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Investigating Chemotherapy Induced Peripheral Neuropathy (CIPN) and its treatment, using functional Magnetic Resonance Imaging (fMRI).

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Preface

I Marta Seretny, author of this thesis declare that:

(a) this thesis has been composed by the me, and
(b) that this work is my own, and
(c) this work has not been submitted for any other degree or professional qualification except as specified, and
(d) included publications are my own work, except where indicated throughout the thesis.

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Abstract

Background

Chemotherapy Induced Peripheral Neuropathy (CIPN) is a debilitating neuropathy caused by commonly used chemotherapeutics. Clinically, the problem of CIPN is compounded by difficulties with diagnosis and limited treatment options. The pathophysiology of CIPN remains elusive, with current mechanistic postulates focused mainly on the peripheral nervous system. However, animal and human models of non-CIPN neuropathic conditions have shown the brain to be central to the development and maintenance of painful neuropathy. Moreover, evidence suggests that aberrant activity in key regions of the brain and brainstem could denote individual vulnerability for chronic pain states. The impact of the brain on CIPN development is unknown. Assessment of drug efficacy using brain imaging can provide sensitive readouts and is increasingly used in clinical trials.

Aims

Firstly, to prospectively explore the structure and function of the brain in cancer patients prior to chemotherapy administration, using functional magnetic resonance imaging (fMRI), in order to determine whether baseline differences exist between patients who progress to CIPN as compared to those who do not. Secondly, to develop a pilot study using fMRI to investigate a topical treatment for CIPN, in order to assess the feasibility of setting up a study with this kind of design.

Methods

To address the first aim of this thesis a prospective cohort study (the CIPN fMRI Study) was developed. Cancer patients scheduled to receive neurotoxic chemotherapy treatment including oxaliplatin, carboplatin, carbotaxol, or cisplatin, were recruited from three NHS trusts in Scotland, to undergo a high resolution (3 tesla) functional MRI scan, at a single time point prior to

commencement of chemotherapy. During the scan structural, resting state and functional data were collected. Functional data involved the presentation of punctate stimuli (using a 256mN von Frey filament), above the patients' right medial malleolus. While receiving the punctate stimuli, patients viewed images that had neutral or positive emotional content or a baseline coloured image with no content. Sample size was based on previously successful pain fMRI studies and pragmatic estimates. Acute CIPN was defined clinically by common toxicity criteria as necessitating a chemotherapy dose reduction or cessation. Data were analysed using FMRIB's Software Library (FSL) version 5, 2015. Standard data pre-processing (brain extraction, registration, B0 unwarping, motion correction, and denosiing with FIX) was carried out. Structural analysis was conducted using FIRST. Resting state analysis utilised FSL's MELODIC tool, and a non-parametric group comparison was made following a dual regression approach. FEAT was used for both first and second level functional analyses. Group comparisons were made using a mixed effects analysis (z threshold 2·3 and 2, regions considered significant at $p<0·05$, cluster corrected). The group was split by sex to explore known sex differences in pain processing. To address the second aim of this thesis, a pilot fMRI randomised controlled trial (MINT3 Study) was designed. Approvals from ethics and research and development were sought and obtained. Data collection forms were developed. An fMRI experiment was proposed and a single pilot scan was conducted and analysed.

Results

30 patients were recruited for the CIPN fMRI study (mean age 60·4 years, [95% Confidence Interval: 57.4-63.4, 17 women). Two patients had lung cancer, nine had gynecological malignancies and 18 had colorectal cancer. 17 patients developed acute CIPN. Structural analysis showed that patients who developed CIPN had a smaller volume of the Nucleus Accumbens (NAc). Resting state analysis did not show clear differences between those who developed CIPN and those who did not. Finally, functional analysis showed that patients who did not develop CIPN had greater activation in the superior frontal gyrus when viewing positive emotional images as compared to those who did progress to CIPN. Region of interest analysis showed that female patients who developed CIPN had

greater activity in their mesencephalic pontine reticular formation (MPRF). Male patients who progressed to CIPN had decreased activity in their thalamus. Feasability of the MINT3 study set up and fMRI paradigm was assessed.

Interpretation

Differences in brain structure and function are evident between patients who developed CIPN and those who did not. Crucially, the regions identified, in particular the NAc, have been postulated to denote a vulnerability for progression to pain states. Although the findings need further confirmation they suggest a paradigm shift in terms of CIPN as a clinical problem. Specifically, it appears that certain individuals can be considered as having increased risk of CIPN development prior to chemotherapy administration. This risk relates to the baseline structure, and function of their brains. Finally, the set up of the MINT3 fMRI study showed that this kind of study design is acceptable in terms of ethical and R&D approvals and a single healthy volunteer pilot.

Publications arising from this thesis

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1. **Seretny M,** Currie GL, Sena ES, Ramnarine S, Grant R, MacLeod MR, Colvin LA, Fallon M: Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis. *Pain. 2014 December; 155(12): 2461-70*

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2. **Seretny M**, Colvin L, Fallon M: Therapy for chemotherapy-induced peripheral neuropathy. *JAMA 2013 Aug 7;310(5): 537-8*

List of Abbreviations

ACC	anterior cingulate cortex
ACCORD	Academic and Clinical Central Office for Research and Development
ACTTION	Analgesic, Anaesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks
AE	adverse event
AMP	adenosine monophosphate-activated
ANOVA	analysis of variance
ASL	arterial spin labelling
B_0	constant magnetic field in a given magnetic resonance system
BBR	Boundary Based Registration
BET	Brain Extraction Tool
BMI	Body Mass Index
BOLD	blood oxygen level dependent
BPI	brief pain inventory
CI	confidence interval
CI	chief investigator
CIPN	Chemotherapy induced peripheral neuropathy
CNS	central nervous system
COPEs	corrected parameter estimates
CRFs	case report forms
CRIC	Clinical Research Imaging Centre
CRPS	complex regional pain syndrome
CRUK	Edinburgh Cancer Research UK Centre

CS	Central sensitisation
DeOxyHb	deoxygenated haemoglobin or deoxyhaemoglobin
DH	dorsal horn
DPMS	Descending Pain Modulatory System
dlPFC	dorsolateral prefrontal cortex
DMN	default mode network
DN	diabetic neuropathy
DNA	Deoxyribonucleic acid
DRG	dorsal root ganglion
DRT	dorsal reticular nucleus
DSST	Digital Symbol substitution Test
ECTU	Edinburgh Clinical Trials Unit
EudraCT	European Union Drug Regulating Authorities Clinical Trials Database
EORTC	The European Organization for Research and Treatment of Cancer
EPI	echo planar imaging
FIX	FMRIB's ICA-based Xnoiseifier
fMRI	Functional magnetic resonance imaging
FMRIB	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain
FOV	field of view
FSL	FMRIB Software Library
GCOS	General Causality Orientation Scale
GLM	general linear modelling
GMV	grey matter volume
GWAS	genome wide association studies
HADS	Hospital Anxiety and Depression Scale
Hb	haemoglobin

