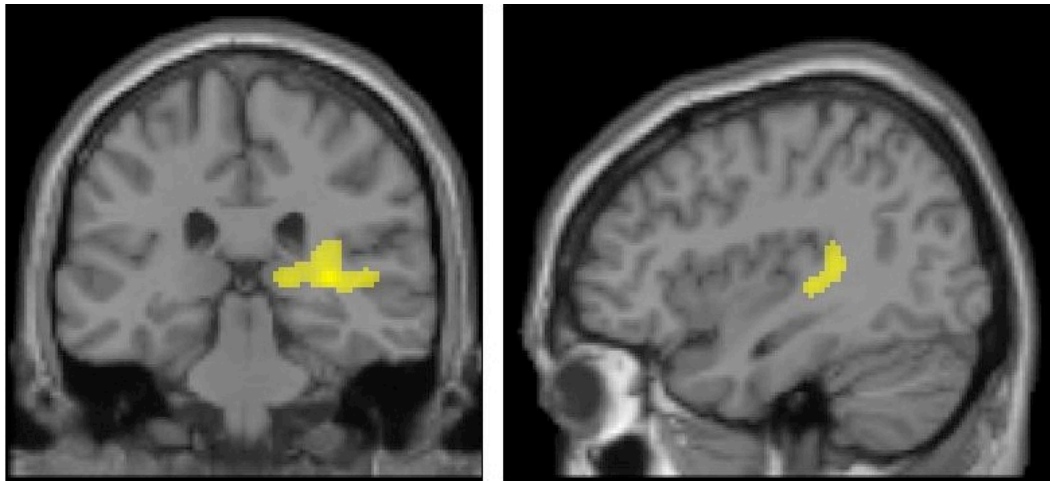
Figure 2.60: Healthy volunteers demonstrated significant activation of the temporal lobe during the first three painful heat stimulations at the thigh when compared with CIPN-myeloma patients.

Images are displayed against sections through a canonical single subject T1-weighted image. For display purposes, the voxel-level threshold is p<0.001, uncorrected at threshold level (k_E) of 592.
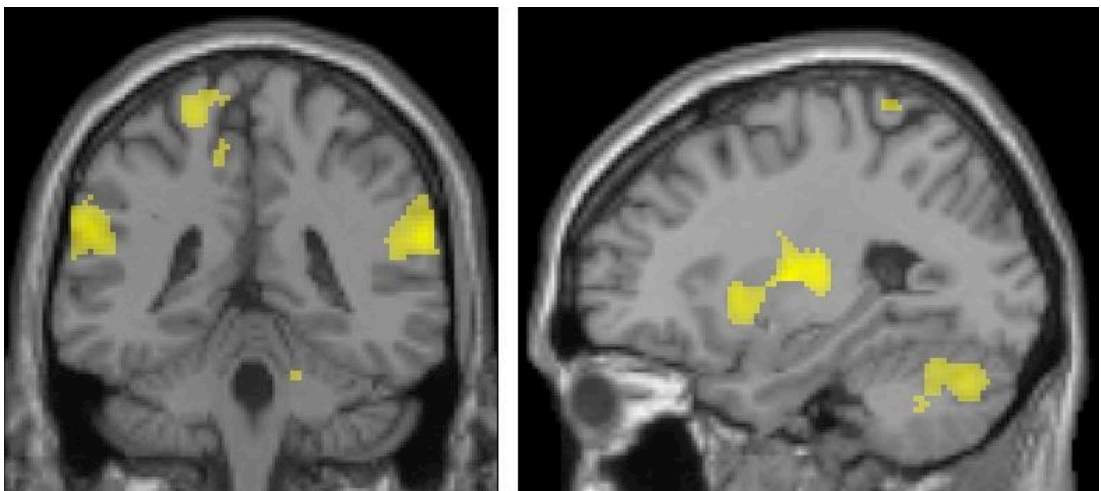
Table 2.38: Significant brain activation on application of the thermal stimuli to the thigh in healthy volunteers vs CIPN-myeloma patients: first three stimuli

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Rt temporal lobe	-	34 -31 7	6.03	0.010	592

From the contrast estimates, it is suggested that CIPN-myeloma patients demonstrated hypo-activation at the temporal lobe, whilst healthy volunteers showed activation (Figure 2.61).
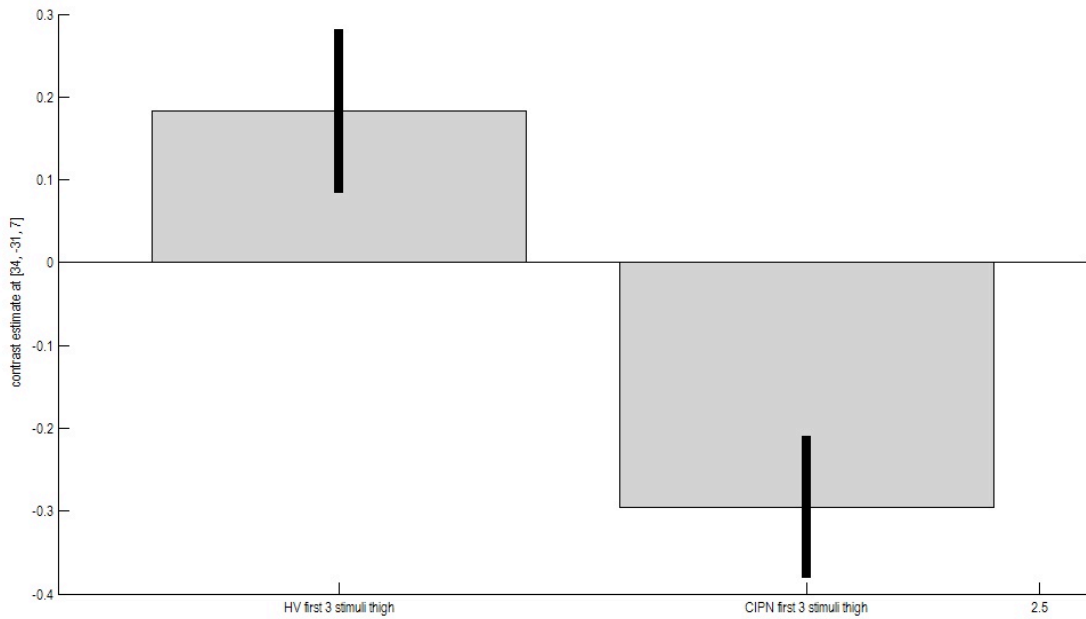
Figure 2.61: Contrast estimates and 90% CI when comparing the first three thermal stimuli delivered to the thigh in healthy volunteers and CIPN-myeloma patients at the right temporal lobe (co-ordinates 34 -31 7).

During last three thermal stimuli to the thigh, there was a significant activation in the putamen in healthy volunteers compared to CIPN-myeloma patients (Figure 2.62 and Table 2.39).
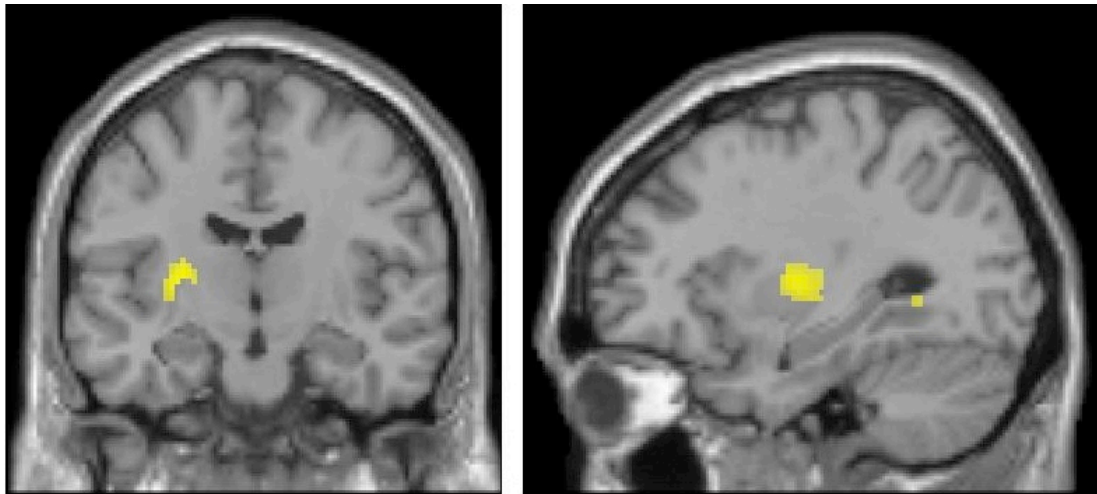
Figure 2.62: Healthy volunteers demonstrated significant activation of the putamen during the last three thermal stimuli at the thigh when compared with CIPN-myeloma patients

Images are displayed against sections through a canonical single subject T1-weighted image. For display purposes, the voxel-level threshold is p<0.001, uncorrected at threshold level (k_E) of 192.

Table 2.39: Significant brain activation on application of the thermal stimuli to the thigh in healthy volunteers vs CIPN-myeloma patients: last three stimuli

Region	BA	Talairach co-ordinates	Peak t	p (FWE-corr)	Voxels
Lt putamen	-	-26 -7 8	6.09	0.008	192

From the contrast estimates, it is suggested that CIPN-myeloma patients demonstrated hypo-activation at the putamen, whilst healthy volunteers showed activation (Figure 2.63).
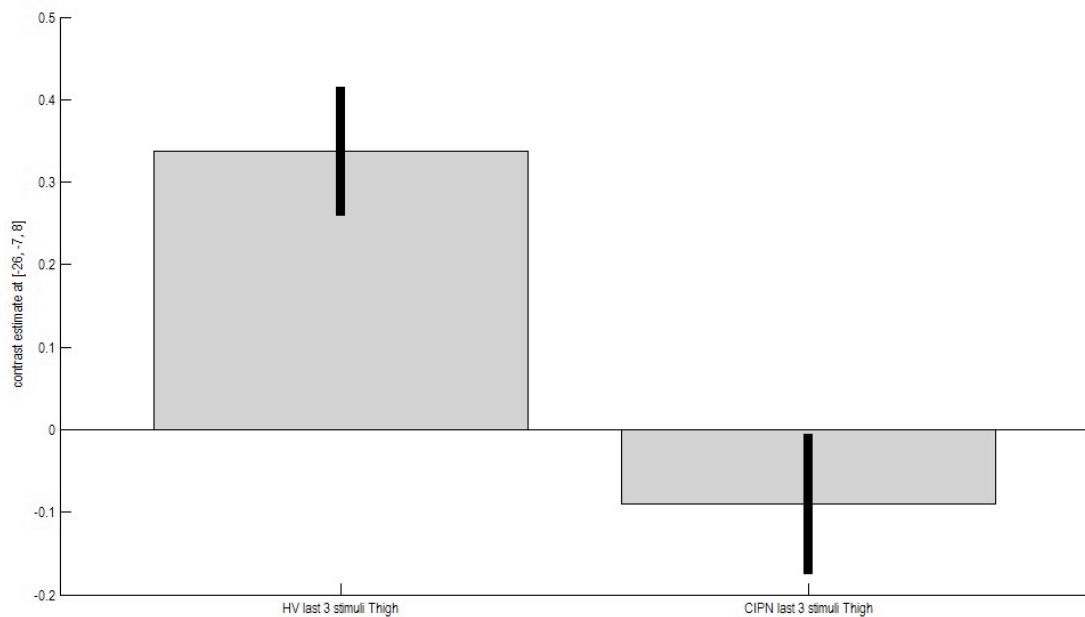
Figure 2.63: Contrast estimates and 90% CI when comparing the last three stimuli deliverd to the thigh in healthy volunteers and CIPN-myeloma patients at the left putamen (co-ordinates -26 -7 8)

2.7.9 Reproducibility

Four healthy volunteers were re-scanned a year later to test for reproducibility. The thermal stimuli to the foot and the finger tapping were repeated. Statistical parametric activation maps were constructed for the individual healthy volunteers. The mean BOLD activation in the same healthy volunteers was obtained for the first scanning session and the second scanning session (a year later) for the thermal stimuli to the foot and the finger tapping. Afterwards, using a 2-sample t-test in SPM, the mean BOLD activations were compared. There were no areas of significantly different activations when comparing the first scans to the second scans in the healthy volunteers both during the painful stimulation (Figure 2.64) and during the finger tapping (Figure 2.65).
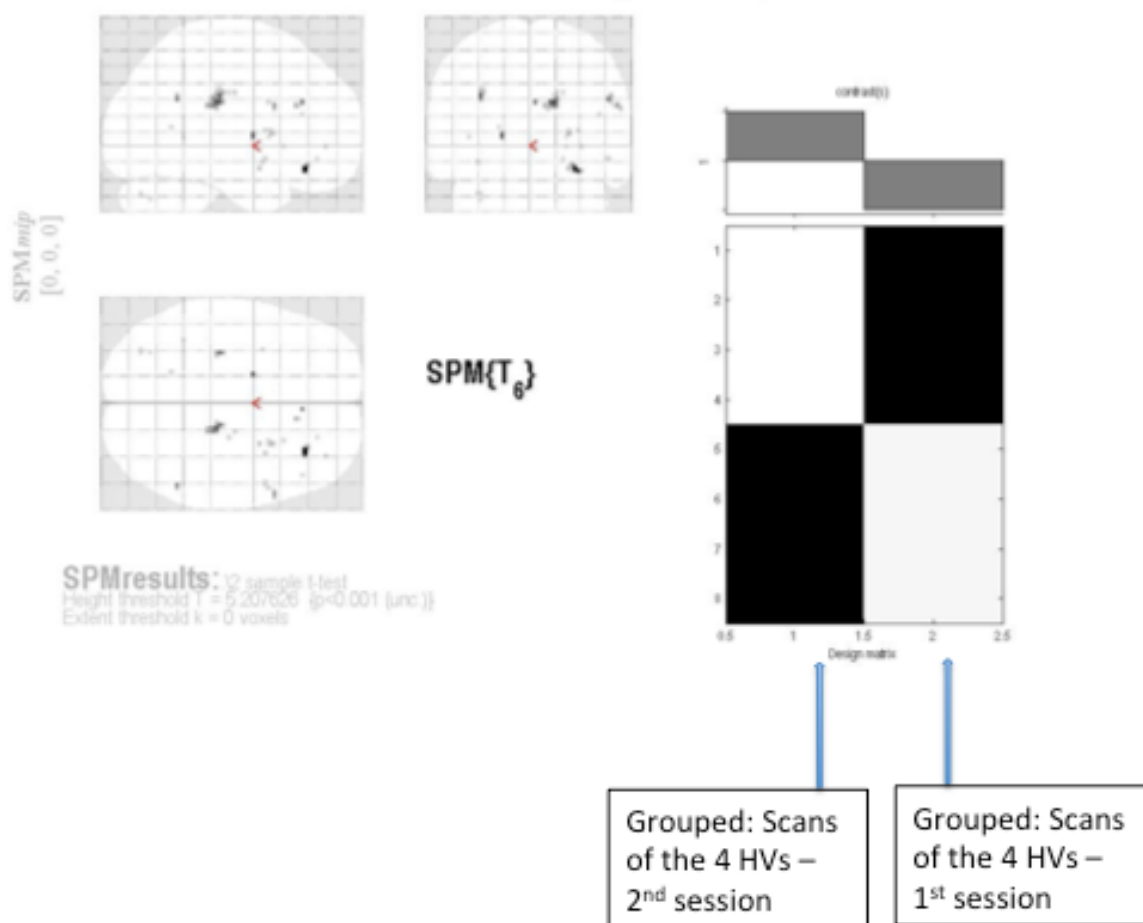
Figure 2.64: Comparison of the fMRI images of the 2nd and 1st sessions of scanning 4 healthy volunteers during thermal stimuli at the foot

The design matrix on the right is showing the contrast of MR images of the 4 HV when comparing the 2nd session with the 1st session scanning. The image on the left displays glass brains in a coronal, sagittal and axial view with no significant activations.
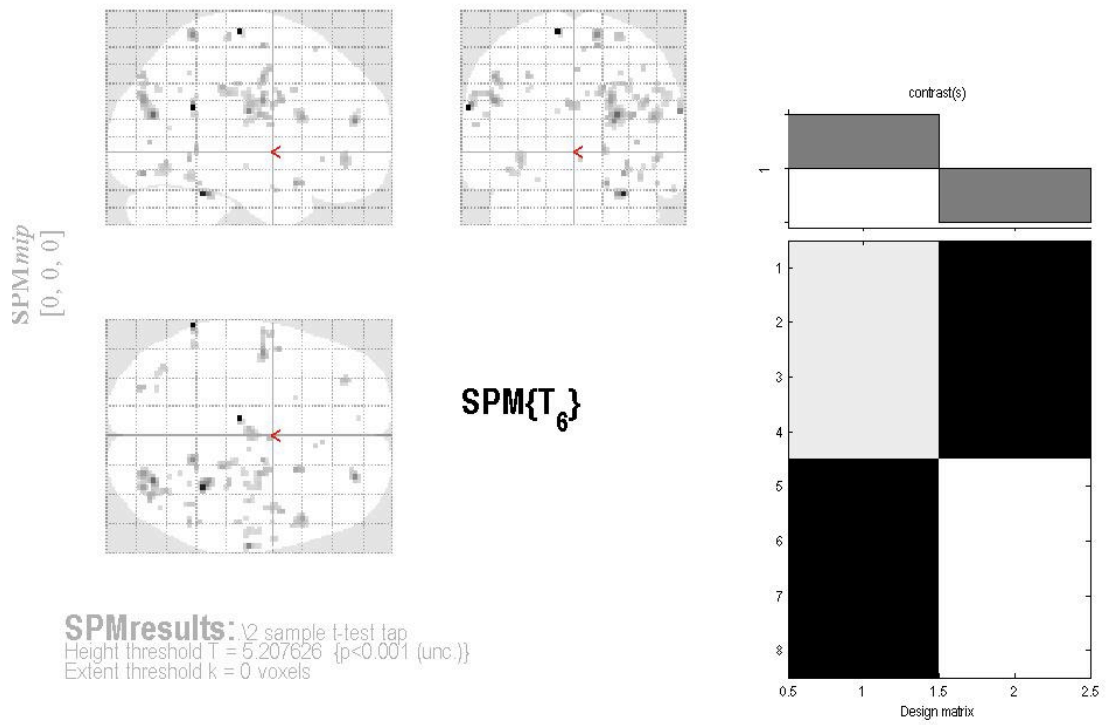
Figure 2.65: Comparison of the fMRI images of the 2nd and 1st sessions of scanning 4 healthy volunteers during finger tapping

The design matrix on the right is showing the contrast of MR images of the 4 HV when comparing the 2nd session with the 1st session scanning. The image on the left displays glass brains in a coronal, sagittal and axial view with no significant activations.

2.8 Discussion

Chemotherapy-induced peripheral neuropathy is thought to be caused by drug-induced damage to components of the peripheral nervous system: including neurons in the dorsal nerve root ganglia, axons, and Schwann cells. It is characterised by abnormal spontaneous discharge in A-fibre and C-fibre primary afferent neurons (Balayssac et al. 2011). Neuropathic symptoms are usually more common and severe in the lower extremities than in the upper extremities as the longest nerves are first affected, with sensory changes affecting the toes and later the fingers, before potentially progressing proximally in a glove and stocking distribution (Wolf et al. 2011). The sample of patients used in this study, who were selected specifically with signs and symptoms of CIPN, reported that numbness and prickling sensations were the most common symptoms from the NTSS-6 questionnaire. This is consistent with the published literature, where the use of the EORTC CIPN20 questionnaire, which is specific for CIPN including three subscales assessing sensory, motor and autonomic symptoms, demonstrated that patients reported 'quite a bit' to 'very much' numbness (57%) or tingling (63%) in the hands and similar results were observed in the feet; numbness was highly correlated with tingling in the hands (r=0.69) (Wolf et al. 2011). We have not used this tool in this study as this was being examined in a large, international clinical trial at the time. In another cross-sectional study of screened patients who were treated with vinca alkaloids, taxanes or platinum derivatives, and using the National Cancer Institute Common Toxicity Criteria, it was found that 71% reported tingling, 58% had numbness, 46% complained of impaired sensory function and 40% reported pain in their hands and feet whilst 37% of patients who developed neuropathic symptoms scored these as the most troublesome side effect (Kautio et al. 2011).

Structural damage to the peripheral nervous system results in abnormal somatosensory processing in the central and peripheral nervous system (Bhagra and Rao 2007). Most of the current concepts regarding the pathophysiology of neuropathic pain originated from experimental work in animal models indicating peripheral and spinal cord reorganization of nociceptive pathways (Baliki et al. 2007). Animal models of neuropathic injuries that result in persistent pain suggest that peripheral and spinal cord pathways transmitting nociceptive signals towards the cortex undergo major reorganization (Woolf 2006; Apkarian et al. 2009). A recent study on two different strains of rats showed variable expression of experimental neuropathic pain after an injury and changes in peripheral nerve injury

did not differ in the DRG or spinal cord, thus suggesting that the behavioural difference between the rat groups lay within the brain and ultimately being dependent on the descending modulation that controls the spinal outcome of peripheral nerve injury (De Felice et al. 2011). Diabetic neuropathy which is characterised peripherally by axonal loss and a reduction in myelinated fibre density has been shown to have central nervous system involvement; MRI data has shown spinal cord shrinkage, thalamic neuro-chemical abnormalities and regional alterations in brain activations reflecting the involvement of the CNS in abnormal sensory perception and pain (Selvarajah et al. 2011).

Although the main symptoms expressed in this group of MM patients were arising from CIPN, these patients also were fatigued and had poor physical and social functioning as reported from the EORTC QLQ-C30. The EORTC MY20 also showed they had issues around body image and this has been previously reported where body image significantly decreased over time in MM patients (Cocks et al. 2007). It is well known that pain is subjective and influenced by emotions, mood, memories, genetic and behavioural stimuli (Tracey and Mantyh 2007). Studies have shown that sad mood, anticipation and visualisation of a painful event can lead to an intensified pain experience (Shimo et al. 2011; Seifert et al. 2012; Yang and Symonds 2012).

We identified regions of activation in the pain matrix during painful stimulation in both the healthy volunteers and the CIPN-myeloma patients and this is comparable to other studies and pathologies (Becerra et al. 1999; Gelnar et al. 1999; Coghill et al. 2003; Albuquerque et al. 2006). Activation in the right superior frontal gyrus has been previously reported in studies using painful stimuli in healthy volunteers. Activation in this area is associated with the subjective experience of the painful stimuli and with negative affective or unpleasant experiences (Fulbright et al. 2001). In CIPN-myeloma patients, there was no activation in this region and the explanation behind this could be that these patients having had neuropathic pain for two years and scoring low in the chronic pain accepting questionnaire and they were not the catastrophizing type (low score in the PCS). Thus, it might imply that they had adapted to the pain over the long time-span. This is further consolidated by the group-by-site interaction where CIPN-myeloma patients demonstrated significantly greater activation at the prefrontal cortex in response to thigh compared with foot thermal stimulation. This has been shown in previous studies where there was deactivation of the prefrontal cortex in response to pain in cluster headaches (Hsieh

et al. 1996) and in repetitive transcranial magnetic stimulation on acute pain induced by capsaicin (Tamura et al. 2004).

There was activation of the precuneus in the CIPN-myeloma group and it has been reported in patients with chronic neuropathic conditions (Hsieh et al. 1995; Witting et al. 2001; Albuquerque et al. 2006; Buvanendran et al. 2010). There was deactivation of the precuneus in the healthy volunteers during thermal stimulation and this is consistent with the literature in normal individuals (Koyama et al. 2005; Kong et al. 2010) The precuneus, which lies on the posteromedial aspect of the parietal lobe, plays a role in conscious pain perception, and is involved in a broad range of higher order cognitive functions. Activation of the posterior precuneus exhibits the strongest correlation with successful retrieval of remembered episodes (Cavanna and Trimble 2006). In CIPN-myeloma patients the resultant activation could suggest that the enhanced response to painful stimulation in the precuneus was driven by abnormally enhanced pain-induced activation at the neuropathic site. This could imply that it stimulated the episodic pain memory retrieval. Furthermore, when investigating the group by site interaction, the current work found that CIPN-myeloma patients demonstrated significant activation at the cuneus (found on the medial surface of the occipital lobe), with greater activation in response to foot thermal stimuli than in response to thigh stimulation. Healthy volunteers exhibited greater activation in response to thigh than foot thermal stimuli at this focus. This may potentially be explained by delivering the thermal stimulation at the foot site first in all cases (not randomised) in this experiment. Therefore when the thigh was stimulated in healthy volunteers, a pain experience had already occurred and perhaps been learnt and thus the cuneus was involved in the anticipatory role of pain. Such an effect has previously been shown in a fMRI study where right cuneus activation was attributed to the anticipation and subjective experience of pain (Fulbright et al. 2001).

In this study, cerebellar activation during noxious stimulation was also noted and this has been reported previously in the literature (Peyron et al. 2000; Apkarian et al. 2005; Kong et al. 2010). The cerebellum comprises two hemispheres that are joined at the midline by the vermis, with an anterior, a posterior, and a flocculonodular lobe; the three lobes are further divided into ten lobules, designated I to X (Schmahmann 2000). Noxious heat stimuli in healthy volunteers can produce increased activation in areas that are believed to be play a part in cognitive processing together with sensory-motor integration (Moulton et al. 2010). It was thought that cerebellar

activation during painful stimulation was due to the motor response of moving away from pain and this has been associated with contralateral primary motor cortex activation (Peyron et al. 2000). However, unconscious subjects (under general anesthesia) also showed cerebellar activation when noxious heat stimuli where delivered (Hofbauer et al. 2004). A fMRI study showed that noxious stimuli activated different areas in the cerebellum compared to non-noxious stimuli namely the deep cerebellar nuclei, anterior vermis and bilaterally the cerebellar hemispheric lobule VI. There was also activation in ipsilateral hemispheric lobule III–VI, deep cerebellar nuclei and in the anterior vermis (lobule III) depending on the perceived pain intensity and these were associated high pain intensity ratings, which reflects a potential relationship between nociceptive activity in the cerebellum with pain perception (Helmchen et al. 2003). A further fMRI study showed that the cerebellum seemed to be capable of distinguishing active from passive painful stimuli (the painful stimulus delivered by a thermode was fixed to a lever, controlled by two ropes which could either be pulled by the investigator's or the volunteer's left hand in order to apply active or passive stimuli to the volunteer's right hand) (Helmchen et al. 2004). The cerebellum has also been activated during the anticipation of pain in humans and in pain empathy (Moulton et al. 2010). Thus the role of the cerebellum in pain states is potentially complex.

In CIPN-myeloma patients, there was a positive correlation between increasing TNSr scores and BOLD response in the operculo-insular cortex. This region includes parts of the insula deep inside the lateral sulcus, and parts of the frontal and parietal lobes that cover the insula, called the opercula, and plays a part in the cortical processing of painful stimuli (Baumgartner et al. 2010). There is evidence to show that the intensity of activation of the operculo-insular cortex correlates with the perceived pain intensity in the human brain (Iannetti et al. 2005). On the other hand, there was no correlation between Dyck's score and brain activation, even though all CIPN-myeloma patients scored ≥ 4.5, which indicates neuropathy. However, dyck's score is specific to diabetic patients and has not been used and validated in CIPN. Dyck's scoring system measures neuropathy using a detailed methodology, with each individual component measures only one aspect of peripheral nerve function. The development of a composite measure of peripheral nerve function, the Total Neuropathy Score, can provide an comprehensive and easily obtained measure of the detection and quantification of peripheral neuropathy due to chemotherapy (Cornblath et al. 1999).

Central nervous system inputs resulting from painful stimuli undergo substantive modulation through spinal cord and brain neuroplasticity as well as modification from descending pathways. The type of the stimulation and the subject's condition play important determining factors for the development of habituation or sensitization to the noxious repetitive stimulus. In healthy volunteers, repetitive nociceptive stimulation usually lessens the pain perception over time by causing habituation and also changes to the structure of the brain in somatosensory areas and mid-cingulate area (Teutsch et al. 2008). A study investigating habituation of heat pain (48°C) over eight days in healthy controls, in patients suffering from major depression and in patients suffering from chronic low back pain showed that habituation to repetitive heat pain was comparable between the three groups. Chronic pain patients reported similar pain ratings to healthy controls on the first day, however these patients showed higher pain thresholds than healthy controls over the subsequent days whilst patients with depression demonstrated significantly higher pain thresholds and showed a trend towards higher pain ratings per session. Furthermore, within each daily session, it was noted that the intensity ratings were significantly increased indicating sensitisation in all three groups (May et al. 2011). Sensitisation to a repetitive noxious stimulus seems to put the sensory system on the alert thus leading to higher pain ratings upon repetition whilst habituation is thought to protect the sensory system from non-important information (May et al. 2011). From neuroimaging on healthy volunteers, habituation has been demonstrated in areas including the primary and secondary somatosensory cortices, the insula and the ACC (Mobascher et al. 2010). In our study, both healthy volunteers and CIPN-myeloma patients showed patterns of habituation when comparing the first three stimuli with the last three stimuli at the foot site; fMRI BOLD habituation was found in the right claustrum and cerebellum in healthy volunteers and in the precentral gyrus, sub-lobar insula and right medial temporal gyrus in CIPN-myeloma patients. However, there was no significant brain activation when looking for habituation at the thigh stimulation site in both groups. This could be explained since the stimuli in both groups were first delivered to the foot and afterwards to the thigh and therefore when the stimuli to the thigh were delivered, the subjects were already habituated since they had the experience of pain on their foot prior to the thigh.

It is known that opioid analgesia attenuates cerebral responses to painful stimulation (Wagner et al. 2007) and opioid exposure causes functional and structural changes in the affect-processing and the reward circuitry (Younger et al. 2011). In our sample of patients, 58% were on opioids, which included oral codeine, morphine and

oxycodone and transdermal buprenorphine and fentanyl patches. Despite being on opioids, the neuropathic pain was still not well controlled as these patients reported high scores for neuropathic pain suggesting that this class of analgesia was not being very effective on decreasing the brain activation due to pain. On dividing the CIPN-myeloma cohort into 2 groups depending on whether or not they were on a regular, stable dose of an opioid, fMRI data demonstrated significant activation in the cerebellum in those patients receiving opioids. There are mu-opioid receptors in the cortex, vermis, and dentate nuclei of the cerebellum (Schadrack et al. 1999). In previous neuroimaging studies, after opioid administration, there have been decreases (Firestone et al. 1996; Wagner et al. 2001) or increases (Petrovic et al. 2002; Leppa et al. 2006) in regional cerebral blood flow within the cerebellum. Furthermore, the cerebellum is known to be involved in motor functions but also in emotional and cognitive processing and thus opiodergic activation in the cerebellum in the current study could have a range of causes that would need further experiments to elucidate (Schmahmann and Sherman 1998).

Clinical assessment has demonstrated that around 20% of MM patients have neuropathy at diagnosis, and up to 75% may experience treatment-emergent neuropathy. (Richardson et al. 2011) It is vital to detect neuropathy early, exclude reversible causes such as spinal cord compression and identify any predisposing factors. The degree of neuropathy should be monitored using a recognised scale and include neurological assessment. Despite its clinical importance, there is no consensus as to the best way to assess CIPN and none of the available tools were developed specifically for MM (Osborne et al. 2012). Whilst common toxicity scales can rapidly give an indication of CIPN severity, they rarely provide useful information regarding the detailed clinical and pathological aspects of CIPN (Cavaletti et al. 2007). The TNSr has been shown to be a reliable tool with a high sensitivity in the context of CIPN (Cavaletti et al. 2007) and was used to assess neuropathy in this current study. In a study of 27 MM patients who received bortezomib and thalidomide therapy the median TNSr was 10 and this is comparable with our cohort of CIPN-myeloma patients where the median of TNSr was 14 (Chaudhry et al. 2008).

Involvement of the CNS by MM is reported to be very rare (Nieuwenhuizen and Biesma 2008). Myeloma patients with CIPN who took part in this study had received different forms of anti-myeloma therapy: vincrisitine, thalidomide and bortezomib. Several chemotherapy-induced CNS disorders are well described and it is known

that vincristine is associated with encephalopathy, cortical blindness, seizures, ataxia and parkinsonian-like symptoms (Quant 2010). Most drugs used as an anti-myeloma treatment cannot permeate the blood–brain barrier (Nieuwenhuizen and Biesma 2008), however Hattori and Iguchi showed that thalidomide penetrates the blood–brain barrier and that it has a transient effect (Hattori and Iguchi 2004). To our knowledge, there have been no studies, specifically looking at the neurotoxic effects on the brain with the anti-myeloma drugs used in this study. This difference of anti-cancer drug sensitivity between the central and the peripheral nervous system is that the CNS has a less permeable blood-brain barrier compared to the blood-nerve barrier protecting the peripheral system and thus the peripheral nervous system is more readily affected by drug neurotoxicity than CNS structures (Balayssac et al. 2005). The anti-myeloma treatment in our cohort was associated with a length-dependent sensory axonal, large>small-fibre neuropathy. This type of neuropathy has also been shown in a study by Chaudhry et al in 2008, where bortezomib and thalidomide combination therapy induced a length-dependent sensory>motor, axonal, large>small-fibre polyneuropathy (Chaudhry et al. 2008).

Multiple myeloma is increasingly being considered as a chronic disease state in which progressive damage from the disease is compounded by cumulative treatment related toxicities (Brenner et al. 2009; Bird et al. 2011; Snowden et al. 2011).

Limitations of the study

This study was a cross sectional study in which CIPN-myeloma patients were selected who already had neuropathy thought to be caused by vincristine, thalidomide and bortezomib and this was done to facilitate patient recruitment. We tried to ensure that the cause of the neuropathic pain in the MM patients was due to chemotherapy as we excluded those with a history of any other known risk factors for peripheral neuropathy, including diabetes, acquired immunodeficiency syndrome, chronic alcoholism, amyloidosis or renal failure and included only those patients whose pain started after they received any of the anti-myeloma therapies described above. Another possible limitation was the duration of thermal stimulation used. For the purposes of this study, a 30 second long heat-pain protocol with seven stimulation epochs was employed. When compared to shorter stimulation periods, this longer stimulation is more natural and perhaps reflects a general real life

experience of pain. This design was based on a pilot study that was undertaken prior to the main study. Despite the efficacy of producing pain-related functional activation, the long stimulation duration also allowed the opportunity for non-pain related functional changes to be detected. All the stimuli were delivered first to the foot and then to the thigh in both groups, thus confirming that habituation develops, however the stimuli sites could have been randomised with half the group receiving the thermal stimuli first to the thigh and then to the foot. The active and control groups were different in at least four ways: diagnosis of MM, diagnosis of neuropathy, medications, and age. The healthy volunteers were younger as the recruitment was mostly from hospital and university staff although there were no significant differences between the temperatures that were delivered and their resultant pain rating. The age difference makes it difficult to confidently attribute fMRI group differences to CIPN. Also the MM group reported poor social and physical functioning, which might have attributed to the central pain processing. We compared the cohort of MM patients with neuropathy to healthy volunteers; a more relevant control might have consisted MM patients who are receiving the same anti-myeloma therapy but did not develop neuropathy. This might have led to better groups to compare, as the two groups would be MM patients receiving the same treatments. Therefore, we cannot be certain that the fMRI differences are due to the CIPN, although it remains the most likely explanation.