HRF	haemodynamic response function
IASP	International Association for the Study of Pain
IAPS	International Affective Picture System
IC	independence component
ICA	independent component analysis
ICH GCP	International Conference on Harmonisation Good Clinical Practice Guidelines
IMMPACT	Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials
ISRCTR	International Standardised Randomised Controlled Trials Registry
LTF	lost to follow up
MAO	monoamine oxidase
MELODIC	Multivariate Exploratory Linear Optimized Decomposition into Independent Components
MeSH	medical subject headings
MDASI	MD Anderson Symptom Inventory
MINT3	Menthol IN Treatment
MHRA	Medicines and Health Products Regulatory Authority
MNI	Montreal Neurological Institute
MPRF	mesencephalic pontine reticular formation
MRI	magnetic resonance imaging
NAc	nucleus accumbens
NART	National Adult Reading Test
NCF	Nucleus Cuneiformis
NCT-CTC	National Cancer Institute – Common Toxicity Criteria
NES	Neurological examination
NHS	National Health Service

NPS	Neurophysiological examination
NR	not reported
NTS	nucleus tractus solitarius
OxyHb	oxygenated haemoglobin or oxyhaemoglobin
PAG	periaqueductal grey
PBN	parabrachial nucleus
PCC	posterior cingulate cortex
PCS	Pain Catastrophizing Scale
PET	position emission tomography
PI	principle investigator
PNM	physiological noise monitoring
PNS	peripheral nervous system
PoC	proof of concept
PS	Peripheral sensitisation
QST	quantitative sensory testing
RCT	randomised controlled trial
R&D	Research and Development
RF	radiofrequency
ROI	region of interest
RSN	resting state network
RVM	rostraventral medulla
SEQ	side effects questionnaire
SFG	superior frontal gyrus
SNPs	single nucleotide polymorphisms
SNR	signal to noise ratio
SPM	Statistical Parametric Mapping
STROBE	Strengthening the Reporting of Observational studies in Epidemiology

T1	recovery time
T2	signal decay time
T2*	T2 star
TE	echo time
TNSc	Total Neuropathy Score
TR	repetition time
TRP	transient receptor potential
TRPM8	Transient Receptor Potential Cation Channel, Subfamily M, Member 8
TSC	Trial Steering Committee
VAS	visual analog scale
VBM	Voxel based morphometry
WBV	whole brain volume

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1. Introduction

Chemotherapy Induced Peripheral Neuropathy (CIPN) is a neuropathic condition affecting the increasing number of cancer survivors. Insight into why some patients develop CIPN and others do not, is lacking. Treatments for established CIPN are limited. The main aim of this thesis is to investigate the development of CIPN and establish pilot work to assess a novel treatment for this condition. This introductory chapter describes pain and its mechanisms, with a focus on neuropathic pain and the issues related to its treatment. Subsequently, CIPN and the postulated pathophysiological mechanisms underpinning its development are discussed. Key questions, which remain unanswered in CIPN, are highlighted. A description of why functional magnetic resonance imaging (fMRI) is a useful tool for understanding CIPN, and a brief description of how fMRI works, follows. The chapter concludes with an outline of the aims and research questions of this thesis, and a thesis overview.

1.1. Background

1.1.1 Pain

The International Association for the Study of Pain (IASP) defines pain as:

> "An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage."(IASP, 2011)

This definition highlights the complex interplay of somatosensory, cognitive and emotional factors in the maintenance and generation of acute and chronic pain (Colvin and Fallon, 2011). At a systems level pain can be described in terms of a physiological progression from peripheral input, via the somatosensory system to central processing within the brain (Fig 1.1). Although there are multiple components of the pain processing system (described subsequently), one of its key features is its dynamic plasticity (Kuner, 2010). This plasticity is central to both the adaptive and maladaptive pain response.

The somatosensory system - made up of a continuum of peripheral nociceptors, primary afferent neurons, and the dorsal root ganglia - is the principal pathway by which peripheral information is conveyed to the central nervous system. Nociceptors are heterogeneous cells, typically found in the skin and walls of organs, which respond to chemical, thermal and mechanical stimulation. They express an array of receptor types including ligand gated ion channels, free ion channels and G-Protein coupled receptors (Reichling et al., 2013).

Mechanical and thermal nociceptors act as transducers. Specifically thermal pain is transduced via specialised transient potential (TRP) ligand gated ion channels. Mechanical transduction is still being elucidated and might involve multipass transmembrane proteins called piezos (Reichling et al., 2013). Chemical nociception occurs via direct simulation of acid sensing ion channels, and other ligand gated receptors and G-Protein coupled receptors. Following nociceptor stimulation depolarisation of the afferent neuron occurs.

An important function of nociceptors is their ability to alter their responsiveness through sensitisation (Reichling et al., 2013). This ability to enhance responsiveness or decrease the threshold needed for depolarisation is key to the plasticity observed in the pain pathway. The molecular mechanism by which nociceptive sensitisation occurs involve two secondary messenger signalling pathways involving AMP/protein kinase A and protein kinase C interacting with calcium, sodium and potassium ion channel families (Reichling et al., 2013).