Chapter 3

Living with advanced but stable multiple myeloma: a study of the symptom burden and cumulative effects of disease and intensive treatment on health-related quality of life

3 Introduction

In the large majority of patients, MM is an incurable disease, but treatment of episodes may result in repeated phases of control. The number of treatment modalities in MM has increased significantly over the last several decades, and has been associated with significant increases in life expectancy (Child et al. 2003; Klepin and Hurd 2006; Smith et al. 2006; Bird et al. 2011) with an extension of the median survival by 50% at all ages (Kumar et al. 2008) and more profound improvements in younger patients, with predicted 5-year and 10-year survival estimates of over 50% and 30% respectively in patients under 60 years. (Pulte et al. 2011).

 Prior to the widespread use of novel agents, patients with MM were recognised to have the highest level of symptoms and the lowest HRQoL among haematological cancers (Johnsen et al. 2009). Despite enhanced disease control, none of the current novel agents are free of significant toxicity, which frequently persists after completing treatment. Myeloma is thus increasingly being considered as a chronic disease state in which progressive damage from MM is potentially compounded by cumulative treatment related toxicities (Brenner et al. 2009; Johnsen et al. 2009; Bird et al. 2011; Snowden et al. 2011).

Despite the advances in treatment, and increasing numbers of patients achieving prolonged survival, the state of living with advanced MM in the era of novel agents is still poorly characterised. Many patients with MM have irreversible physical symptoms from the outset, particularly pain and fatigue, and, in the course of the disease, this is increased by progressive damage directly from MM itself and indirectly by the toxicity of treatments. Research has shown that pro-inflammatory cytokines can signal the CNS to induce symptoms of fatigue and several

inflammatory mediators have been linked to altered CNS activity, including IL-1β, IL-6, and TNF-α (Collado-Hidalgo et al. 2006). In a haematological cancer study, 34 patients aged 50 or older with acute myeloid leukaemia within 1 year of diagnosis showed potentially clinically important correlations between global QoL and IL-2, IL-5, IL-8 and TNF-α. A similar correlation was observed between IL-6 and fatigue (Panju et al. 2009). In particular, IL-6 has been shown to play an important role in the inflammatory process after a nerve injury and has been linked to the commencement and maintenance of neuropathic pain (De Jongh et al. 2003; Lee et al. 2004).

Although some toxicities may be acute, reversible and short-lived, others, such as peripheral neuropathy, may be insidious in onset and result in permanent damage. Drug toxicities and side effects may be additive or synergistic with other cytotoxic and supportive agents used sequentially or in combination, and to tissue/organ damage occurring by other means (Klepin and Hurd 2006). None of the novel agents are free of significant toxicity, and, perhaps ironically, many agents used in symptom control have significant side effects.

In addition to physical problems that accumulate in patients with MM, psychological and social factors impact on HRQoL (Larsen et al. 2003; Gulbrandsen et al. 2004; Sherman et al. 2004; Straus et al. 2006; Frodin et al. 2010; Molassiotis et al. 2011). Although HRQoL has been explored previously in relation to specific treatments in MM patients, including transplantation (Larsen et al. 2003; Sherman et al. 2004; Straus et al. 2006) or more generally (Gulbrandsen et al. 2004), there is little information relating to the overall impact of modern clinical management strategies in patients with MM, particularly in the context of co-morbidities of an ageing population (Klepin and Hurd 2006). However, compared with some other cancers with prolonged survival (Mols et al. 2005), relatively little is known in MM about patients' psychosocial and broader holistic needs, particularly with modern clinical management strategies.

Thus, perhaps due to the tempo of improvements in survival in recent years, living as a long term 'survivor' with MM is inadequately defined. In this study, we identified a cohort of patients with advanced heavily pre-treated MM. In order to exclude the effect of active disease, we identified patients with stable disease off active treatment or on only maintenance. The aim of this exploratory study was therefore to characterise HRQoL, symptom burden and the state of living in advanced, intensively treated MM and find relationships between HRQoL, pain and

demographic variables. In addition, based on correlations between serum cytokine levels including IL-1β, IL-6, TNF-α, and symptoms in other cancers, (Collado-Hidalgo et al. 2006) we used the opportunity to investigate the relationship between symptoms using the EORTC QLQ-C30 and BPI-SF and cytokines relevant to MM.

Defining the elements of compromised health status on QoL will inform interventional studies for supportive and palliative care needs in patients with advanced relapsed MM to be used alongside the evolving treatments in this population.

3.1 Overview of this study

3.1.1 Hypothesis

In the modern management of advanced MM, patients are living longer and their lives are compromised due to a complex set of complications related to the disease and its treatment and these involve mainly physical and psychosocial issues.

3.1.2 Primary Aims

1) To characterise late effects in patients with advanced but stable MM, clinically and in terms of health-related quality of life and specifically define the physical consequences of MM and its treatment, including pain, neuropathy and other symptoms.

2) To investigate the relationship between symptoms and functioning using the EORTC QLQ-C30 and BPI-SF and cytokine levels including IL-6, and tumour necrosis factor (TNF)-α, relevant to MM.

3.1.3 Secondary Aim

1) Comparison of the symptoms and functioning using the EORTC QLQ-C30, EORTC MY20 and BPI-SF between MM patients with neuropathy and those without using s-LANSS.

The study was conducted in accordance with International Conference on Harmonisation-Good Clinical Practice and formally approved by Sheffield Teaching Hospitals NHS Foundation Trust and the local NHS Research and Development following local research ethics committee review.

3.2 Methods and Materials

3.2.1 Patient Selection

A diagnosis of MM was made using the criteria proposed in 2003 by the International Myeloma Working Group (Table 3.1). These criteria distinguish between MM and monoclonal gammopathy of undetermined significance principally on the basis of M-protein concentration, percentage of bone marrow plasma cells and presence or absence of myeloma-related organ and tissue impairment which include increased calcium levels (corrected serum calcium >0.25 mmol/l above the upper limit of normal or >2.75 mmol/l), renal insufficiency (creatinine >173 µmol/l), anaemia (haemoglobin 2g/dl below the lower limit of normal or haemoglobin <10g/dl), bone lesions (lytic lesions or osteoporosis with compression fractures) and other features like symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections (>2 episodes in 12 months) (Bird et al. 2011).

Table 3.1: Diagnostic criteria for monoclonal gammopathy of undetermined significance, asymptomatic myeloma and symptomatic myeloma
adapted from (International Myeloma Working Group 2003)

Monoclonal gammopathy of undetermined significance	Asymptomatic Myeloma	Symptomatic Myeloma
M protein in serum <30g/l	M protein in serum ≥30g/l and/or	M protein in serum and/or urine
Bone marrow plasma cells <10% and if trephine biopsy done, low level of plasma cell infiltration	Bone marrow clonal plasma cells ≥10%	Bone marrow plasma cells or biopsy proven plasmacytoma
No related tissue or organ impairment	No related tissue or organ impairment	Myeloma-related tissue or organ impairment

Patients recruited had received initial treatment with induction chemotherapy consolidated by at least one transplant procedure, followed later by treatment with at least one additional line of treatment for progressive or relapsed disease. Importantly, in order to exclude the impact of active disease and acute toxicity of treatment, patients were in stable plateau phase defined as a <25% change in serum or urine M-protein or, in patients with low serum M-proteins (<5g/L), no evidence of progressive disease (i.e. a rise in M-protein >5g/L), and either off active

cytotoxic re-induction treatment or on maintenance treatment for at least 3 months. Exclusion criteria included uncontrolled disease, inability to give informed consent and lack of fluency in English. The haematology team approached patients meeting the inclusion criteria when they attended for their follow-up appointment, and informed them of the study. The research associate, research fellow or the haematologist carried out the assessments. Recruitment started in July 2008 and ended in November 2010. The STROBE guidelines were applied for the reporting of this study (Osborne et al. 2003).

3.2.2 Assessments

3.2.2.1   Demographic, clinical and employment data

Patients self completed questionnaires describing demographic and employment information. Clinical history, treatment and current medications were obtained from medical case notes. Neurological and medical examination was performed by myself or by another haematology doctor who was available to also recruit the patients.

Serum tumour necrosis TNF-α was assayed by Immunotech TNF-α enzyme immunoassay whilst serum IL-6 was assayed using the electrochemilluminescence immunoassay (ECLIA). When blood was taken in the appropriate containers, these were stored immediately in freezing conditions until the samples were sent to the laboratory for testing.

3.2.2.2 Health-related quality of life (HRQoL)

For HRQoL measures, different questionnaires were used. A generic HRQoL measure was suitable for evaluating the impact of the common elements of health, well-being and functionality and also pain.

The generic Short Form-12 health survey v2 (SF-12) is a short health survey which is a subset of the SF-36 and utilises only 12 items drawn from each of the eight subscales of the SF36 (Ware et al. 1996; Jenkinson et al. 1997). SF-12 measures eight health domains and provides psychometrically-based physical component summary (PCS SF12) and mental component summary (MCS SF12) scores and therefore provides a glimpse into the overall HRQoL. It reflects sub-domains including general health perceptions, bodily pain, physical functioning, role physical, role emotional, social functioning (SF), mental health and energy/vitality. The PCS SF-12 and MCS SF-12 are computed using the scores of twelve questions and range from 0 to 100, where a zero score indicates the lowest level of health measured by the scales and 100 indicates the highest level of health (Detmar et al. 2002). The PCS SF-12 and MCS SF-12 scores correlate 0.95 and 0.96 with the SF-36 counterparts (Ware et al. 1996). Published reliability coefficients range from 0.73 to 0.87 across all eight subscales of the SF-12 and test-retest reliability for PCS SF-12 is 0.89 and for MCS SF-12 is 0.76 (Ware 2002).

The European Organization for Research and Treatment of Cancer core quality of life questionnaire (EORTC QLQ-C30) (Aaronson et al. 1993) and the myeloma specific module EORTC QLQ-MY20 (Cocks et al. 2007) were used. The EORTC QLQ-C30 comprises nine multi-item scales: five functional scales (physical, role, cognitive, emotional and social); three symptom scales (fatigue, pain, and nausea/vomiting); and a global health and quality of life scale, three symptom scales, a global health and quality-of-life scale and single items assessing symptoms. Patients respond in a yes/no format and also a likert-scale and patients answer to items framed 'the past week'. Scores for each scale were calculated as advised by the EORTC Study Group on QOL (Fayers et al. 2001) and linearly transformed.

The principle for scoring these scales is the same in all cases:
1) The average of the items that contribute to the scale is estimated and known as the raw score.

The raw answers are transformed to a raw score by:

RS= (answer 1+ answer 2+…+ answer n)/n

n= number of questions within a scale

2) Then a linear transformation is used to standardise the raw score to score, S, so that scores range from 0 to 100

For functional scales: S= (1- ((raw score-1)/range) *100

Global quality of life and symptom scales/items: S= ((raw score -1)/range) *100

Range is the difference between the maximum possible value of raw score and the minimum possible value. Therefore, the range of raw score equals the range of the item values. Most items are scored 1 to 4, giving range = 3.

Higher scores on the functioning and global QOL scales represent better functioning and QOL whereas a high score for a symptom scale or item represents more symptoms/problems. The EORTC QLQ-C30 questionnaire has been shown to be a valid and reliable tool to measure QoL in research settings. The internal consistency of the items produced reliability coefficients of 0.52-0.89 with the exception of role function status (Aaronson et al. 1993).

The EORTC MY20 has four scales (two symptom scales: disease symptoms, side-effects of treatment; one function scale: future perspective, social support) and one single item: body image. All scores were linearly transformed to a 0–100 scale. A higher score on the symptom scales indicated a higher level of symptoms, whereas a higher score on future perspective, social support and body image indicated good functioning or good support. All scales except side-effects scales showed good convergence since the correlations between items and their hypothesised scale were greater than 0.4 and internal consistency was greater than 0.7 in all scales (Cocks et al. 2007). The EORTC MY20 is a good measure for additional aspects of QoL, especially issues around body image and future perspective and is a reliable and valid instrument for measuring QoL in MM patients. It is now recommended that the QLQ-C30 and QLQ-MY20 are used together to measure QoL in international clinical trials in MM.

The Profile of Concerns questionnaire was adapted and used to assess concerns about MM (Spencer et al. 1999). This adapted version consisted of 21 items each naming a specific potential concern. The adapted version consisted of 21 items each naming a specific potential concern (range 0-5). Factor analysis restricted to items which were responded to by the majority of respondents, resulted in identification of three factors addressing 1) life and pain (8 items), 2) rejection (3 items) looking at adverse reactions from family and friends and 3) sexuality looking

at dealing with being less attractive and less sexual (4 items). We included these 15 items plus 7 additional ones thought relevant to MM patients. Each item named a specific potential concern and was rated on a series of 5-point Likert scales. In our study, Cronbach's alpha for items in pain and life issues was 0.86, for rejection issues was 0.89 and for sexuality issues was 0.89.

3.2.2.3 Pain and peripheral neuropathy

Pain was assessed with the Brief Pain Inventory short form (BPI-SF) (Cleeland and Ryan 1994) and peripheral neuropathy was assessed by using the self report Leeds Assessment of Neuropathic Symptoms and Signs (s-LANSS) (Bennett et al. 2005) and clinical neurological examination.

The BPI-SF is a self-report measure of pain that measures the severity and location of pain and pain's interference in domains of daily functioning and pain medications and amount of pain relief in the past 24 hours or the past week. This includes the three pain severity items (pain worst, pain average and pain now) and the seven interference items (how pain interferes with activity, mood, relations with others, walking ability, work, enjoyment of life and sleep). It uses simple numeric rating scales from 0 to 10. Pain varies considerably over a day and thus, the BPI-SF asks patients to rate their pain at the time of responding to the questionnaire. Also, the questionnaire asks the patient to specify the pain at its least, worst and average over the previous week. The reliability Cronbach's alpha ranges from 0.77 to 0.91. BPI-SF has been shown to be psychometrically sound in its reliability and validity in pain and is also sensitive to changes in pain severity or pain interference resulting from pain treatment (Mendoza et al. 2006).

The s-LANSS is a screening tool that aims to identify pain of predominantly neuropathic origin, as distinct from nociceptive pain (Bennett et al. 2005). The LANSS pain scale has been used widely, but as clinician examination is needed, this limits the instrument's use in large-scale research. Therefore the s-LANSS is a modified version of the LANSS pain scale to make it capable of self-completion. The domains assessed are: prickling/tingling, mottled/red/pink skin, sensitive skin, bursting/sudden pain, hot/burning, allodynia, and numbness/tenderness. If a patient registers twelve or more on this scale then there is a strongly indicative that neuropathic pain is present to some degree. The S-LANSS has a Cronbach's α of 0.76 when completed unaided, rising to α =0.81 when completed at interview,

demonstrating a good level of internal consistency. S-LANSS score is a valid and reliable self-complete instrument for identifying neuropathic pain in clinic settings and research (Bennett et al. 2005).

To minimise measurement error, research instruments should meet validity and reliability criteria. Validity is the degree to which a test measures what it claims to measure. It is important for a test to be valid in order for the results to be accurately applied and interpreted. There are three main types of validity. *Content* validity is based on the extent to which a measurement reflects the specific intended domain of content. *Criterion related* validity (instrumental validity) is used to demonstrate the accuracy of a measure or procedure by comparing it with another measure or procedure, which has been demonstrated to be valid. *Construct* validity is the extent to which a test measures the concept or construct that it is intended to measure. On the other hand, reliability refers to the consistency of a measure and it examines the amount of random error in the measurement technique, rather than random error. A test is considered reliable if the same result is obtained repeatedly. In group-level comparisons, the reliability coefficient should be 0.70, that is, 70% of the measured variance is reliable (Nunnally and Bernstein 2006). The reliability coefficient is the correlation coefficient between the two sets of scores. Measuring the reliability of tools occur in different ways.

3.3 Statistical analysis

Standardised measures were scored as directed in manuals and published reports.

Two types of incorrect conclusions can be drawn when testing a statistical hypothesis. The null hypothesis is accepted or rejected on the basis of the value of the test-statistic. A type I error occurs when the null hypothesis is inappropriately rejected (false positive), indicating a test of poor specificity. A type II error occurs when the null hypothesis is falsely accepted (false negative), indicating a test of poor sensitivity (Table 3.2). Conventionally, it has been accepted that the probability of a type I error will be less than 5% and this is the reason for the threshold for *P* values: *P* must be less than 0.05 before we conclude that a study is positive. Type II errors are generally the result of a small sample size. To avoid the error, a sample size calculation is performed before beginning a study and as part of the calculation

asserts what a "true difference" is and accepts that this will miss it 10% to 20% of the time (i.e., type II error rate of 0.1 or 0.2) (American-College-of-Physicians 2001).

Table 3.2: Type I and Type II errors

	Null hypothesis (H_0) is true	Null hypothesis (H_0) is false
Reject null hypothesis	Type I error False positive	Correct outcome True Positive
Fail to reject null hypothesis	Correct outcome True Negative	Type II error False negative

To check if the data was normally distributed or not, the skewness and histogram plots with normal curve overlay were performed. The age of the patients, the years since diagnosis and the years since their first transplant were tested for normal distribution, as these characteristics are what define this cohort (Table 3.3, Figure 3.1, 3.2, 3.3). The results show that the data was not normally distributed.