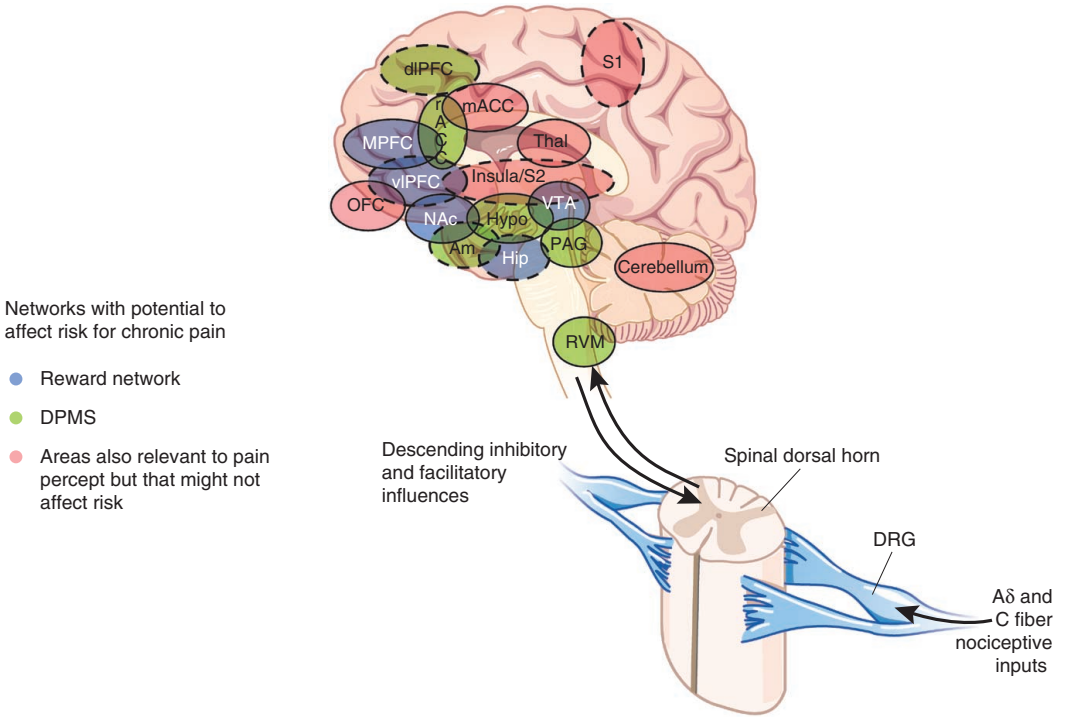


Fig 1.1: The pain pathway. Noxious stimuli are converted by specialized receptors (nociceptors) to electrical signals and conveyed via two nerve fibre types (A-Delta and C) to the dorsal horn of the spinal cord. Prior to entering the spinal cord the impulse passes through the dorsal root ganglion (DRG). Plasticity, known to be important in conversion from acute to chronic pain states, is demonstrable in the entire primary afferent nociceptor complex. Changes can occur anywhere from the neuronal terminals, through to axons and the DRG. Signal is then transmitted up to the brainstem and brain where it is processed by the thalamus, insula, anterior cingulate cortex and somatosensory cortices amongst many other cortical and subcortical regions. Descending signal, which can facilitate or inhibit pain perception is generated in regions denoted here in green. Figure adapted from (Denk et al., 2014) .

Two types of nerve fibres are involved in pain signal transmission C and A-Delta. A-Beta fibres play a role in development of allodynia (see later) but are not classified as part of the pain neuro-axis. These contain nociceptors, which after activation initiate depolarization. Primary afferent neurons synapse in the dorsal horn of the spinal cord. Prior to entering the spinal cord the nerve impulse passes through the dorsal root ganglion. The ganglion is made up of afferent nerve cell bodies. This is a key area where dynamic changes occur and pain

processing may alter from acute to chronic states, including progression to neuropathic pain states (see 1.1.1.1).

The circuitry of the dorsal horn is complex and understanding of the exact connections is continually evolving see figure 1.2 for diagrammatic representation (Todd, 2010). Broadly, within the dorsal horn the primary afferent neuron synapse occurs in one of six laminae (Rexed's laminae). Lamina I and II receive input from A –Delta fibres and C fibres. These laminae contain cells specific to nociceptive input, as well as cells able to respond to both nociception and innocuous stimuli (Spoors and Kiff, 2010). Specifically, within laminae I A-Delta fibres synapse with projection neurons and some small interneurons, which contribute to the spinothalamic tract. Projection neurons cross the midline and ascend, relaying information up to the thalamus and then onto the somatosensory cortex (Todd, 2010). C fibres synapse within laminae II (substantia gelatinosa). Signal is conveyed from this laminae to laminae I, IV, V, by small interneurons which then join the spinothalamic tract. Additionally, axons of these neurons decussate to ascend in the contralateral spinothalamic and spinoreticular tracts to reach the brainstem, thalamus and somatosensory cortex (Spoors and Kiff, 2010). Lamina III through to VI mostly receive innocuous input from A-Delta and A- Beta fibres. However, this region, like other laminae in the dorsal horn, can undergo dynamic changes leading to chronic pain states, these changes are only now becoming more clearly understood (see 1.1.1.1)(Levine et al., 1993).

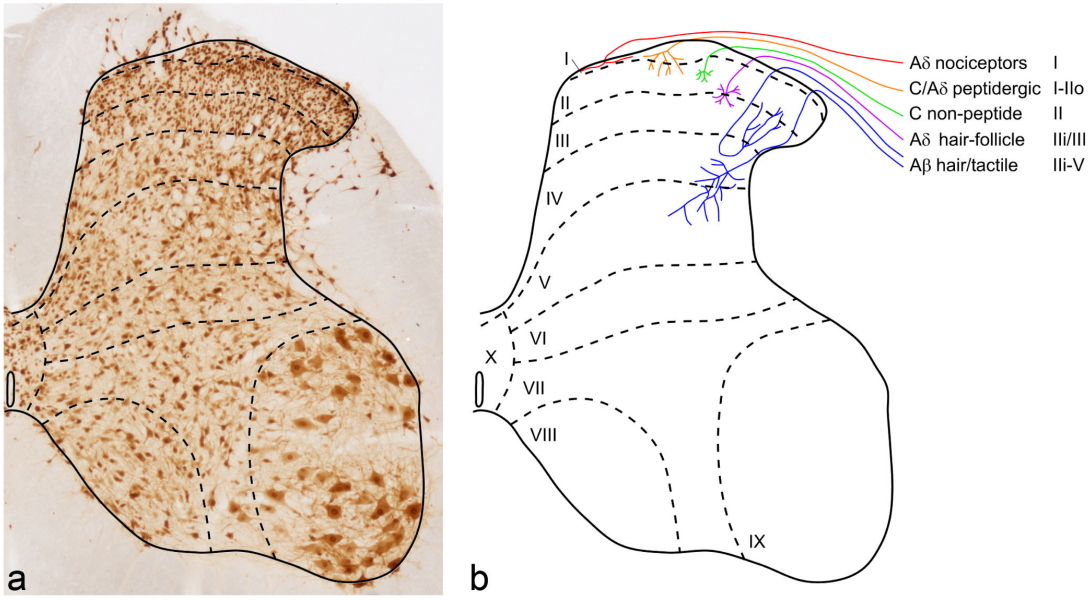


Figure 1.2 Organisation of the Dorsal Horn. Left hand panel (a) showing organisation of the mid lumbar rat spinal chord. Panel on right (b) showing a diagrammatic depiction of the stained slide, dashed line denoting laminae with details of nerve fibres synapsing in each. Figure adapted from (Todd, 2010).

Various spinal tracts propagate pain impulses via the brainstem up to the brain. It is important to note that naturally occurring pain modulation occurs at the level of the brainstem, dorsal horn and also via the endogenous opioid system. Although descending modulatory pathways originate in multiple regions of the cortex and brainstem, a number of brainstem nuclei warrant special mention. Specifically the periaqueductal grey (PAG), rostraventral medulla (RVM), nucleus tractus solitaries (NTS), parabrachial nucleus (PBN), and the dorsal reticular nucleus (DRT) are of key importance in descending modulation, due to their bidirectional communication with cortical, and subcortical structures as well as the dorsal horn (fig 1.3) (Dunckley et al., 2005, Saade and Jabbur, 2008). Fibres from these nuclei descend to the dorsal horn where they exert either presynaptic or postsynaptic inhibition. Presynaptic inhibition consists of blockage of calcium channel opening and decreased neurotransmitter release. Post synaptic inhibition hyperpolarizes neurons via potassium channel opening (Spoors and Kiff, 2010). A key modulator of these changes is substance P acting on the neurokinin 1 receptor. Brainstem nuclei also connect to higher brain regions. Endogenous opioids act both at the brainstem level and at the dorsal horn level,

where they cause membrane hyperpolarization and calcium channel inhibition. Aberrant descending facilitation and inhibition has been shown in both human and animal experiments to be a key factor contributing to the generation and maintenance of mechanisms such as central sensitisation, relevant in chronic (in particular neuropathic) pain states (Zambreanu et al., 2005, Lee et al., 2008, Yarnitsky et al., 2008, De Felice et al., 2011). Additionally, modulation at brainstem level allows pain signals to be integrated with autonomic, homeostatic and arousal processes allowing the signal to be conveyed as coherent whole to higher cortical centres (Tracey and Mantyh, 2007).

Beyond the brainstem the hippocampus, amygdala, cerebellum and thalamus are involved in nociceptive signal transmission. The thalamus appears to serves a specific role as a relay centre. Similarly to the brainstem areas described above thalamic nuclei have a bidirectional spinal and supraspinal connectivity that enables varied nociceptive transmission to higher centres (Tracey and Mantyh, 2007).

Higher centres involved in pain processing are heterogeneous and reflect the complex and subjective nature of the pain experience. Key regions activated in human and animal pain studies include, but are not exclusive to, the primary and secondary somatosensory cortex, amygdala and the different sub-regions of the insula cortex, anterior cingulate cortex (ACC), and prefrontal cortex. These regions are not unique to pain processing and their pattern of activation varies depending on the specific individual context of the pain experience (Lee and Tracey, 2013). Consequently, to date no single ‘pain’ region akin to the visual cortex has been identified, highlighting the complexity and individual nature of the pain pathway.

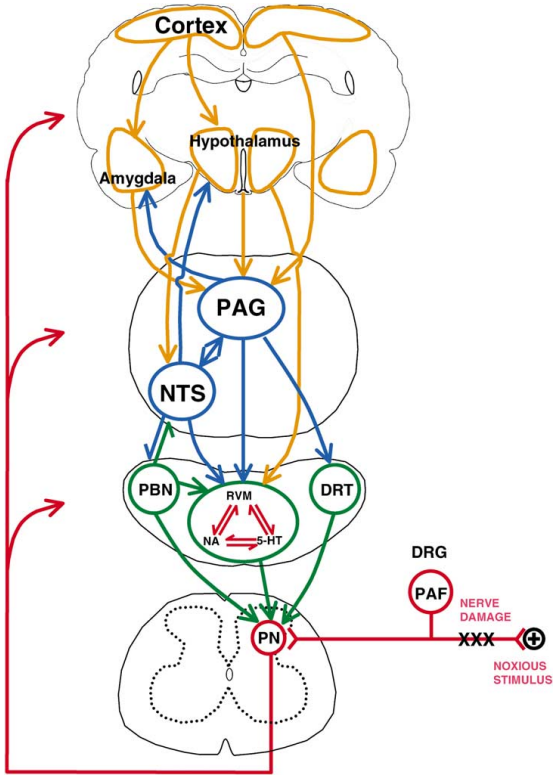


Fig 1.3 Descending Pathways involved in the Control of Pain. Cortical and subcortical structures (such as the amygdala and hypothalamus), along with brainstem nuclei govern descending inhibition. Some brainstem nuclei: RVM, NTS and less so PAG communicate directly with the dorsal horn of the spinal cord. These are key regions that have been suggested as important in the development and maintenance of neuropathic pain. Figure adapted from (Millan, 2002).

1.1.1.1 Progression from Acute to Chronic Pain

Physiologically, pain serves an important evolutionary role in species survival. Pain indicates impending or on going tissue damage and drives behaviour modification aimed at limiting and or avoiding this. Clinically, acute pain can be defined by it temporal and causal relationship with tissue injury or disease (Spoors and Kiff, 2010). In contrast chronic pain, which persists beyond the period of tissue injury and healing, is maladaptive. Progression from acute to chronic pain remains incompletely understood but is a field of active research. What is clear is that a complex interplay of factors at peripheral, spinal and supraspinal level lead to changes which maintain and exacerbate chronicity.

Chronic pain states can be sub-classified into inflammatory pain, neuropathic and idiopathic or functional pain (Costigan et al., 2009). Inflammatory pain results from tissue injury and the ensuing inflammatory cascade, as in for example rheumatoid arthritis. In contrast dysfunctional pain, such as fibromyalgia occurs in the absence of any (as yet) identifiable nociception, tissue or nervous system damage or inflammation(Costigan et al., 2009). Neuropathic pain (as discussed in detail below) results from lesions or disease in the peripheral and or central nervous system (IASP, 2011). Although the aetiology of these chronic pain subtypes differs, some of the mechanisms underpinning the changes leading to chronicity are shared. These are outline below and discussed subsequently in the context of neuropathic pain.

1.1.1.1.1 Central Sensitization

Central sensitization (CS) is defined as the increased responsiveness of central nociceptive neurons to their normal or sub-threshold afferent input (IASP 2011). The processes that underpin CS include; alterations in synaptic modulators, increase in excitatory amino acids, and changes in ion channel architecture, density and kinetics (Costigan et al., 2009). These result in increased synaptic strength, with central nociceptors becoming and remaining more reactive to sub-threshold input. Importantly, afferents from areas discreet from initial stimuli are co-recruited, increasing the receptive area of the central nociceptors.