Table 3.3: The mean, median and skewness of the MM cohort

Statistics	Age	Years since diagnosis	Years since transplant
Mean	60.03	5.68	5.03
Median	61.00	5.50	4.96
Skewness	-.457	.476	.663
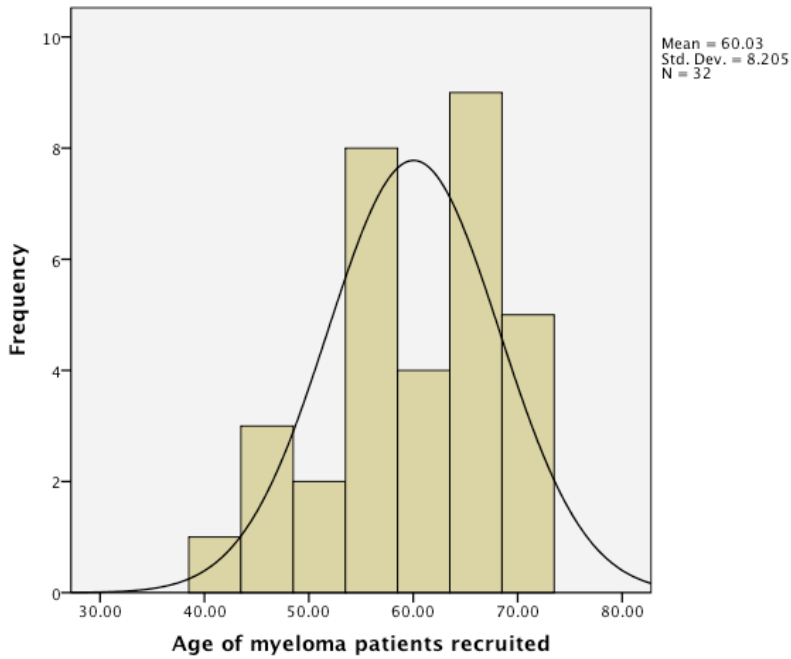
Figure 3.1: Histogram plot with overlay for age of MM patients

The data is moderately skewed to the left: the left tail is slightly longer and more data of the distribution is at the right.
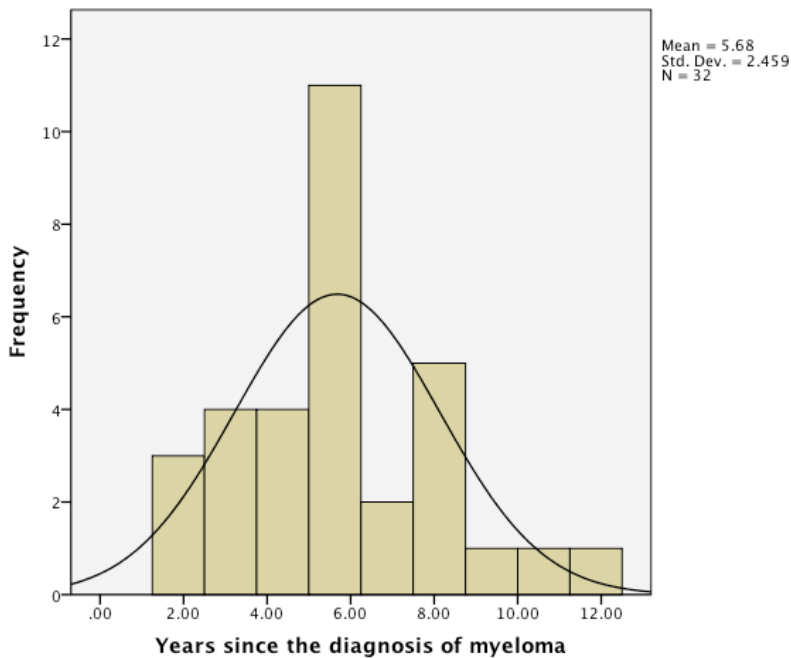
Figure 3.2: Histogram plot with overlay for years since the diagnosis of MM

The data is moderately skewed to the right: the right tail is longer and more data of the distribution is at the left.
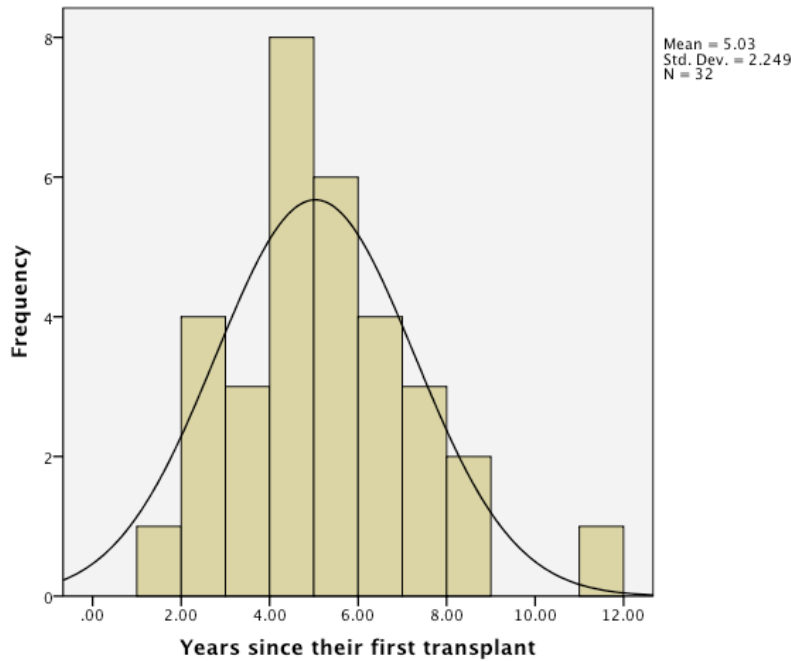
Figure 3.3: Histogram plot with overlay for years since the the first transplant

The data is moderately skewed to the right: the right tail is longer and more data of the distribution is at the left.

Non-parametric tests were used for descriptive statistics generally and correlation testing. One way t-tests were used to compare mean HRQoL scores with the general population (Oxford Healthy Lifestyles survey) based on published recommendations (Campbell et al. 2007; Walters 2009). A Mann-Whitney's U test was used to evaluate the symptoms and functioning using the EORTC QLQ-C30, EORTC MY20 and BPI-SF between MM patients with neuropathy and those without using the s-LANSS. A Mann-Whitney's U test counts the number of times an observation from sample 1 is greater than an observation from sample 2, this is denoted by U. Quantitative data were analysed using PASW version 18 (SPSS 2009).

Since the collected data had ordered categorical characteristics, the median and interquartile ranges were used. Nonparametric methods are most appropriate when the sample sizes are small and this test has the advantage of not requiring the assumption of normality or the assumption of homogeneity of variance. Non-parametric tests are preferable when few assumptions can be made about the distribution of the data and if it is not normally distributed or contains outliers (Pappas and DePuy 2004). The median is a more robust measure of the centre of distribution, as it is not influenced by outliers and skewed data. The interquartile

range often represents the spread, which is simply the difference between the first and third quartiles. Spearman's rank correlation coefficient was used for correlations between questionnaires and demographic and clinical data. Given the exploratory hypothesis-generating nature of the study, we used a Spearman's rho (r) to test the direction and strength of the relationship between two variables and considered r of 0.4 and below to be of low likelihood of clinical relationship, 0.4-0.6 moderate, and above 0.6 high clinical relationship (Swinscow and Campbell 2002). All tests were two-tailed and $p < 0.05$ was used as significance level.

3.4 Results

3.4.1 Demographic, clinical and employment data

Basic demographic and clinical data are summarised in table 3.4. All patients were of Caucasian. Thirty-two patients (17 males, 15 females) were enrolled with a median age of 55 years (range 36-69) at diagnosis and 60 years (range 41-71) at assessment and a median 5.5 years from diagnosis (range 2-12) with a median 3 lines (range 2-6) of previous treatment.

Twenty-nine patients had been treated with high dose melphalan (140-200mg/m^2) and autologous HSCT, and three patients had previous allogeneic HSCT, but all had disease progression post-transplant and none had evidence of graft-versus-host disease. In line with UK and local practice at the time of the study, none of the patients in this study had received a concurrent combination of drugs commonly considered to cause peripheral neuropathy i.e. bortezomib, thalidomide, or vincristine were never used concurrently, only sequentially, in combination with dexamethasone +/- other conventional chemotherapy agent (alkylating agent or anthracycline). At the time of assessment, twenty-nine (91%) patients were off all anti-myeloma treatment (except bisphosphonates) and three (9%) patients were receiving maintenance treatment with lenalidomide and one with interferon-alpha. All patients had received bisphosphonate treatment from diagnosis.

At the time of diagnosis, 19 (59%) were employed whilst 4 (12%) were not working due to ill health, and 9 (28%) were retired or a home-maker. At the time of the assessment, 6 (19%) of this cohort were either actively employed whilst 7 (22%) were not working due to ill health and 16 (50%) were either retired or a home-maker (two (6%) missing data). The median hours worked per week had fallen from 39 at diagnosis (range 13-80) to 25.5 at assessment (range 11-40). Compared with when first diagnosed, 50% reported loss of earnings, 37% changed their job responsibilities and 44% felt they were less able to do their job well.

Compared with when they were first diagnosed, 50% reported loss of earnings, 37% changed their job responsibilities and 44% became less able to do their job well. Those patients who had given-up paid work or were on long-term sick leave due to their illness, reported that they had been able to rethink what they wanted to do with their life (56%), fifty-nine percent reported that they now have a worse QoL whilst

30% were unsure. On the other hand, 76% were enjoying spending more time with their family. Forty-seven percent felt isolated at home and 72% missed their friends at work.

Table 3.4: Demographics and clinical data of the cohort

Median age at diagnosis	55 (range 36 - 69)
Median age at assessment	60 (range 41 – 71)
Median duration from diagnosis	5.5 years (range 2-12)
Sex	17 males, 15 females
Lines of treatment received (initial induction + HSCT counted as a single line)	3 (range 2-6)
HSCT	29 (91%) autologous HSCT 3 (9%) allogeneic HSCT 10 (31%) 2 HSCT procedures
Other relevant anti-myeloma therapy	26 (81%) thalidomide (repeated courses) and/or lenalidomide 27 (84%) vincristine 22 (69%) bortezomib (all intravenous) 21 (65%) doxorubicin 32 (100%) high dose steroids 4 (13%) interferon-alpha

The majority of patients were married or living with a partner (87.5%). 25% of patients had a university or vocational degree. Patients were asked if they were informed of the benefits they were entitled to and 41% reported that hospital staff had informed them, only 9% were informed by the social worker, 16% were informed by their family and friends whilst 16% were not informed by anyone. Forty-two percent received no help in filling in the benefits form (24 patients answered, 75%) and 48% found it stressful completing the benefits claim.

3.4.2 Health-related QoL

3.4.2.1   Generic QoL (SF-12)

The sub-domains for the SF-12 are shown in Table 3.5.

Table 3.5: SF-12 sub-domain health survey in the cohort of MM.

SF 12; (n=32)	Median (IQR)	Mean (SD)
Mental health	51.7 (50.5 – 52.4)	51.6 (1.3)
Role emotional	49.2 (48.8 – 50.5)	49.6 (1.1)
Social functioning	50.5 (49.4 – 50.5)	50.1 (0.5)
Role physical	47.6 (45.9 – 49.0)	47.8 (2.3)
General health	48.0 (46.8 – 48.5)	48.2 (1.4)
Bodily pain	48.5 (48.2 – 49.4)	48.8 (1.1)
Vitality	50.3 (49.5 – 51.0)	50.3 (0.7)
Physical functioning	46.5 (45.8-48.8)	47.4 (2.5)

Median, interquartile, mean and standard deviation for the SF-12 sub-domain
A zero score indicates the lowest level of health measured by the scales and 100 indicates the highest level of health

The main sub-domains reported as low by patients were physical functioning and the fatigue score in the vitality domain (Figure 3.4).

How much of the time during the past four weeks did you have a lot of energy?
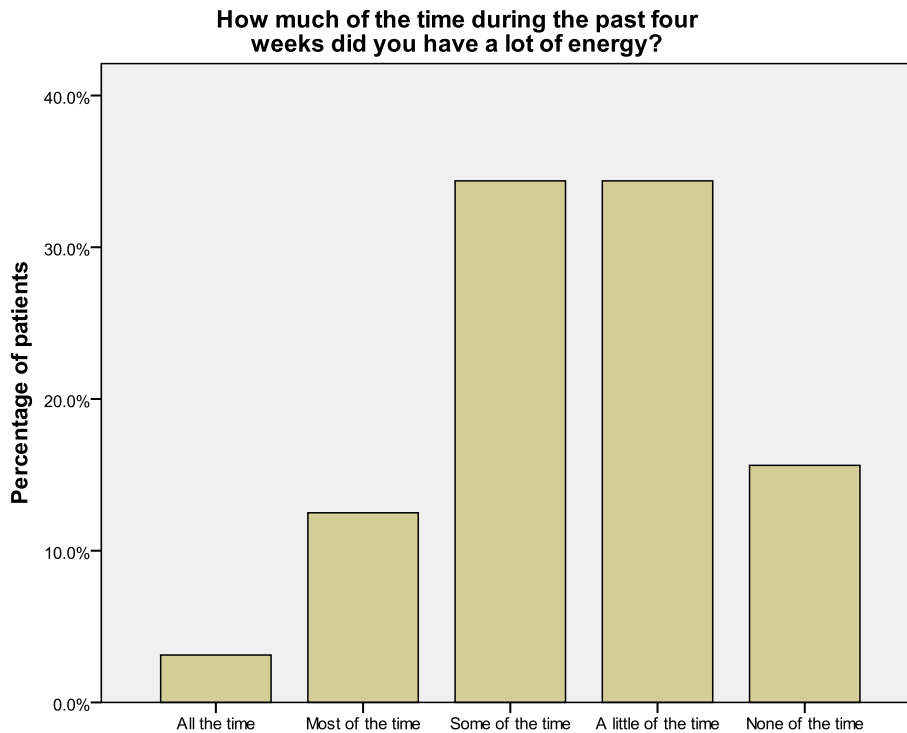
Figure 3.4: Sub-domain of the SF-12 looking specifically at fatigue

Data from the SF-12 questionnaire was compared to the Oxford Healthy Lifestyles survey using a one sample t-test (Jenkinson and Layte 1997). The average score in the PCS SF-12 (47) of the advanced MM cohort patients were significantly lower than the population mean (p<0.001) but the average score for MCS SF-12 for our cohort (51) was similar to that of the population (50).

3.4.2.2   Disease specific QOL

EORTC QLQ-C30 and EORTC MY20 scores are summarised in Tables 3.6 and 3.7.

From the EORTC QLQ-C30, All domains relating to functioning and global health were impaired, particularly social functioning. High levels of fatigue and pain were reported.

Table 3.6: Quality of life from the EORTC-QLQ-C30

Functional Scales	Median (IQR)	Mean (SD)
Physical Functioning	60 (41.7-80.0)	60.6 (25.4)
Role Functioning	67 (33.0-79.0)	55.2 (31.2)
Emotional Functioning	71 (44.0-92.0)	68.8 (23.9)
Cognitive Functioning	83 (50.0-95.7)	71.8 (25.9)
Social Functioning	50 (33.0-67.0)	46.9 (28.3)
QoL/Global Health Status		
QoL/Global Health Status	57.5 (50.0-67.0)	58.8 (17.4)
Symptom Scales/Items		
Pain	33 (17.0-67.0)	45.6 (31.9)
Fatigue	44 (33.0-67.0)	48.9 (29.0)
Insomnia	33 (0-67.0)	35.4 (36.5)
Appetite loss	33 (0-33.0)	27.0 (31.0)
Dyspnoea	33 (0-67.0)	33.3 (30.6)
Nausea and vomiting	0 (0-17.0)	11.1 (17.3)
Constipation	0 (0-33)	14.4 (24.2)
Diarrhoea	0 (0-25)	10.4 (19.7)

The median and interquartile range (IQR) and the mean and standard deviation (SD) reported. A high score (100) in the functional scales indicates better HRQoL but a high score (100) in the symptom scales indicate a high level of symptomatology / problems

Individual items of the EORTC QLQ-C30 functioning scales reported as most bothersome (>25% of patients reporting 'very much' or 'quite a bit') included 24 patients (75%) reporting inability to do strenuous activities and 21 patients (66%) reported having trouble taking long walks; 12 patients (37.5%) felt limited in doing either work or other daily activities and 15 patients (47%)felt limited in pursuing hobbies or other leisure time activities and 16 patients (50%) felt their physical function interfered with family life and 17 patients (53%) with social activities.

From the EORTC QLQ-C30, over one-third of the patients reported that they had pain ('quite a bit' or 'very much'; 40.7%), whilst from the EORTC QLQ-MY20 34.4% reported bone aches and pain and 28.1% reported back pain ('very much' or 'quite a bit'; 28%).