An important phenomenon, which can feature as part of CS, but is also a cause of it, is known as 'wind up'. Wind up is the continued increase in response to a series of repeated stimuli (Latremoliere and Woolf, 2009, Woolf, 1983). The key fibres involved are C fibres and the resulting increase in output despite an unchanging input is an important manifestation of CS in chronic pain states (Herrero et al., 2000).

1.1.1.1.2 Peripheral Sensitization

Peripheral sensitization (PS) similarly to CS is a heightened responsiveness of the peripheral nervous system to normal or sub-threshold stimuli. The mechanisms driving these changes relate to mediators up regulating intracellular transduction pathways, resulting in the increased production and

insertion of nociceptor proteins into peripheral nerve terminals (Costigan et al., 2009). This leads to fewer stimuli causing activation and general hyper-responsiveness of the nerve.

1.1.1.1.3 Influence of Immune Mediators

Immune mediators generated by immune cells such as bradykinin, nitrous oxide, interleukins and tumour necrosis factor stimulate peripheral nociceptors directly (Grace et al., 2014). These mediators increase in response to both inflammation and neuronal damage. They play a key role in the generation and propagation of inflammatory and neuropathic pain. Various immune mediators also influence central changes. Injured glia release cytokines and chemokines promoting central sensitisation and central driver of chronic pain development (Ji et al., 2014).

1.1.1.2 Neuropathic Pain

IASP defines neuropathic pain as: "Pain caused by a lesion or disease of the somatosensory nervous system" (IASP, 2011). Neuropathic pain may affect the CNS, peripheral nervous system (PNS) or both. Neuropathy is defined by IASP as: "A disturbance of function or pathological change in a nerve: in one nerve, mononeuropathy; in several nerves, mononeuropathy multiplex; if diffuse and bilateral, polyneuropathy" and, or neuritis "a special case of neuropathy, caused by processes affecting nerves". Neuritis and neuropathy may be a part of but is not a prerequisite for neuropathic pain states.

Changes governing the development of neuropathic pain involve CS, PS and reactivity to inflammatory mediators as described above (see 1.1.1.1.1 to 1.1.1.1.3). These occur in response to neuronal damage of varied aetiology. Alterations in the molecular architecture and function of peripheral nerves, the dorsal root ganglion (DRG), dorsal horn (DH), glia and CNS all play a part in neuropathic pain development and propagation. Importantly, once these changes take place, they typically persist well beyond the duration of the etiological cause (e.g.: surgical nerve damage or herpetic infection).

Consequently, it has been proposed that general neuropathic pain mechanisms rather than etiological factors should be the focus of research aimed at progressing diagnosis and treatment of neuropathic pain (Costigan et al., 2009, Colvin and Dougherty, 2014). However aetiological understanding of neuropathic pain enables development of preventive strategies and preventive drugs. This is in contrast to mechanistic insights, which allow for drug target identification. Arguably therefore, both etiological and mechanistic research is needed to allow for a holistic approach to the prevention, diagnosis and treatment of neuropathic pain states. Indeed recent guidelines suggest using more detailed measures to enable clearer characterisation of etiological factors in order to better delineate responder profiles for drug targets (Attal et al., 2010, Haanpaa et al., 2011)

1.1.1.2.1 Problems with Neuropathic Pain Treatments

Current neuropathic pain treatments include antidepressants, antiepileptic medications and a number of topical agents including lidocaine and capsacin (Dworkin et al., 2007). Unfortunately, most of these treatments have significant limitations. The side effect profile suffered by many patients receiving oral anti-neuropathic pain drugs is broad. Moreover, systematic reviews investigating the efficacy of neuropathic pain treatments suggest limited effectiveness (Wiffen Philip et al., 2013). Indeed in the case of carbamezapine there is insufficient data to draw any concrete conclusions regarding benefit (Wiffen Philip et al., 2014). This pattern of modest effect is also applicable to antidepressants, topical analgesics and tramadol in neuropathic pain (Saarto and Wiffen Philip, 2007, Derry et al., 2013, Duehmke Rudolf et al., 2006).

The reasons for these limitations are multifold, reflecting problems with how analgesics are tested, their effectiveness measured and how they are chosen in a clinical setting. Specifically, response to neuropathic analgesics is varied amongst neuropathic pain sufferers. Despite this, data from non-responder and responders in clinical trials, are often pooled in statistical summaries, leading to skewed results. Standardized methodological guidance has attempted to address this (see 1.1.1.2.2). Secondly, measurement of neuropathic pain, particularly in a

time limited clinical setting remains non-standard. This results in varied intensity of pain being treated with possibly non-optimal drug regimens. Finally, understanding of how the various maladaptive changes in the pain pathway lead to the clinical pain response continues to evolve. However, translation of this understanding into a unified treatment regimen is lacking and has necessitated a trial and error approach to treatment implementation. This has also contributed to the moderate clinical efficacy of neuropathic analgesic strategies.

1.1.1.2.2 Challenges with Neuropathic Treatment Trials

Assessment of novel drugs for neuropathic pain is marred with difficulties. Specifically, the subjective, varied nature of individual pain and the influence of an active placebo response, results in analgesic randomized controlled trials (RCTs) often describing small effect sizes, difficult to interpret clinically (Moore et al., 2010, Quessy and Rowbotham, 2008, Dworkin et al., 2010). Moreover, measurement tools used to assess pain - including quantitative sensory testing (QST), a non-invasive method of assessing pain and sensory dysfunction in peripheral nerves (see 3.1), and pain questionnaires - are often inadequate at standardizing pain experiences in the context of neuropathic analgesic RCTs (Attal et al., 2011, Maier et al., 2010). The culmination of these influences has resulted in clinically inconclusive outcomes from many large, robustly designed analgesic clinical trials (Moore et al., 2013).

To address these problems with pain RCTs the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT) was set up (Dworkin et al., 2005). The initiative has drawn up evidence-based guidelines and written reviews aimed at optimizing the design and execution of pain trials. Additionally, recognizing the need to translate basic mechanistic findings in pain research into effective treatments more promptly and successfully, the United States Food and Drug Administration (FDA) has also launched a new partnership to optimize the design and efficiency of analgesic clinical trials (Dworkin and Turk, 2011). The partnership has incorporated the efforts of IMMPACT and the later now works under the auspices of Analgesic, Anaesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks (ACTTION).

It has also been recognized that use of new research tools, such as functional magnetic resonance imaging (fMRI), as adjuncts in pain RCTs may also help improve the effectiveness of assessing novel analgesic medications (Wise and Tracey, 2006, Schwarz et al., 2011, Wanigasekera et al., 2016) (see 1.1.2.5).

1.1.2.What is Chemotherapy Induced Peripheral Neuropathy (CIPN)

Chemotherapy induced peripheral neuropathy (CIPN) is a debilitating condition resulting from chemotherapy treatment. The incidence of CIPN is around 40% (Gutierrez-Gutierrez et al., 2010). Estimated prevalence ranges from 60% within the first month of chemotherapy, to 30% six months after treatment completion (Seretny et al., 2014). With a postulated 5 million cancer survivors worldwide, the problem of CIPN is an important healthcare concern (Cancer Research UK, 2014). CIPN can manifest with or without severe pain.

CIPN affects patients receiving treatment for multiple cancer types including: colorectal, testicular, breast, lung, ovarian, and haematological malignancies(Park et al., 2013). Implicated chemotherapy types include taxanes (e.g. Paclitaxel), vinca alkaloids (e.g. vincristine), platins (e.g. Oxaliplatin), alkylating agents (e.g. Procarbazine), thalidomide, bortezomib, as well as other newer drugs some of which remain experimental (e.g. Cetuximab and Suramin). Many of these agents cause CIPN at standard dose while others require higher treatment quantities (Weimer, 2013).

1.1.2.1 Clinical Presentation

Onset of CIPN is clinically insidious, with subtle neurological changes abruptly progressing to symptoms arising from large sensory nerve fibres. Motor and autonomic nerve involvement is less common and often results from use of specific drug types including thalidomide for motor and vincristine for autonomic symptoms (Cavaletti et al., 2011a). Sensory symptoms include paresthesia (pins and needles), allodynia (pain following non-painful stimuli) especially to cold, hyperalgesia (increased pain following painful stimuli) and numbness (Park et al., 2013, Fallon, 2013). In some cases pain may not be a

major presenting feature of CIPN. Distribution of symptoms is symmetrical, principally in the hands and feet, reflecting a 'glove and stocking' presentation. Sensory symptoms may exist without associated pain. However, once present symptoms often limit chemotherapy dose and sometimes require complete cessation of chemotherapy treatment (Cavaletti et al., 2011a). This has implications for patient morbidity and mortality.

Due to the seemingly 'all or nothing' presentation of CIPN, diagnosis remains elusive. Difficulties in diagnosing CIPN are compounded by a non-standardized clinical approach (Cavaletti, 2012). CIPN assessment methods include an assorted combination of: physical examination, detailed neurophysiological testing and use of multiple diagnostic scales with varying degrees of sensitivity and specificity (see 2.1.1). Consequently, CIPN development has remained inadequately characterised and understood. Recently however, there have been important efforts to standardize the approach to CIPN diagnosis (Cavaletti et al., 2013).

The clinical course of CIPN is varied. Broadly, CIPN may present early in the course of treatment or after multiple chemotherapy doses. Equally, in some cases CIPN symptoms may not become apparent until after chemotherapy cessation, in a phenomenon known as 'coasting' (Cavaletti et al., 2011a). It is clear that chemotherapeutic-drug specific characteristics of CIPN exist (Park et al., 2013). For instance, oxaliplatin induced neuropathy has a distinct acute presentation (Argyriou et al., 2012b), with a variable progression to chronic CIPN. In contrast, bortezomib related CIPN presents with a distinct small fibre neuropathy, which is often reversible after treatment cessation (Dimopoulos et al., 2011, Park et al., 2013).

Clinically the terms acute and chronic CIPN have been adopted. Acute CIPN refers to CIPN occurring during chemotherapy treatment, whilst chronic CIPN denotes the condition continuing after chemotherapy has ceased. It appears that the pathophysiology underpinning these presentations is varied and is likely, as discussed above, drug dependent (Addington and Freimer, 2016). There is

evidence that in the paediatric population acute vincristine related CIPN is linked to genetic factors (Diouf et al., 2015).

A number of risk factors for CIPN have been postulated, however there is no overall consensus as to their importance. Risk also varies according to chemotherapy type with treatment duration, cumulative dose and single dose administration all inducing different risk(Park et al., 2013). Some of the non-chemotherapy related risk factors include: factors predisposing to other neuropathies (alcohol excess, diabetes mellitus, smoking), sensory changes during chemotherapy treatment, and genetic status (Seretny et al., 2014). The majority of these risk factors are derived from statistical propensity score modelling, with likely influences of bias (see 2.2).

In summary, extensive variance in the clinical course of CIPN has been observed. This partly reflects the non-standardized approach to its diagnosis (see 2.1.1). However, another important consideration in this clinical variation, are the heterogeneous mechanisms by which different chemotherapeutics impact on peripheral nerves (Weimer, 2013).

1.1.2.2 Postulated Pathophysiological Mechanisms

Mechanisms underpinning the pathophysiology of CIPN have been investigated at genetic, molecular and cellular level (fig 1.4) (Cavaletti et al., 2011b, Argyriou et al., 2013, Park et al., 2008, Robinson et al., 2014). No one clear pathophysiological pathway leading to CIPN development has been described. Reported mechanisms most likely reflect aspects of a complex, multistage pathophysiological process or set of processes. Mechanisms documented to date are varied and likely dependent not only on the actions of specific chemotherapy drugs, but also the interactions of concomitant medications and underlying neoplastic processes. Therefore the aim here will be to provide an overview of the known genetic, molecular and cellular changes associated with CIPN development, and where possible broadly describe how these may link to the clinical presentation discussed above.

1.1.2.2.1 Genetic Mechanisms

Multiple single nucleotide polymorphisms (SNPs) have been associated with increased incidence of CIPN (Cavaletti et al., 2011b). Some SNPs have been replicated in repeat studies others have not (Custodio et al., 2014). The lack of reproducible associations likely reflects the heterogeneous patient populations studied as well as varied technical approaches to assessing SNPs (Cavaletti et al., 2011b). Importantly, the variety of proteins related to the many identified SNPs highlight the complexity and diversity of mechanisms underpinning CIPN development. To date proteins associated with identified SNPs include those related to DNA repair, cell cycle progression, multidrug efflux pumps as well as enzymes that catalyse detoxification reactions (e.g. conjugation of hydrophobic compounds with glutathione)(Cavaletti et al., 2011b, Custodio et al., 2014). Additionally, ion channel proteins, proteins involved in: neuronal development, Schwann cell function, inflammation and immunity, mitochondrial actions, as well as apoptosis have all been associated with CIPN related SNPs.