Table 3.7: Quality of life from the EORTC MY20

EORTC QLQ-C20	Median [IQR]	Mean [SD]	Range
Subscales			
Disease specific [a]	22.0 [17.0-51.7]	29.0 [22.5]	0-100
Have you had bone aches or pain?	2.0 [2.0-3.0]	2.38 [1.05]	1-4
Have you had pain in your back?	2.0 [2.0-3.0]	2.03 [1.03]	1-4
Have you had pain in your hip?	1.0 [1.0-2.0]	1.61 [1.05]	1-4
Have you had pain in your arm or shoulder?	1.0 [1.0-1.0]	1.16 [0.37]	1-4
Have you had pain in your chest?	2.0 [1.0-3.0]	2.16 [0.97]	1-4
If you had pain did it increase with activity?	2.0 [1.0–3.0]	2.16 [0.97]	1-4
Side effects of treatment [a]	22.0 [15.0-35.2]	25.0 [16.7]	0-100
Do you feel drowsy?	2.0 [1.0-2.0]	1.87 [0.87]	1-4
Do you feel thirsty?	2.0 [1.0-3.0]	2.03 [0.98]	1-4
Have you felt ill?	1.0 [1.0-2.0]	1.56 [0.67]	1-4
Have you had a dry mouth?	2.0 [1.0-3.0]	2.06 [1.06]	1-4
Have you lost any hair?	1.0 [1.0-1.75]	1.37 [0.79]	1-4
Did you have any tingling hands or feet?	3.0 [1.0-3.75]	2.47 [1.19]	1-4
Did you feel restless or agitated?	2.0 [1.0-2.75]	1.91 [0.86]	1-4
Have you had acid indigestion or heartburn?	1.0 [1.0-2.0]	1.41 [0.61]	1-4
Have you had burning or sore eyes?	1.0 [1.0-2.0]	1.37 [0.61]	1-4
Body Image [b]	33.0 [0-67.0]	41.0 [34.7]	0-100
Have you felt physically less attractive as a result of your disease or treatment?	2.0 [1.0-3.0]	2.15 [1.04]	1-4
Future Perspective [b]	67 [44.0-78.0]	61.0 [21.7]	0-100
Have you been thinking about your illness?	2.0 [2.0-3.0]	2.31 [0.82]	1-4
Have you been worried about dying?	2.0 [1.0-2.0]	1.72 [0.68]	1-4
Have you worried about your health in the future?	2.0 [2.0-3.0]	2.50 [0.84]	1-4

[a] High scores indicate worse symptoms; [b] High scores indicate better support/functioning.
Individual items score: 1 = not at all; 2 = a little; 3 = quite a bit; 4 = very much

Based on EORTC MY20, the most common symptom was reported by 17 patients as tingling in the hands and feet (53% reported quite a bit or very much). Thirteen patients also reported that they worried about their health in the future and 11 patients thought about their illness (41% and 34% respectively reported quite a bit or very much).

3.4.2.3   Pain and peripheral neuropathy

Pain scores in the Brief Pain Inventory short form (BPI-SF) showed a median of 3 (range 0 -10) for the cohort. The average pain was reported as mild (0-3) by 50% of patients, as moderate (4-6) by 38% and severe (>6) by 10% (1 missing data). As shown in Table 3.8, there were no significant differences between males and females in the pain scores in the BPI-SF.

Table 3.8: BPI-SF for males and females

	Males	**Females**	**P-value**
Worse pain	4.0 (2-7.5)	4.0 (2-5)	0.29
Least pain	1.0 (0-2.5)	1.0 (1-3)	0.65
Average pain	3.0 (2-5)	4.0 (1-5)	0.35
Pain right now	2.0 (1-5)	3.0 (1-4)	0.86
Pain Interference	3.0 (1-5)	3.0 (2-4)	0.92

The Mann-Whitney U Test to compare the medians (p-value); (Pain range 0 -10).

There was no significant association between average pain severity scores and sex, years since diagnosis and years since the first transplant.

On the s-LANSS, 16 patients (50%) scored ≥12 consistent with a predominantly neuropathic origin of pain. This was supported by the neurological examination, which showed signs of sensory neuropathy in 20 patients (67%).

Twenty-two (69%) patients were prescribed different types of analgesia for pain: 15 out of the 22 (68%) patients were on opioids - six patients being on tramadol and codeine (40%), three being on fentanyl patches (20%), five on oxycodone (33%) and one on methadone (7%). Out of the 22 patients, 15 (68%) were on calcium channel blockers as a form of analgesia namely, six being on gabapentin (37%) and nine being on pregabalin (63%).

3.4.2.4   Concerns

Analysis from the adapted concerns questionnaire revealed that patients were worried that they will lose their independence and self-sufficiency, the cancer will come back and their life with their partner will be cut short (median 5; range 0-5). The least concerns for our group were that their family will be angry at them, they will argue more with their partner and they will be rejected by their friends or that their illness was contagious (median 1; range 0-5). The data was analysed for sex differences and there were no real correlations to factors for life and pain issues, sexuality issues and also for rejection issues. In older patients, their concerns of losing their independence increased (r=0.379, p=0.02). There was a positive correlation between concerns about life and pain issues and rejection issues (r=0.889, p<0.001) and a positive correlation between concerns about sexuality issues and rejection issues (r=0.436, p=0.01). Cronbach's alpha for items in pain and life issues was 0.86, for rejection issues was 0.89 and for sexuality issues was 0.89.

3.4.3 Correlations between clinical demographics, HRQoL, pain and cytokines

There were significant correlations between QoL by EORTC QLQ-30 and the BFI-SF scores for 'pain on average' (p=0.001) and pain interference with general activity, mood, walking, work, relations, sleep, and enjoyment of life (p<0.001). Physical functioning from EORTC QLQ-30 was significantly correlated with 'pain on average' based on the BPI-SF (p=0.006) and fatigue from the SF-12 (p=0.001). However, there were no correlations between HRQoL and age, sex, years from diagnosis and lines of treatment received.

Serum IL-6 was positively correlated with pain (p=0.03) and pain interference (p=0.003) based on the BPI-SF and inversely correlated with physical functioning (p=0.03) from the EORTC QLQ C30. Also serum IL-6 was positively correlated with insomnia (p=0.02) and appetite loss (p=0.02). However, there were no significant relationships with TNF-α for these measures and no relationships were demonstrated between fatigue, overall HRQoL or other patient related factors and cytokine levels (Tables 3.9 and 3.10).

Table 3.9: Correlations of cytokines with EORTC QLQ C30

Functional Scales	IL-6 (p value; r)	TNF-α (p value; r)
Physical Functioning	*0.03; -0.38*	0.62; -0.91
Role Functioning	0.07; -0.33	0.63; 0.09
Emotional Functioning	0.85; 0.03	0.93; -0.02
Cognitive Functioning	0.61; -0.09	0.39; 0.16
Social Functioning	0.81; -0.04	0.22; 0.22
QoL/Global Health Status		
QoL/Global Health Status	0.23; -0.22	0.63; -0.09
Symptom Scales/Items		
Pain	0.02; 0.41	0.84; 0.04
Fatigue	0.37; 0.16	0.89; 0.25
Insomnia	*0.02; 0.40*	0.47; 0.13
Appetite loss	*0.02; 0.41*	0.09; 0.30
Dyspnoea	*0.20; 0.23*	0.66; 0.08
Nausea and vomiting	0.28; 0.20	0.31; 0.18
Constipation	0.57; 0.34	0.16; 0.26
Diarrhoea	0.37; -0.16	0.51; -0.12

r = Spearman's rho correlation coefficient

Table 3.10: Correlations of cytokines with BPI-SF

BPI-SF	IL-6 (p value; r)	TNF-α (p value; r)
Average pain	*0.03; 0.38*	0.15; 0.27
Pain interference	*0.003; 0.516*	0.46; 0.14

r = Spearman's rho correlation coefficient

3.4.4 Comparison between myeloma patients with neuropathy and myeloma patients without neuropathy

Using s-LANSS, 16 patients (50%) scored ≥12 consistent with a predominantly neuropathic origin of pain (1 missing data) (Table 3.11).

Table 3.11: Males and females with and without neuropathy according to s-LANSS

s-LANNS ≥12	s-LANNS ≤12
Neuropathy group	*Non-neuropathy group*
Males: 8	Males: 8
Females: 8	Females: 7

A Mann-Whitney's U test was performed to evaluate the differences in symptoms and functioning using the EORTC QLQ-C30, EORTC MY20 and BPI-SF between the two groups. It was found there was a significant difference (myeloma patients with neuropathy having worse symptoms) with regards to the physical, role and social functioning from the EORTC QLQ-C30 (Table 3.12); side-effects of treatment and future perspectives from the EORTC MY20 (Table 3.13) and average pain and pain interference from the BPI-SF (Table 3.14) between the 2 groups.

Table 3.12: Mann-Whitney's U test comparing neuropathy and non-neuropathy groups for the EORTC QLQ-C30

Functional Scales	Mann-Whitney U	p value
Physical Functioning	54.0	*0.009*
Role Functioning	65.0	*0.027*
Emotional Functioning	81.5	0.125
Cognitive Functioning	97.0	0.35
Social Functioning	78.0	*0.09*
QoL/Global Health Status		
QoL/Global Health Status	90.5	0.232
Symptom Scales/Items		
Pain	83.5	0.227
Fatigue	111.5	0.734
Insomnia	77.0	0.126
Appetite loss	113.5	0.783
Dyspnoea	116.5	0.884
Nausea and vomiting	105.0	0.50
Constipation	78.0	0.056
Diarrhoea	107.5	0.518

Table 3.13: Mann-Whitney's U test comparing neuropathy and non-neuropathy groups for the EORTC QLQ-MY20

EORTC QLQ-MY20 subscales	Mann-Whitney U	p value
Disease specific	86.0	0.175
Side effects of treatment	56.0	*0.011*
Body Image	107.0	0.587
Future Perspective	65.0	*0.026*

Table 3.14: Mann-Whitney's U test comparing neuropathy and non-neuropathy groups for the BPI-SF

BPI-SF	Mann-Whitney U	p value
Average pain	58.5	*0.024*
Pain interference	51.5	*0.012*

The mean ranks for physical, role and social functioning from the EORTC QLQ-C30 and future perspectives from the EORTC MY20 were higher in the non-neuropathy group (s-LANSS ≤12) meaning that this group had better functioning whilst the mean ranks for side-effects of treatment from the EORTC MY20 and average pain and pain interference were higher in the neuropathy group (s-LANNS ≥12) meaning that this group had more pain symptoms. However there was no significant difference between groups in age or sex, years since diagnosis, QoL, and emotional functioning (Table 3.15).

Table 3.15: The mean ranks from the Mann-Whitney's U test

	s-LANSS	Mean Rank
Physical functioning	≤12	20.40
	≥12	11.88
Role functioning	≤12	19.67
	≥12	12.56
Social functioning	≤12	18.80
	≥12	13.38
Side effects of treatment	≤12	11.73
	≥12	20.00
Future perspectives	≤12	19.67
	≥12	15.19
Average pain	≤12	11.68
	≥12	18.84
Pain interference	≤12	11.18
	≥12	19.28

3.5 Discussion

In previous decades, when the average survival of patients with MM was relatively short and treatment options limited, the emphasis was on providing suppression of disease along with symptom relief and ultimately terminal care. In the modern management of advanced intensively treated MM, patients are living longer and are compromised due to a complex set of physical and psychosocial complications. Perhaps due to the tempo of improvements, QoL in long-term survivors of MM has been poorly characterised especially when compared to some other cancers where patients have prolonged survival.

In this study we identified a cohort of patients with advanced, multiply relapsed and intensively pre-treated, but stable MM. Despite disease stability, the absence of acute treatment-related toxicity and standard use of supportive care measures (such as analgesics and bisphosphonates), our findings confirm significant compromise of physical and social functioning as well as QoL, which was closely related to a persistent and cumulative symptom burden. Although the influence of fatigue and pain on HRQoL has long been recognised in MM, (Gulbrandsen et al. 2004) the novel finding of this study is that on-going fatigue and pain are significant symptoms in longstanding MM patients, even in stable phase disease. Moreover, in our study, half of the patients had neuropathic pain. Clinical assessment has shown that up to 20% of MM patients have clinical or sub-clinical evidence of neuropathy at diagnosis and as many as 75% may experience neuropathy during treatment (Richardson et al. 2011) but our study suggests continued suffering in patients with stable disease. Also our study showed that MM patients with neuropathy had a worse physical, role and social functioning; increased pain and pain interference when compared to MM patients who did not report neuropathy. In a study by Johnsen et al, patients with MM generally had the highest level of symptoms and problems compared to patients with other haematological diseases (Johnsen et al. 2009), and this was also supported by an earlier study in Nordic patients with MM six months of treatment had similar functioning and physical symptoms to our group of patients (Wisloff et al. 1996). The influence of fatigue and pain on HRQoL has long been recognised in MM (Gulbrandsen et al. 2004).

These findings in chronic stable MM cohort build on to other studies which have used broader more heterogeneous MM patients from all stages of disease, including those on acute treatment, where functioning is significantly impaired with fatigue, pain and peripheral neuropathy, insomnia and future health worries in around 30-

40% of patients (Molassiotis et al. 2011). Compared with early stage disease assessed with EORTC QLQ C-30 and EORTC MY-20 (Cocks et al. 2007), our more advanced, heavily treated cohort had greater symptom burden, reduced body image, and more impairment of future perspectives. These are compounded by concerns of loss of independence, disease recurrence and death, despite control of their disease. Compared to a recent study, in which 25 MM patients were followed up 3 years after their autologous stem cell transplantation, our group of patients were five years post transplantation, and were found to have worse physical functioning, social functioning, emotional functioning and cognitive functioning whilst there was no difference in the QoL; also our group had worse pain and insomnia (Frodin et al. 2010). This might indicate that the longer these patients survive, the more troubled they are with symptoms thus follow-up at regular intervals is crucial to identify and manage these. In our study, overall mental functioning (measured by MCS SF-12) was preserved, suggesting effective coping mechanisms as these patients have been living with MM for a number of years. In a review by Pidala et al, it was suggested that patients who undergo autologous bone marrow transplantation return to their baseline emotional functioning by 3–6 months (Pidala et al. 2010).

IL-6 is an important cytokine in the pathophysiology of MM, and is related to disease activity (Vito Michele 2001). Interestingly, correlations were detected between pain, physical functioning, insomnia and appetite loss and the cytokine IL-6, in this stable advanced cohort. However, as the correlation coefficients were generally low to moderate strength, we do not regard this as definitive data, but more a basis for future investigation as to whether a causative or other relationship (such as a biomarker) exists between IL-6 levels and these symptoms. A study investigating levels of pro-inflammatory cytokines IL-2 and TNF-α together with anti-inflammatory cytokines IL-4 and IL-10 in patients with painful and painless neuropathy and healthy volunteers showed that patients with painful neuropathy had increased levels of IL-2 and TNF-α, whilst patients with painless neuropathy had significantly higher levels of IL-4 and IL-10 (Uceyler et al. 2007). This indicates that interactions between these cytokines are vital for the pathophysiological changes associated with pain and additional cytokines may also be involved in the development of painful CIPN. We had specifically recruited patients with chronic stable MM and the relationship with pain and reduced physical functioning in this setting warrants further investigation. In other cancers inflammatory mediators have been linked to altered CNS activity, potentially influencing the perception of pain and other symptoms (Collado-Hidalgo et al. 2006).

As to whether novel strategies used in other cancers can improve HRQoL in the expanding population of long surviving myeloma patients requires further investigation. A randomised controlled trial of HRQoL assessments in general oncology practice demonstrated that these led to the encouragement of the discussion of QoL issues and increased the physicians' awareness of their patients' QoL together with greater patient satisfaction (Detmar et al. 2002). Other evidence suggests that cancer patients do not always report QoL problems during clinical appointments (due to time constraints, or sensitive nature of the problems) (Velikova et al. 2008) and compared to patients and their partners, physicians tend to overestimate patients' QOL and underestimate symptoms (Hendriks and Schouten 2002). Therefore, approaches to improve HRQoL may be economically beneficial, both individually and societally and have implications for a multi-disciplinary approach. Our study confirmed significant work disability and early retirement, along with restricted physical and social activities in patients with an average age of presentation of 55 years. In other settings, returning to or maintaining employment after cancer is important for survivors' QoL, including mental and physical health (Aaronson et al. 1993). Furthermore, employment earnings can improve standards of living and a return to normal life activities (van der Wouden et al. 1992).

Given the range of late-effects from MM treatment, management demands a multi-faceted approach. Previously patients have been managed in a variety of settings, from general haematology clinics in a busy district general hospital to specialist clinics in tertiary referral centres, where, for example, there may be immediate availability of transplant services. Availability of specialist palliative and supportive care services also may vary considerably between units. However, there is a balance between local services, which might lack disease specific expertise but are convenient for the patient, versus centralised specialist services working alongside the haematologist, which may not always be desirable in terms of patient travelling. It might be better for patients with early diagnosis to be treated in centralised specialist services and localised services might be more appropriate for relapsed patients approaching end of life although this will depend on the patient and their carers' preference and is locality dependent.

Limitations of this study

We recognise that this exploratory, cross-sectional study has limitations, including relatively small numbers, and the fact that it included some patients who had previous allogeneic HSCT. However, all these patients had at least one episode of disease progression post transplant, and such patients would feature in specialised myeloma clinics in tertiary care centres. One further limitation is that our inclusion criteria also included patients on maintenance treatment. These included a minority who received maintenance treatment with lenalidomide or interferon-alpha, which have recognised side effects, and may have influenced the results of this analysis.