It is clear that some of the above heterogeneity is due to varied methodological approaches to genome wide association studies (GWAS). Therefore prior to genetic data being routinely used clinically to individualize chemotherapy regimes, methods and populations will need to be standardized in order to avoid spurious associations governing clinical decision.

1.1.2.2.2 Molecular & Cellular Mechanisms

Knowledge regarding the molecular and cellular mechanisms underlying, CIPN has come from animal models developed over the last 20 years (Authier et al., 2009). Injection of mice and rats with CIPN inducing chemotherapeutics has allowed characterisation of neuro-pathological (e.g. axonal swelling), neurophysiological (reduced conduction velocities, reduced action potentials), altered nerve fibre density and behavioural changes caused by these agents (Hoke, 2012). More recently use of genetic knock out species has also enabled the elucidation of receptor pathways in CIPN development (Authier et al., 2009). In short animal models have provided the description of the pathways detailed

below. These models continue to be refined in order to more clearly translate animal findings to clinical realities (Hoke, 2012).

Most of the chemotherapeutics causing CIPN affect molecular pathways, in turn leading to cellular changes. However, even when the anti-cancer mechanisms of chemotherapeutics are well understood, the actual pathways leading to neurotoxicity remain unclear and are based on postulates (Argyriou et al., 2012a, Miltenburg and Boogerd, 2014, Park et al., 2008). As an example this is true for even the most clearly described taxane and vinca alkaloid chemotherapeutics. These compounds interact with tubulin, a molecule integral to the formation and stability of microtubules- the architectural scaffolding of cells.

Taxanes prevent the destabilization of microtubules, a function important in axonal transport and cell division (Miltenburg and Boogerd, 2014). Even though neurons are not cells undergoing mitosis, the interference with anterograde axonal transport leads to axonopathy (Park et al., 2008). Secondary to this injury macrophages are activated peripherally and microglia centrally, leading to secondary inflammatory cytokine activation and further neuronal damage (Argyriou et al., 2012a, Miltenburg and Boogerd, 2014, Park et al., 2008). There is also evidence that taxanes exert a toxic effect on neuronal mitochondria, leading to inadequacies in axonal energy supply and subsequent sensory neuropathy (Flatters and Bennett, 2006).

Although like taxanes, vinca alkaloids interact with tubulin, unlike taxanes their interaction prevents tubulin polymerization into microtubules. In neurons, via an unclear mechanism, this leads to altered axonal arrangement, orientation and length(Argyriou et al., 2012a). This in turn causes impaired axonal transport and ultimate axonal degeneration(Miltenburg and Boogerd, 2014). Subsequently, a decrease in abutting myelin thickness and even segmental demyelination is observed(Argyriou et al., 2012a). Again clear pathophysiological pathways remain elusive.

Similarly, to taxanes and vinca alkaloids, bortezomib binds to a specific intracellular molecular structure. Although this drug is known to be a proteasome inhibitor, neither its tumouricidal nor its neurotoxic mechanisms are clear (Miltenburg and Boogerd, 2014). Following proteasome inhibition accumulation of neurofilaments in neuronal cytoplasm has been demonstrated. Additionally, mitochondrial dysfunction has also been described.

In contrast to the above three groups the platinum based chemotherapeutics interfere with DNA synthesis leading to cellular apoptosis. In the neuron, changes in the nuclei of DRG neurons precedes anterograde axonal degeneration. The exact mechanism by which this neurotoxicity proceeds is unclear (Argyriou et al., 2012a, Park et al., 2008). Interestingly, Oxaliplatin a key compound in the platinum group has been shown to have important effects on ion transport channels. This effect occurs specifically via gene up regulation and it is known to increase the excitability of sodium channels leading to neuronal hyper excitability and the clinical phenomenon of hyperalgesia (Miltenburg and Boogerd, 2014, Argyriou et al., 2013).

Like the compounds described above, thalidomide – a more recently used chemotherapeutic - has poorly described neurotoxic mechanisms. Current postulates include speculation about its interference with angiogenesis around the DRG as well as DRG neuronal cell body degeneration and subsequent axonopathy (Park et al., 2008).

Interestingly, the DRG is particularly sensitive to the changes induced by all the chemotherapeutic drug groups mentioned above. It is postulated that this is due to the high permeability of DRG capillaries, which allow transit of large molecules including chemotherapeutic agents (Argyriou et al., 2012a). Due to the importance of the DRG in the pain pathway: its high plasticity and central role in neuropathic pain development, the vulnerability of this structure in CIPN is likely of key importance to further understanding of pathophysiological mechanisms of this condition.

Finally, recent evidence suggests that the development and maintenance of CIPN is dependent on more than just neuronal damage. Activation of astrocytes and microglia in response to chemotherapeutics has been documented and postulated to play a role in the neurotoxicity underlying CIPN (Robinson et al., 2014, Di Cesare Mannelli et al., 2013). Although mechanisms by which these activations lead to CIPN remain unclear, and authors debate the importance of microglia versus astrocyte activation, it is likely that these changes will be of growing interest in future CIPN research (Di Cesare Mannelli et al., 2013).