This study was exploratory and was not designed to arrive at a firm conclusion regarding the issue of overlapping toxicities or the effect of cumulative dose from different chemotherapeutic agents. In line with UK and local practice at the time of the study, none of the patients in this study had received a combination of drugs commonly considered to cause peripheral neuropathy i.e. bortezomib, thalidomide, or vincristine were never used concurrently, and only sequentially, mainly in combination with dexamethasone +/- alkylating agent. We recognise the cumulative doses of individual agents may have varied between individuals, and that toxicities (such as peripheral neuropathy) from one drug may have persisted and then overlapped with later toxicities from other drugs, and a more complex analysis is desirable. We do not feel it is achievable with the present study, but future prospective studies designed to elucidate the impact of cumulative doses and overlapping toxicities are warranted.

Furthermore, it is important to point out that this study selected younger, intensively treated myeloma patients, all of whom had at least one HSCT procedure and is not representative of all MM patients, particularly those in older age groups who have less intensive treatments.

Chapter 4

4 Conclusions from both studies

Chemotherapy-induced peripheral neuropathy is becoming increasingly common as patients are living longer and being exposed to more toxic treatment that will give them a longer life expectancy but with the deleterious consequences of side effects. Neurotoxicity can be so severe that it can limit the dose of chemotherapy or even potentially stop curative cancer treatment thus having an impact on survival. It has been well established that the incidence of CIPN can be variable, ranging from 0% to 70% of patients receiving chemotherapy, but generally occurring in 30–40% of patients. This variability is associated with several factors that can influence the development of CIPN especially, the patient's age, dose intensity, cumulative dose and the duration of treatment, administration of other neurotoxic agents, and pre-existing conditions such as diabetes (Barton et al. 2011) as well as the diagnostic criteria used and if neuropathy was measured using tools and neurophysiological testing. Once CIPN develops, especially with a subgroup of chemotherapy agents, it is usually long lasting and irreversible causing great suffering and disability to patients affecting their QoL as has been demonstrated in my studies. Unfortunately, clinical practice has shown that the current analgesics that are used for patients suffering from CIPN only provide pain relief in a small number of patients and this is sometimes only achieved at the expense of often, intolerable side effects. Although several therapies have been studied for the prophylaxis of CIPN; none have been effective and there are no clear guidelines regarding the use of prophylaxis. On the other hand, pharmacological treatment for neuropathic pain using various drugs, including antidepressants, calcium channel blockers, opioid agonists, and topical lidocaine, have been shown in randomised controlled clinical trials to be efficacious (Attal et al. 2010; Baron et al. 2010; Dworkin et al. 2010). However, to date, there have not been any trials of the use of opioids in patients with CIPN. Several of our patients in this fMRI study have been on opioids although their neuropathic pain linked to chemotherapy was not well controlled. There are concerns with the use of long-term opioids, such as physical dependency, misuse or abuse, and immunological changes; however if patients are selected properly, opioids may be a good option. As there is no effective prophylaxis for CIPN, the aim is to manage symptoms using drugs targeted at known chronic pain mechanisms including

calcium channel blockers and serotonin–norepinephrine reuptake inhibitors. The management of peripheral neuropathy should include symptom control, and dose adjustment of the anti-myeloma therapy alongside treatment of any potentially reversible causes.

Due to the heterogeneity of neuropathic pain mechanisms and the coexisting psychological and emotional aspects of pain, treatment can be difficult and therefore needs a multidisciplinary therapeutic approach. This includes not only multimodal pharmacology, but also and non-pharmacological treatment regimens, such as psychological, physical, and occupational therapy. It is imperative in the development a holistic approach to neuropathic pain management that a correct diagnosis of the cause of pain is made, the type of pain is determined, and an appropriate treatment plan is made. Symptoms of neuropathy in the feet should be evaluated distinctly from those in the hands as they have been shown to differ and tend to be more severe in the lower extremities for individual patients. A better understanding of CIPN and its pathophysiological mechanisms into sensory signs will lead to a more effective and specific mechanism-based treatment approach (Baron et al. 2010).

Structural damage to peripheral nerves causes abnormal somatosensory processing of the peripheral and possibly of the central nervous system (Windebank and Grisold 2008). When there is damage to the small nerve fibres this leads to burning and lancinating pain, cutaneous hyperesthesia, and loss of pain and temperature senses whilst involvement of large nerve fibres results in loss of vibration sense, loss of proprioception, loss of reflexes, muscle weakness, and slowed nerve conduction (Wolf et al. 2011). Chemotherapy-induced peripheral neuropathy has traditionally been considered a disease of the peripheral nerve only, with not much emphasis at how the brain interprets this pain and whether changes occur at the level of the central nervous system. Our results indicate that fMRI is capable of providing novel information with respect to the processing of pain within the CNS in CIPN. Our results also show that painful stimuli delivered to neuropathy-affected and symptom-free sites in CIPN evoke differential activation of distinct cortical regions and therefore we deduce that the nociceptive system undergoes plastic changes, which appear to reflect abnormal central pain processing. Functional MRI acquired in the context of pain stimuli and CIPN may thus help elucidate pathogenesis and development, stimulate treatment development and target and monitor future treatment to aid the successful survival of patients with treatable MM.

As well as reducing tumour load and prolonging life, it is important to take into account the cumulative burden of the disease and treatment-related symptoms (both acute and long-term), co-morbidities and the large range of associated psychological and social issues in patients with advanced MM (Ahmedzai and Walsh 2000). Stable disease after induction, after transplantation and after post-transplant salvage therapy is quite different and patients have different symptom burdens depending on stage of the disease and the treatment they received (Kvam et al. 2009; Frodin et al. 2010). Supportive care plays an increasingly important part in the modern management of MM in maintaining a good QoL from the initial diagnosis through the different stages of active treatment and towards the end of life. Supportive care aims to maximise the benefits of treatment by ensuring the patients with MM maintain as high a QoL in spite of incurable disease and the cumulative effects of disease and treatment. Optimal comprehensive care in MM therefore requires an on-going multidisciplinary approach, including both nursing and supportive care, to improve an active awareness and appreciation of HRQoL, in both active and inactive disease (Bird et al. 2011). In the recently published British supportive care guidelines for MM, routine systematic assessments, such as holistic needs questionnaires and/or care plans, and specific interventions for fatigue, pain and rehabilitation have been recommended (Snowden et al. 2011). Further studies are needed to determine the benefit of such intervention with respect to HRQoL and functioning in patients at all stages of MM.

Chapter 5

5 Final discussion and future work

The principal aims of this work were to investigate the involvement of the CNS in MM patients with CIPN and to have a better understanding of what living with MM means to these patients clinically and in terms of health-related quality of life.

In the first study (Chapter 2), the fMRI data reported suggests that painful stimuli delivered to myeloma patients with CIPN evoke differential activation of distinct cortical regions, reflecting different central pain processing when compared to healthy volunteers. In CIPN-myeloma patients, there is a possibility that neural adaptations have occurred secondary to sustained abnormal peripheral input. There was activation in the right superior frontal gyrus in healthy volunteers but not in CIPN-myeloma patients during heat-pain stimulation whilst there was activation of the precuneus in the CIPN-myeloma group and deactivation in the healthy volunteers during this stimulation. In addition, significant correlations were also found between brain activation in the operculo-insular cortex and the reduced version of Total Neuropathy Score. These findings highlight anatomical-functional regions that have been previously shown to be involved in other pain studies; however, this is the first identification of pain function-neuroanatomical correlates in the context of CIPN in MM. This shows that fMRI is capable of providing novel information with respect to the processing of pain within the CNS in CIPN. It could be postulated that cerebral involvement may occur concomitantly with peripheral nerve damage and these findings demonstrate clearly that the neuropathic process associated with CIPN is not confined to the peripheral nerve only but does involve the functioning of the brain. The study was cross-sectional, where CIPN-myeloma patients already had developed neuropathy. Taking this work forward, a sample of MM patients who are on the same chemotherapeutic agents but who do not develop neuropathy is being studied and compared to CIPN-myeloma patients and healthy volunteers. Prospective longitudinal studies would help to determine the natural history of cerebral involvement in CIPN and whether CNS involvement precedes peripheral nerve involvement. Provision of an early objective CNS marker with regards to the development of neuropathy may guide the development and objective assessment of much-needed pharmacological interventions, which could improve therapeutic response with minimal toxicity. Characterisation of the pain matrix

activation in CIPN may also identify novel CNS targets that could drive the development of new pharmaceuticals, or new ways of using current drugs. Furthermore, as CIPN occurs in many other cancers because of cytotoxic drug effects and new biological agents, prospective studies in the future will be of potential benefit not only to MM patients but other cancer patients undergoing anti-cancer treatment to improve and enhance their QoL. It has been suggested that combination of fMRI and drug administration may prove useful in the future development of new analgesic compounds (Schweinhardt et al. 2006).

Recent advances in our understanding of human brain function have been driven by the introduction of fMRI (Boguslawska et al. 1999). The non-invasive, ionising-radiation free aspect of fMRI is useful in studies investigating the perception and modulation of pain using experimental noxious stimuli. Functional MRI has been used to characterise a network of brain areas that activate in response to pain stimuli, identifying a 'pain matrix' (Melzack 1999). Both clinical and experimental studies have shown interactions between pain unpleasantness, pain intensity and emotions, and neuroimaging studies have unravelled structures transmitting pain including the ascending spinal pathways and a central network of brain structures. The spinal pathways converge onto the brain stem, thalamic nuclei, the sensorimotor cortices and the limbic system (amygdala, hypothalamus, insular cortex, ACC) (May 2007). However, the pain matrix cannot be viewed as a single, stand-alone entity, which is implicated in nociception. Instead, it should be seen as a flexible system which is actively modulated by a variety of brain regions depending on inter-individual variability (due to emotion, chronic illness, previous experiences) which influence the perception of pain (Bingel and Tracey 2008). Disruption of the pain matrix, and the pathways between them, are thought to have implications for the pathogenesis and persistence of neuropathic pain (Tracey 2005). Studies have begun to evaluate CNS changes that occur in patients with neuropathic pain but to our knowledge, there have been no fMRI studies in multiple MM patients who have developed treatment emergent CIPN in myeloma.

In the second study (Chapter 3), despite disease stability and absence of acute treatment toxicity in this specific group of younger MM survivors, the results showed that patients were significantly compromised by physical symptoms and psychosocial interactions. Among the strengths of this study was the use of validated measures to assess a range of outcomes amongst a sample of MM

patients at a similar point in treatment. Although care was taken to select psychometrically good instruments, scores on these measures cannot substitute for clinical diagnoses such as major depression, adjustment disorders, and steroid-induced mood disorder. Another important limitation was the cross-sectional design; however it was perceived that there was a strong practical need for information about symptom burden during this phase of treatment, but obviously in clinical care, it is important to assess changes in adjustment and QoL throughout the course of disease and thus future longitudinal evaluations are needed. Future studies should also determine whether interventions such as routine use of systematic evaluations including the use of HRQoL assessments, holistic needs questionnaires and/or care plans, and specific interventions for fatigue, pain and rehabilitation can lead to improvements in HRQoL and functioning at all the stages of MM.

Many patients with MM have a significantly prolonged survival through modern treatments that have improved disease control to the extent that MM now has the potential to be a chronic disease. The majority of these patients are now living with the burden of MM together with the cumulative side effects of treatments. Thus optimal supportive and palliative care is necessary in parallel with disease control to maximise QoL at all stages of the disease. Preservation of a good QoL presents challenges from diagnosis through the multiple phases of active treatment to the end of life. One of the commonest symptoms MM patients experience is pain and this could be the reason for presentation or subsequent relapse and also could be due to the side effects of the treatment. As the National Institute for Health and Clinical Excellence guidance has suggested supportive care is 'care that helps the patient and their family to cope with cancer and treatment of it – from pre-diagnosis and treatment, to cure, continuing illness or death and into bereavement. It helps the patient to maximize the benefits of treatment and to live as well as possible with the effects of the disease. It is given equal priority alongside diagnosis and treatment' (NICE 2004). In recently published supportive care guidelines for MM, it has been recommended that all patients should be assessed for unmet holistic needs at key times (at diagnosis, after initial treatment, during follow-up) in the disease trajectory (Snowden et al. 2011).

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List of Appendix

Appendix 1:   Presentations, publications and manuscripts in progress

Appendix 2:   Study title: Central Pain Processing in Diabetic Neuropathy, Chemotherapy-induced painful neuropathy subgroup

Appendix 3:   Study Title: Central Nervous System Involvement in Diabetic Neuropathy, Healthy Volunteers

Appendix 4:   Functional Pain Imaging Study: Case Report Form

Appendix 5:   Normal ranges for nerve conduction study

Appendix 6:   Definitions in context of this study

Appendix 1

Presentations, publications and manuscripts in progress

Key Abstract publications:

- E. Cachia, D. Selvarajah, M. D. Hunter, J. Snowden, S. H. Ahmedzai, and I. D. Wilkinson. Central Pain Processing in Chemotherapy Induced Peripheral Neuropathy. European Journal of Palliative Care, (2011), Abstract number: FC6.3, Abstract type: *Oral*.

- E. Cachia, D. Selvarajah, M. D. Hunter, J. Snowden, S. H. Ahmedzai, and I. D. Wilkinson. Intracranial pain processing on fMRI in chemotherapy-induced peripheral neuropathy. (2011). European Society for Magnetic Resonance in Medicine, Abstract number: 356, Abstract type: *Oral*.

- E. Cachia, D. Selvarajah, M. D. Hunter, J. Snowden, S. H. Ahmedzai, and I. D. Wilkinson. Central pain processing in chemotherapy induced peripheral neuropathy. Proc. Intl. Soc. Mag. Reson. Med. 19 (2011); 4167; Abstract type: *Electronic poster.*

- E.Cachia, D. Selvarajah, R. Gandhi, A, Sankar, J. Snowden, SH Ahmedzai, S.Tesfaye, ID Wilkinson. Investigating the neural basis of pain processing in diabetic peripheral neuropathy and chemotherapy-induced peripheral neuropathy. Palliat Med 24: S5 (2010); Abstract number: 362, Abstract type: *Poster.*

Oral presentations:

The data generated in this thesis has been presented at local, national and international meetings including:

- The British Pain Society Annual General Meeting (April 2012) - Central pain processing in chemotherapy-induced peripheral neuropathy.

- European Society for Magnetic Resonance in Medicine, Leipzig (October 2011) - Intracranial pain processing on fMRI in chemotherapy-induced peripheral neuropathy.

- The University of Sheffield Radiology Symposium (October 2011) - Chemotherapy & the 'pain matrix.

- European Association of Palliative Care, Lisbon (May 2011) - Central pain processing in chemotherapy-induced peripheral neuropathy in myeloma.

- APM AGM Mini conference, Dublin (March 2011) - Central pain processing in chemotherapy-induced peripheral neuropathy in myeloma.

- Advanced Course in Pain and Symptom Management, Nottingham and Oxford (June, July 2010) - Pain, Neuropathy & Imaging.

- Specialised Medicine and Specialised Cancer Services Joint Research Symposium, Sheffield (January 2010) - A study of pain, peripheral neuropathy and psychosocial late effects in patients with intensively treated advanced multiple myeloma.

Manuscripts in preparation:

- Elaine Boland, Christine Eiser, Yousef Ezaydi, Diana M Greenfield, Sam H Ahmedzai, John A Snowden. Living with advanced but stable multiple myeloma: a study of the symptom burden and cumulative effects of disease and intensive treatment on health-related quality of life. Submitted to the Journal of Pain and Symptom Management.

- Elaine Boland, Dinesh Selvarajah, Mike Hunter, Yousef Ezaydi, Sam H. Ahmedzai, John A. Snowden, Iain D. Wilkinson. Central pain processing in chemotherapy-induced peripheral neuropathy: a functional magnetic resonance imaging study. In preparation and planned for submission to the Journal of Clinical Oncology.

Sheffield Teaching Hospitals **NHS**
NHS Foundation Trust

STUDY TITLE: Central Pain Processing in Diabetic Neuropathy, Chemotherapy-induced painful neuropathy subgroup

Principal Investigator; Professor Solomon Tesfaye MD

You are being asked to take part in an <u>educational</u> study. Before you decide to take part, it is important for you to understand why the research is being done and what it involves. Please take the time to read the information sheet carefully and discuss it with friends, relatives and if you feel it necessary your GP. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

WHAT IS THE PURPOSE OF THE STUDY?

Peripheral neuropathy is a term used to describe damage to nerves that are outside of the brain and spinal cord (peripheral nerves). Chemotherapy-induced peripheral neuropathy describes damage caused by chemotherapy to the peripheral nervous system, the system that transmits information between the central nervous system (e.g. the brain and spinal cord) and the rest of the body. Some anti-cancer drugs can cause nerve damage. This is the most common cause of peripheral neuropathy in people with cancer. This can lead to a change in the sensation in the hands and feet with increased sensitivity and pain. In some patients this results in severe intractable pain. Despite lots of research, the exact cause of this damage is unclear.

The diabetes research department in this hospital is carrying out a study looking at how nerves in the brain may be involved. One aspect of this study is looking at another group of patients, that is; multiple myeloma patients who have been treated with chemotherapy and developed this nerve damage (neuropathy). Functional Magnetic Resonance Imaging (fMRI) - (*MRI brain scan*) is being performed on these patients with nerve damage looking specifically for changes within the brain caused by neuropathy. In this study, we will apply heat (like running your hand over a candle) to the top of the foot, thigh and forearm. In addition, we also propose to

study nerve activation to a soft brush stroke stimulus to the top of your foot and to flashing lights.

The aim of this study is to allow us to gain a better understanding of how the processing of pain signals is altered in this group of patients and also to compare the results with the diabetes group. This may ultimately lead to better treatments, which could alleviate the problem.

WHY HAVE I BEEN CHOSEN TO TAKE PART IN THE STUDY?

You have been chosen to take part in the study as you have the condition that fit the criteria, which we are studying:

 - People who have been treated with one of the following chemotherapy agents (thalidomide, bortezomib-velcade, vincrisitine) for multiple myeloma and now has established neuropathy (numbness and painless nerve damage).

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without having to give a reason. This will NOT affect any medical care you receive

WHAT WILL HAPPEN IF I AGREE TO TAKE PART IN THE STUDY?

All of these tests will be carried out at the Royal Hallamshire Hospital. It will involve coming for up to 2 visits:

First Visit:
At the first visit a Doctor will document your medical history and carry out some tests to examine your nerves. These will include standard clinical tests of your ability to feel different sensations (vibration, cold etc.) and also measurements of the speed at which your nerves transmit impulses. We will also perform the standard clinical

tests that are used to test autonomic nerve function. This will involve connecting you to a heart monitor and taking a heart tracing (ECG) and monitoring blood pressure at rest and whilst doing different breathing exercises and lying down and standing up. At this visit we will also apply a heat stimulus to the top of your right foot, thigh and forearm to demonstrate the stimulus that will be applied during your second MRI visit (detailed below) and to also determine the temperature necessary to delivery an acceptable level of pain necessary for the study. This sensation could be likened to heat experienced when running your hand over a candle. These tests will be carried out in the diabetes research room on M floor.

This will take about 1.5 hours.

Second Visit

During this visit you will have a fMRI scan of your *brain*. The machine uses a magnet to look at water atoms in your body. The scan takes 60 minutes. This will be performed in the MRI department, and involves lying flat and very still on a bed and moving through a scanner. It can be very noisy as the magnets moving makes a sound rather like a washing machine, you can be provided with ear plugs to lessen the noise. Some patients can feel claustrophobic but the procedure can be stopped at any time should you wish.

During the MRI scan we will apply the heat stimulus (previously demonstrated at the first visit) to the top of your foot and upper thigh. Part of this examination will be assessing how your brain responds to heat stimulus, which you may find uncomfortable. Altogether this visit will last 1.5 hours.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

The study may allow us to learn more about the nature of nerve damage due to chemotherapy, and whether the nerves in the brain are involved and be able to compare this to the diabetes group. At present early neuropathy is very difficult to diagnose and involves several time consuming tests. If it is shown that fMRI scan can detect a unique pattern of activation in painful chemotherapy induced neuropathy, then it may be a way forward to making diagnosis easier (more objective) and aid the development of novel new treatments.

WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART?

The MRI scan is a safe procedure that is used frequently in normal medical practice. It does not use ionising radiation or X-rays. Some people find the scan claustrophobic but the test can be stopped if you wish at any time. If you know that you suffer from claustrophobia we request that you do not volunteer to take part in this study for your own comfort. Application of heat stimulus will cause discomfort but we intend to only apply this for a short duration during fMRI scanning, on and off. The nerve tests are safe, but some people may experience slight discomfort during the nerve conduction test.

Patients with neuropathy will be asked to discontinue pain relieving medications for 48 hours prior to fMRI scanning. This is an important requirement for the study, as it will allow us to accurately assess the brain's response to the heat stimulus applied without attenuation by medication taken. Discontinuing your pain relieving medication may cause an exacerbation of your painful symptoms and if these were potentially intolerable, we would advise that you do not take part in this study. Once the fMRI scanning is complete you can continue taking you medications as prescribed.

WHAT IF SOMETHING GOES WRONG?

In the unlikely situation of something going wrong you may complain via the normal NHS complaints procedure. You will also be able to take legal action if there is any medical negligence on behalf of the doctors seeing you.

Should you have any cause for concern about the running of the study please contact the Lead Investigator Professor Solomon Tesfaye on 0114 2712709 or contact Professor Welsh in writing.

Professor C.L Welsh

Medical Director

Sheffield Teaching Hospitals NHS Foundation Trust

8 Beech Hill Road

Sheffield

S10 2SB

CAN I WITHDRAW FROM THE STUDY AT ANY POINT?

If you do consent to take part in the study, it will be purely on a voluntary basis, and you may withdraw from the study at any time if you wish. You do not have to give a reason for withdrawing, and it will NOT affect your future medical care with us. If you do withdraw, we will request permission to retain any data collected prior to withdrawal.

WILL MY TAKING PART IN THE STUDY BE KEPT CONFIDENTIAL?

All information that we obtain during the course of this study will be kept strictly confidential; the only people who will have access to your results will be the medical staff running the study. All the information form the MRI scan will be kept in the MRI department. We will be informing your GP that you are taking part.

WHAT WILL HAPPEN TO THE RESULTS OF THIS STUDY

The results of this study may be published in medical journals, and may be presented at medical conferences.
We will be happy to provide you with a copy of the results when they are published, if you contact us. The results should be available early 2012.
Any publication of the results of this study will not include any information, which may disclose the identity of anyone taking part.

WHO IS ORGANISING AND FUNDING THE RESEARCH?

The study is being carried out by the Diabetes Research Department at the Royal Hallamshire Hospital and is being funded.

WHO HAS REVIEWED THE STUDY?

This study has undergone a detailed scientific review, and it has also been reviewed by the South Sheffield Ethics Committee.

WHAT IF I WISH TO COMPLAIN ABOUT THE WAY THE STUDY HAS BEEN CONDUCTED?

If you have any cause to complain about any aspect in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study.

If you have any complaints or concerns, please contact either the project co-ordinator:

Name: Professor Solomon Tesfaye   Tel: 0114 2711900

Otherwise, you can use the normal hospital complaints procedure and contact the following person:

Name: Andrew Cash Chief Executive   Tel: 0114 2712358

WHO DO I CONTACT FOR FURTHER INFORMATION?

If you would like to find out more information please contact:

Professor Solomon Tesfaye

Royal Hallamshire Hospital

Glossop Road

Sheffield, S10 2JF

0114 2712709

Thank you very much for taking the time to read this information sheet. If you agree to participate in the study, you will be asked to sign a consent form to confirm that you are satisfied with the information provided and have had adequate opportunity to discuss the information provided.

If you do kindly agree to take part in this study you will be obliged not to take part in any other study nor for the 3 months following the completion of your participation.

Central Pain Processing in Diabetic Neuropathy

Name of Researcher: Prof. Solomon Tesfaye

CONSENT FORM

Please initial box

1. I confirm that I have read and understand the information sheet dated 12 Aug 2009 (version 1.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

4. I give permission for my GP to be informed of my participation in this study

5. I agree to take part in the above study.

_____   _____   _____
Name of Patient   Date   Signature

_____   _____   _____
Name of Person taking consent Date   Signature

Sheffield Teaching Hospitals **NHS**
NHS Foundation Trust

STUDY TITLE: **Central Nervous System Involvement in Diabetic Neuropathy**

Principal Investigator; Professor Solomon Tesfaye MD

You are being asked to take part in an <u>educational</u> study. Before you decide to take part, it is important for you to understand why the research is being done and what it involves. Please take the time to read the information sheet carefully and discuss it with friends, relatives and if you feel it necessary your GP. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

WHAT IS THE PURPOSE OF THE STUDY?

Diabetes can produce nerve damage, leading to loss of sensation in the feet and legs. In some patients this results in severe intractable pain. Despite lots of research, the exact cause of this damage is unclear.

The diabetes research department in this hospital is carrying out a study looking at how nerves in the brain may be involved. Functional Magnetic Resonance Imaging (fMRI)- (*MRI brain scan*) is being performed on volunteers with diabetes and healthy non-diabetic volunteers looking specifically for changes within the brain caused by diabetes. In this study, we will apply heat (like running your hand over a candle) to the top of the foot, thigh and forearm. In addition, we also propose to study nerve activation to a soft brush stroke stimulus to the top of your foot and to flashing lights.

If differences are found then it will allow us to gain a better understanding of how the processing of pain signals is altered by diabetes. This may ultimately lead to better treatments, which could alleviate the problem.

WHY HAVE I BEEN CHOSEN TO TAKE PART IN THE STUDY?

You have been chosen to take part in the study as you are a healthy volunteer without diabetes.

DO I HAVE TO TAKE PART?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you do decide to take part you are still free to withdraw at any time and without having to give a reason. This will NOT affect any medical care you receive

WHAT WILL HAPPEN IF I AGREE TO TAKE PART IN THE STUDY?

All of these tests will be carried out at the Royal Hallamshire Hospital. It will involve coming for up to 2 visits:

First Visit

At the first visit a Doctor will document your medical history and carry out some tests to examine your nerves. These will include standard clinical tests of your ability to feel different sensations (vibration, cold etc.) and also measurements of the speed at which your nerves transmit impulses. We will also perform the standard clinical tests that are used to test autonomic nerve function. This will involve connecting you to a heart monitor and taking a heart tracing (ECG) and monitoring blood pressure at rest and whilst doing different breathing exercises and lying down and standing up. At this visit we will also apply a heat stimulus to the top of your right foot, thigh

and forearm to demonstrate the stimulus that will be applied during your second MRI visit (detailed below) and to also determine the temperature necessary to delivery an acceptable level of pain necessary for the study. This sensation could be likened to heat experienced when running your hand over a candle. These tests will be carried out in the diabetes research room on M floor.

This will take about 1.5 hours.

Second Visit

During this visit you will have a fMRI scan of your *brain*. The machine uses a magnet to look at water atoms in your body. The scan takes 60 minutes. This will be performed in the MRI department, and involves lying flat and very still on a bed and moving through a scanner. It can be very noisy as the magnets moving makes a sound rather like a washing machine, you can be provided with ear plugs to lessen the noise. Some patients can feel claustrophobic but the procedure can be stopped at any time should you wish.

During the MRI scan we will apply the heat stimulus (previously demonstrated at the first visit) to the top of your foot, upper thigh and forearm. Part of this examination will be assessing how your brain responds to heat stimulus, which you may find uncomfortable. Following heat stimulation we will stimulate the top of your foot with a soft brush, and finally stimulate your eyes with a sequence of flashing lights.

Altogether this visit will last 2 hours.

Follow Up Visit

You may be invited to come back for a third visit (6-12 months later) for a second MRI scan when we will be assessing the reproducibility of the technique used. This part of the study is optional and does not stop you taking part in the main study.

WHAT ARE THE POSSIBLE BENEFITS OF TAKING PART?

The study may allow us to learn more about the nature of nerve damage in diabetes, and whether the nerves in the brain are involved. At present early neuropathy is very difficult to diagnose and involves several time consuming tests. If it is shown that fMRI scan can detect a unique pattern of activation in painful diabetic neuropathy, then it may be a way forward to making diagnosis easier (more objective) and aid the development of novel new treatments.

WHAT ARE THE POSSIBLE DISADVANTAGES AND RISKS OF TAKING PART?

The MRI scan is a safe procedure that is used frequently in normal medical practice. It does not use ionising radiation or X-rays. Some people find the scan claustrophobic but the test can be stopped if you wish at any time. If you know that you suffer from claustrophobia we request that you do not volunteer to take part in this study for your own comfort. Application of heat stimulus will cause discomfort but we intend to only apply this for a short duration during fMRI scanning, on and off for approximately 2 minutes each for the 3 areas. Those of you who have contact hypersensitivity will experience some discomfort when your foot are lightly brushed. However, this will only be on and off for approximately 2 minutes. The nerve tests are safe, but some people may experience slight discomfort during the nerve conduction test.

There is a chance of less than one in a 100 that your MR scan will show a significant abnormality of which you are unaware. In such circumstances you will be referred to the appropriate specialist in consultation with your general practitioner, if that is what you like. Such detection has the benefit of starting treatment early but in a small number of cases may have implications for future employment and insurance.

WHAT IF SOMETHING GOES WRONG?

In the unlikely situation of something going wrong you may complain via the normal NHS complaints procedure. You will also be able to take legal action if there is any medical negligence on behalf of the doctors seeing you.

Should you have any cause for concern about the running of the study please contact the Lead Investigator Professor Solomon Tesfaye on 0114 2712709 or contact Professor Welsh in writing.

Professor C.L Welsh

Medical Director

Sheffield Teaching Hospitals NHS Foundation Trust

8 Beech Hill Road

Sheffield

S10 2SB

CAN I WITHDRAW FROM THE STUDY AT ANY POINT?

If you do consent to take part in the study, it will be purely on a voluntary basis, and you may withdraw from the study at any time if you wish. You do not have to give a reason for withdrawing, and it will NOT affect your future medical care with us. If you do withdraw, we will request permission to retain any data collected prior to withdrawal.

WILL MY TAKING PART IN THE STUDY BE KEPT CONFIDENTIAL?

All information that we obtain during the course of this study will be kept strictly confidential; the only people who will have access to your results will be the medical staff running the study. All the information form the MRI scan will be kept in the MRI department. We will be informing your GP that you are taking part.

WHAT WILL HAPPEN TO THE RESULTS OF THIS STUDY?

The results of this study may be published in medical journals, and may be presented at medical conferences.

We will be happy to provide you with a copy of the results when they are published, if you contact us. The results should be available early 2011.

Any publication of the results of this study will not include any information, which may disclose the identity of anyone taking part.

WHO IS ORGANISING AND FUNDING THE RESEARCH?

The study is being carried out by the Diabetes Research Department at the Royal Hallamshire Hospital and is being funded by a grant from xxx. Travel expenses for travel to and from the hospital will be re-enumerated.

WHO HAS REVIEWED THE STUDY?

This study has undergone a detailed scientific review, and it has also been reviewed by the South Sheffield Ethics Committee.

WHAT IF I WISH TO COMPLAIN ABOUT THE WAY THE STUDY HAS BEEN CONDUCTED?

If you have any cause to complain about any aspect in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you and are not compromised in any way because you have taken part in a research study.

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Name: Professor Solomon Tesfaye   Tel: 0114 2711900

Otherwise, you can use the normal hospital complaints procedure and contact the following person:

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If you would like to find out more information please contact:

Professor Solomon Tesfaye

Royal Hallamshire Hospital

Glossop Road

Sheffield, S10 2JF

0114 2712709

Thank you very much for taking the time to read this information sheet. If you agree to participate in the study, you will be asked to sign a consent form to confirm that you are satisfied with the information provided and have had adequate opportunity to discuss the information provided.

If you do kindly agree to take part in this study you will be obliged not to take part in any other study nor for the 3 months following the completion of your participation.

Appendix 4

Sheffield Teaching Hospitals **NHS**
NHS Foundation Trust

Functional Pain Imaging Study: Case Report Form

Study Code: _____

Date: _____

Handedness _____

Employment _____

(1= Yes, 2= Unemployed, 3= Retired on health grounds, 4= Retired other reasons)

If answer above is 3 give the main medical diagnosis: _____

Date of Birth: _____

Age: _____

Sex: _____

Onset of Pain: _____

Myeloma History	Date diagnosed
(please list conditioning therapy and doses, cycle length, number of cycles, start and end date of cycles) (date of first diagnosis) (date and type of transplants)	Date/type of transplant (please state auto/allo)

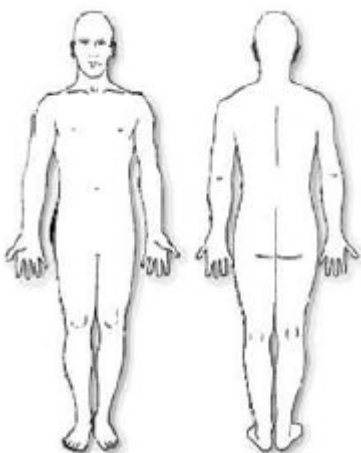|	Conditioning therapy/doses	Cycle length(days/Months)	Number of cycles	Date/first cycle	Date of last cycle

Radiotherapy (site & year): _____

Painful CIPN: NTSS-6

1. A more or less continuous "dead feeling" like anaesthetised without tingling/ pins

and needles: **Yes No**
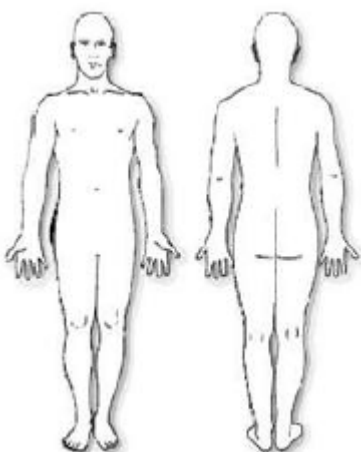
Symptom present at onset: _____ _____

If Yes,

	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

Persists Resolved

Current nature of symptom: _____ _____

If persists,

	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

If no/resolved (when): _____

2. A more or less continuous prickling/ pins and needles feeling with or without a dead leg. **Yes   No**

Symptom present at onset: _____ _____

 If Yes,

	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

Persists   Resolved

Current nature of symptom: _____   _____

If persists,

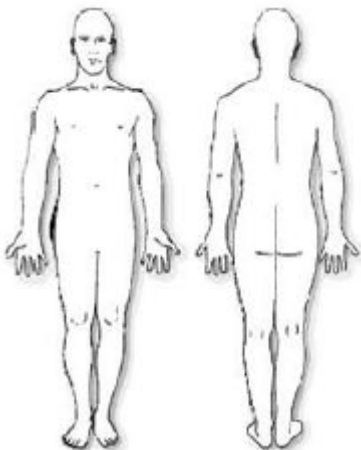|	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

If no/resolved (when): _____

3. Unusual sensitivity or tenderness when regions of body touched:

                                    Yes   No

Symptom present at onset: _____ _____

If Yes,

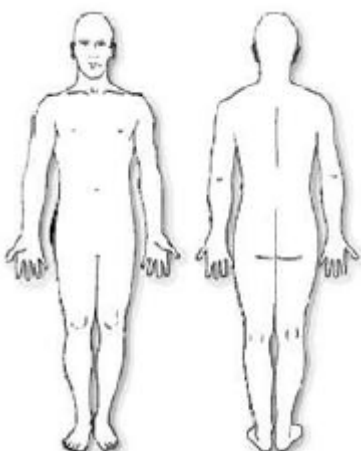|	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

                        Persists   Resolved

Current nature of symptom: _____   _____

If persists,

	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

If no/resolved (when): _____

4. Sharp jabbing needle like/electric shock (lasting seconds or a minute or two):

Yes   No

Symptom present at onset: _____ _____

If Yes,

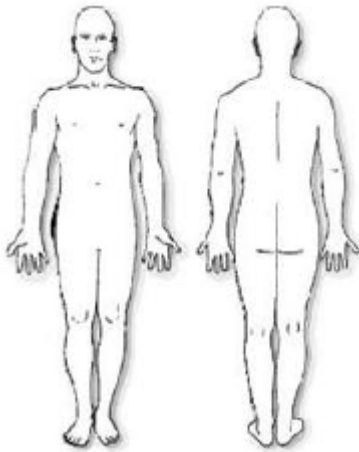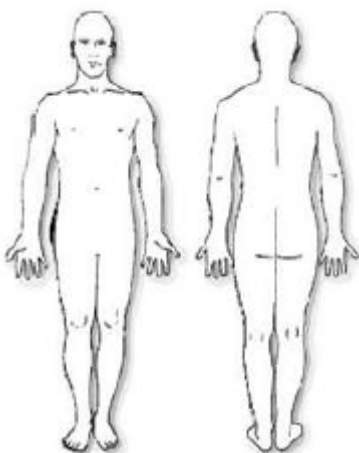|	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

Persists   Resolved

Current nature of symptom: _____   _____

If persists,

	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

If no/resolved (when): _____

5. Burning discomfort:

	Yes	No
Symptom present at onset:	_____	_____

If Yes,

	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

	Persists	Resolved
Current nature of symptom:	_____	_____

If Persists,

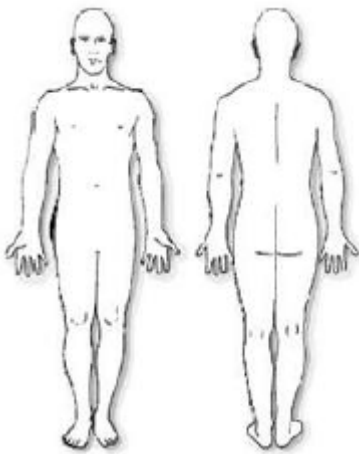|	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

If no/resolved (when): _____

6. Deep aching pain:

	Yes	No

Symptom present at onset: _____ _____

If Yes,
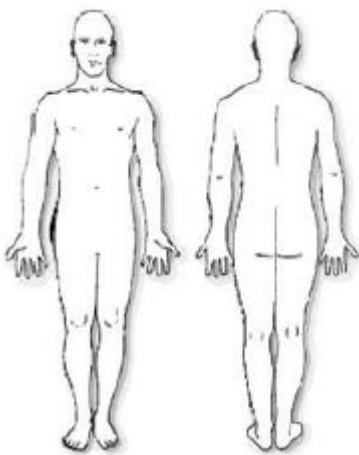
	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

	Persists	Resolved

Current nature of symptom: _____   _____

If persists,

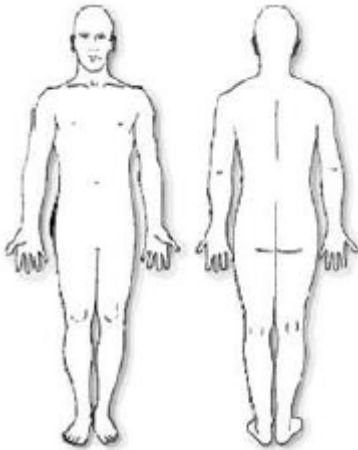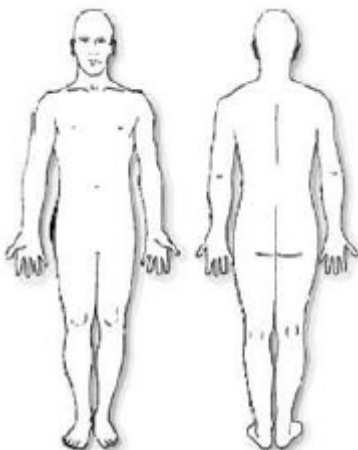|	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

If no/resolved (when): _____

7. Other pain (describe): _____

	Yes	**No**
Symptom present at onset:	_____	_____

If Yes,

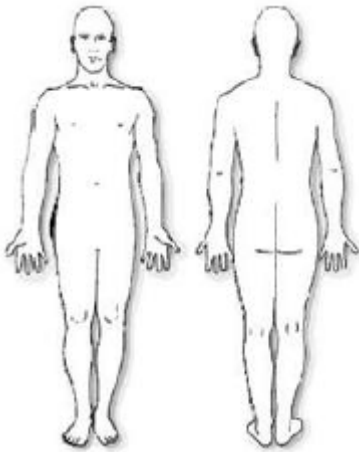|	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

	Persists	**Resolved**
Current nature of symptom:	_____	_____

If persists,

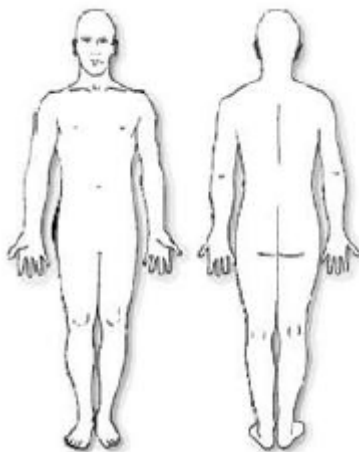|	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

If no/resolved (when): _____

8. Which of the seven descriptions of pain above troubles you most now: _____

Present pain (type): _____

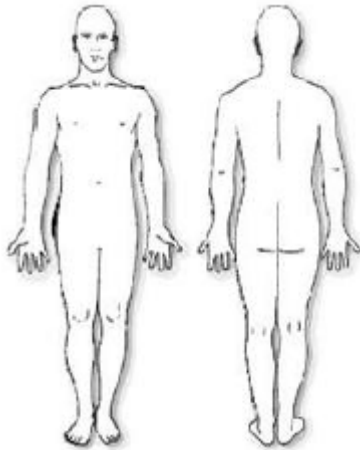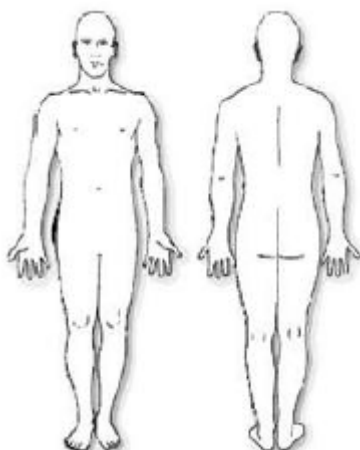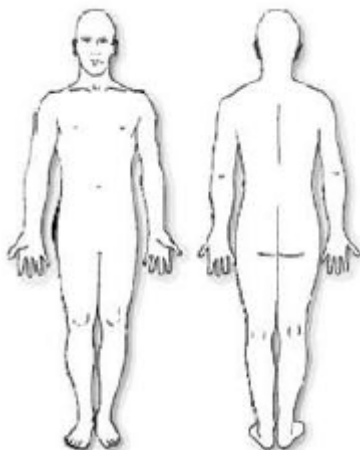|	Absent	Mild	Moderate	Severe
Never	0.00	0.00	0.00	0.00
Occ (<33%)	0.00	1.00	2.00	3.00
Often (<33, >66%)	0.00	1.33	2.33	3.33
Continuous (>66%)	0.00	1.66	2.66	3.66

Past medical history

(1= Yes, 2= No, 3= Don't Know)   If yes (Month, Year)

Have you ever suffered a heart attack?   _____

Have you had coronary bypass grafting?   _____

Do you suffer with diabetes mellitus?   _____

Have you ever had a stroke?   _____

Have you been told you have high blood pressure?   _____

Have you been told you suffer from kidney disease?   _____

Have you had problems like spinal cord compression?   _____

Have you been admitted to hospital before?

_____

_____

_____

_____

Family History
(Age, if passed away document cause of death)

Father:

_____

Mother:

_____

Brothers/Sisters:

_____

Current Medications

Chemotherapy (inc dexa):

_____

Immunosuppressive agents:

_____

Anti platelet agents:

_____

ACE inhibitors:

_____

Beta blockers:

_____

Calcium Channel Antagonists:

_____

Diuretics:

_____

Statins:

_____

Analgesia:

_____

Others:

_____

_____

Alcohol (Units/week):   _____

Have you ever consistently consumed more than 20 units/week: _____

Comments:

_____

_____

Smoking History

Current Smoker: _____

(1= Yes, 2= No, 3= Don't Know)

If no, when did you give up (month, year)   _____

How many cigarettes do/did you smoke per day: _____

How many ounces/week of tobacco do/did you smoke   _____

Physical Activity

Exercise physically impossible: _____

(1= >3x weekly, 2= 1-2x weekly, 3= 1-3x monthly, 4= hardly ever)

Hours per week

Minimal exercise (e.g. walking, household work)   _____

Light exercise (e.g. lawn mowing, swimming cycling)   _____

Heavy exercise (e.g. running, competitive sport)   _____

Physical Examination

Height (cm):   _____

Weight (Kg):   _____

Hip circumference (cm):   _____

Waist circumference (cm):   _____

Blood pressure (mm/Hg):   _____

Questionnaires   (1= Yes, 2= No)

NTSS-6   _____

NIS   _____

NPS   _____

EORTC QLQ C30   _____

EORTC MY20   _____

Pain Catastrophising   _____

HADS   _____

S-LANSS   _____

Quantitative Sensory Assessments

	JND	Percentile	ND
Cooling detection threshold	_____	_____	_____
Vibration detection threshold	_____	_____	_____
Heat pain	_____	_____	_____

Autonomic Function Tests

	RR	SD	BP
Resting supine	_____	_____	_____
Deep breathing	_____		
Valsalva	_____		
Standing	_____		_____

Nerve Conduction Studies

	Velocity	DL
SNAP/CMAP		
Radial	_____	_____	_____
Peroneal	_____	_____	_____
Sural	_____	_____	_____
Tibial	_____	_____	_____

Has the patient been given a pain diary: _____

Has the patient been instructed to stop meds: _____

Results

Hb: _____

Creatinine: _____   Total Protein: _____

eGFR: _____   Immunoglobulin: _____

Alb:Crt: _____   Plasma viscosity: _____

T Chol: _____

Appendix 5

Normal ranges for nerve conduction study

Normal values and ranges used to calculate the percentile abnormalities for Dyck's neuropathy composite score

Common Peroneal Nerve

Velocity (m/s): 53.32 ± 5.6

Amplitude (µV): 20.5 ± 6.1

Latency (ms): 2.9 ± 0.3

Tibial Nerve

Latency at ankle (ms): 4.28 ± 0.74

Sural Nerve

Amplitude (µV): >15 years old: 23.7 ± 3.8

Data shown as normal values ± standard deviation

m/s=metre/second; µV=micro volts; ms=milliseconds

Appendix 6

Definitions in context of this study

Clinical definitions

Nociception: processing and transmitting sensory information about noxious stimuli.

Neuropathic pain: 'pain arising as a direct consequence of a lesion or disease affecting the somatosensory system' as defined by the Neuropathic Pain Special Interest Group of the International Association for the Study of Pain (IASP).

Acute pain: Acute pain is short-term pain of less than twelve weeks duration that is temporarily related to injury and that resolves during the appropriate healing period.

Chronic pain: Chronic pain is continuous, long-term pain of more than 12 weeks or after the time that healing would have been thought to have occurred.

Neuroimaging

Functional MRI: is a technique based on the increase in blood flow that accompanies neural activity in the brain and obtains physiologic information about different parts of the brain; thus providing very accurate static anatomical maps and adds on additional dimensions.

Tesla (T): the unit of measurement quantifying the strength of a magnetic field.

Blood-oxygen-level dependent (BOLD): regional blood flow is valuable in studying brain function as it is closely related to neural activity and depends on the blood oxygenation level. In simple terms, BOLD is used as a contrast mechanism, when a region of the brain is activated there is increased blood flow in a specific area as well as increased use of oxygen and the difference in magnetic susceptibility of deoxygenated and oxygenated haemoglobin is detected as the BOLD signal.

T1-weighted magnetic resonance image: created typically by using short TE and TR times. T1-weighted images that appear dark are due to oedema, tumour, infarction,

inflammation, infection, hemorrhage (hyperacute or chronic) whilst those that appear bright are due to fat, paramagnetic substances such as gadolinium and copper.

T2-weighted magnetic resonance image: created typically by using longer TE and TR times. T2-weighted images that appear dark are due to calcification, fibrous tissue, paramagnetic substances such as deoxyhemoglobin, iron, ferritin, hemosiderin whilst those that appear bright are due to oedema, tumour, infarction, inflammation, infection, subdural collection.

The signal to noise ratio (SNR) is a measure of signal strength relative to background noise. The signal is the voxel brightness and the sources of noise in the image are the MR coils and the patient's body due to thermal motion. Incoherent noise appears on the image as irregular grainy patterns, which can degrade image details.

Axial: a plane, slice or section made by cutting the body or part of it at right angles to the long axis. If the body or part is upright, the cut would be parallel to the horizon.

Coronal: a plane, slice or section made by cutting across the body from side to side and therefore parallel to the coronal suture of the skull.

Sagittal: a plane, slice or section of the body cutting from front to back through the sagittal suture of the skull, and continued down through the body in the same direction, dividing it into two parts, then turning one half to view it from its cut surface.

Data analysis

Statistical Parametric Maps (SPM): images or fields with values that are, under the null hypothesis, distributed according to a known probability density function, usually the Student's *t* distributions known as *t* –maps.

Block design: paradigm based (in my study) on presenting the noxious stimuli sequentially within a condition, alternating this with a rest period.

General linear model (GLM): 4 components – observed data, design matrix, parameters (representing how well it fits the model at that voxel) and error (difference between the observed data and that predicted by the model). The GLM analyses each voxel using any statistical parametric test (t-test paired or un-paired, ANOVA - one-way, two-way, main effect, factorial, simple regression, linear regression, multiple regression, multivariate regression) and the resulting statistics are assembled into an image.

Spatial pre-processing: the aim is to reduce unwanted variance components in the voxel time-series that are induced by movement or shape differences among a series of scans.

Glass brain: Differences in activity may be represented as a 'glass brain', a representation of three outline views of the brain as if it were transparent. Only the patches of activation are visible as areas of shading.

Voxel: volume element represents a value (BOLD signal, density) and a location on a regular grid in 3D space. The MRI scanner produces a map of the brain area being scanned that is represented as voxels.

Z score: Statistical parametric maps have a value for a certain statistic at each voxel in the brain, which is the result of the statistical test done on the scan data for that voxel, across scans. This statistic is a Z statistic. Z scores are a way that SPM uses to display and analyse the p values from the t statistics. The Z scores are the numbers from the unit normal distribution (mean 0 sd/variance 1) that would give the same p value as the t statistic.

T statistic: tests the null hypothesis. The serial t-test defines a resting state baseline, and compares the images acquired at each time point before, during and after the stimulus (in this study heat-pain) with this baseline. For each time point following the heat-pain stimulus, a mean and standard deviation image is constructed, as is a

baseline mean and standard deviation image. Then a set of t-statistical parametric maps are produced by calculating, on a pixel-by-pixel basis, the t-score (difference between mean image one and the mean baseline image, mean image two and baseline, and so on).

Family-wise error (FWE) correction: controlling the chance of any false positives (type 1 errors). The standard conventionally dictates a statistic is significant if it is less than 5% likely to occur by chance: a p-threshold of 0.05. In imaging, each voxel in the brain represents a separate test; therefore thousands of tests for a given subject are being performed. If the conventional p-threshold of 0.05 is applied on a voxelwise basis, then, just by chance hundreds of false-positive voxels will appear. In order to avoid any false positives, p-threshold is corrected to account for how many tests being performed. The standard approach to FWE correction is the Bonferroni correction - dividing the desired p-threshold by the number of tests, and maintaining correct control over the FWE rate. The Bonferroni correction needs all the tests to be independent from each other and assumes there are more spatial degrees of freedom than there really are.

Degrees of freedom (df): the number of values in the final calculation of a statistic that are free to vary. It is the difference between the number of observations or sample size and the number of parameters estimated.

Random-field theory (RFT): body of mathematics defining theoretical results for smooth statistical maps. It attempts to control the FWE rate by assuming that the data follow certain specified patterns of spatial variance; the distributions of statistics mimic a smoothly varying random field. RFT corrections work by calculating the smoothness of the data in a given statistic image and estimating how unlikely it is that voxels with particular statistic levels would appear by chance in data of that local smoothness.

Talairach co-ordinates: based on definition by Talairach and Tournaeux atlas and is the most commonly used system for reporting coordinates in neuroimaging. Talairach brain is the brain dissected and photographed for the Talairach and Tournoux atlas (1988). Therefore, images obtained from SPM are described in MNI and so are then converted to Talairach co-ordinates.

MNI: The Montreal Neurological Institute defined a new standard brain by using a large series of MRI scans on normal controls. The SPM programme uses standard brains from MNI.