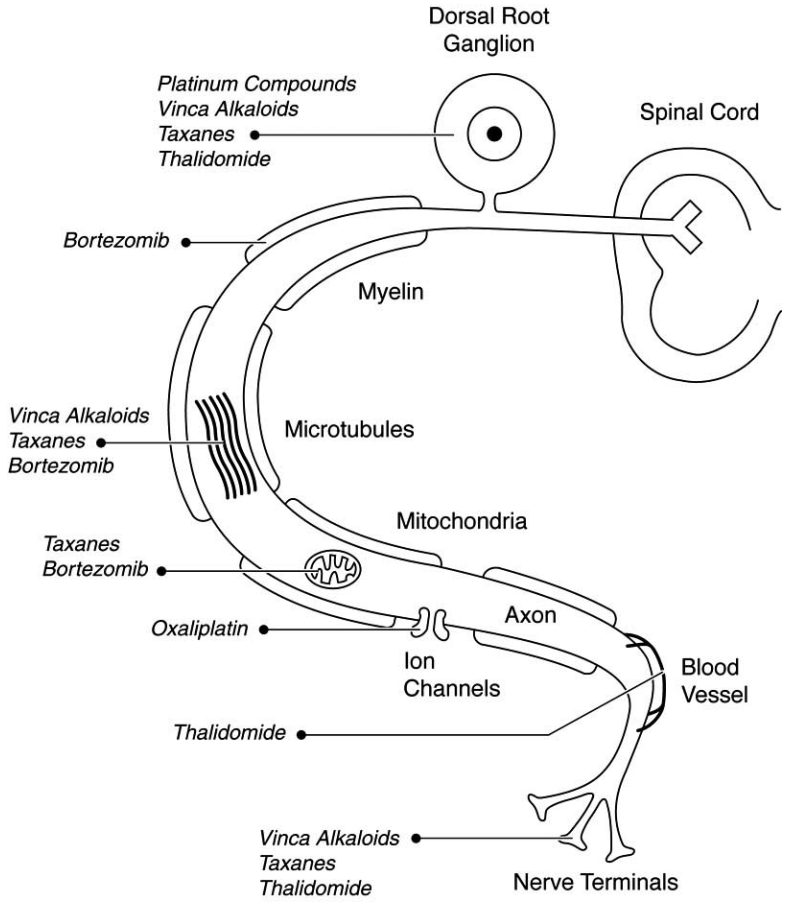


Figure 1.4 Drug targets thought to underpin peripheral nerve damage important in CIPN development. Image depicting the peripheral neuron with the key molecular and cellular structures vulnerable to damage by chemotherapeutics highlighted. Of key importance are: microtubules, mitochondria, ion channels, the dorsal root ganglion and myelin. Figure adapted from (Park et al., 2008).

1.1.2.3 Prevention and Treatment of CIPN

Prevention of CIPN development is a key area of interest in oncology. To date despite extensive investigation of small compounds, vitamins, minerals and topical agents, no CIPN preventing neuro-protective strategy has been identified (Hershman et al., 2014, Albers et al., 2011). Consequently, chemotherapy dose reduction or cessation remains the only effective strategy for limiting CIPN. This has obvious implications for patient morbidity and mortality. Importantly, chemotherapy cessation is only implemented after CIPN symptoms become apparent, serving a containing rather than a preventive role.

Treatment of established CIPN is equally elusive. To date only one randomized controlled trial (RCT) has shown duloxetine to be an effective measure in longstanding CIPN(Smith et al., 2013). Other proposed treatments have been extrapolated from management of disparate neuropathic pain conditions such as trigeminal neuralgia. They include antidepressant and antiepileptic agents (see 2.3). However, these treatments are often only moderately successful in treating CIPN and have important side effects (Rao et al., 2007, Hammack et al., 2002). Nonetheless, in the absence of treatment options, drugs such as gabapentin, nortriptyline and topical ketamine are recommended in existing CIPN management guidelines(Hershman et al., 2014).

Alternative approaches to treatment of CIPN are under evaluation. Specifically, assessment of alternative therapies such as acupuncture are in progress(Garcia et al., 2014). Additionally, translational work suggesting that TRPM8 agonists may have a role in CIPN treatment (Proudfoot et al., 2006), led to several case reports investigating topical menthol gel. These have promising results (Colvin et al., 2008, Storey et al., 2010) and a subsequent phase one study has shown benefit in a small cohort who received topical menthol gel (Fallon et al., 2015). Nonetheless no clear long-term solutions to the problem of post chemotherapy neuropathy are currently available. Arguably until greater understanding of CIPN development is achieved, finding effective treatments will remain elusive.

1.1.2.4 Unanswered Questions in CIPN

Although there are many mechanistic questions that remain unanswered in relation to CIPN development, from a clinical perspective the following key issues are perhaps most pertinent:

1. Why do some patients develop CIPN and others do not?
2. Can CIPN be prevented without decreasing tumouricidal effect of chemotherapy?
3. How can CIPN be treated effectively once it develops?

Arguably, concrete answers to questions two and three, will only be possible with clear insight into question one. Currently it is not apparent why in a cohort of for example 10 patients matched for age, sex, chemotherapy and cancer type, around 6 patients will develop the neuropathy and the remaining 4 will not. To address this question an integrated, patient centred approach is needed. Addressing this problem of CIPN development is one of the key aims of this thesis.

1.1.2.5 Novel Approaches to Investigating CIPN

Methods employed to understand CIPN can broadly be split into laboratory work and clinical research. Laboratory approaches constitute a complex and diverse set of animal and non-animal experimental work aimed at probing the mechanistic basis of CIPN. These are not the focus of the present work and will not be discussed here. It is however worth noting that although animal work is integral to further understanding of CIPN, useful translation of neuropathic pain models to human clinical realities is impacted by a two-fold process. Firstly translation is always reliant on the closeness of outcomes used in animal work to clinically useful and interpretable measures (Sikandar and Dickenson, 2013). Secondly and perhaps more importantly translation of animal work will always be limited by the complex subjective experience of pain in humans that is hard to model in animals (see 1.1.1).

Clinical research investigating CIPN has mostly centred on prospective as well as retrospective, observational work. This is predominantly a consequence of

ethical constraints, whereby interference with patient's chemotherapy regimes is unethical. The mainstay of prospective observational work has focused on tracking the development of peripheral nerve changes, using quantitative sensory testing (QST), neurological examination, nerve biopsies and nerve conduction studies (Argyriou et al., 2012b, Attal et al., 2009). Recently less invasive in-depth examinations of peripheral nerves -aimed at substituting nerve biopsies, such as in vivo laser reflectance confocal microscopy- have also been employed to predict CIPN development(Kosturakis et al., 2014).

A more global approach to understanding CIPN has been reflected in the increasing number of CIPN related genome wide association studies (GWAS). These have aimed to identify genetic markers of CIPN, in the hope of translating findings back to laboratory studies aimed at probing mechanistic pathways involved in the disease (Cavaletti et al., 2011b).

It is interesting that very few studies have focused on the central nervous system (CNS) as a component of CIPN development. It is clear from extensive pain research that peripheral nerve damage leads to central nervous system changes that maintain and exacerbate pain conditions leading to chronicity, with particular emphasis on aberrations in the descending pain modulatory system (DPMS) (Tracey, 2005, Yarnitsky, 2010, Denk et al., 2014). Moreover, animal work has verified that similar changes occur in the CNS with the maintenance of neuropathic conditions (De Felice et al., 2011).

The ability to assess the involvement of the CNS in human pain has been demonstrated in numerous functional magnetic resonance imaging (fMRI) experiments (see 1.1.2.4). To date only one retrospective study has assessed CNS changes in CIPN using fMRI (Boland et al., 2014). No prospective work has been done to appraise the association between baseline CNS pain processing and subsequent CIPN development using fMRI as a tool. This will be the main theme of this thesis that aims to understand whether predisposing vulnerabilities in the brain DPMS relate to the development of CIPN.

1.1.3. Functional Magnetic Resonance Imaging (fMRI)

In 1990 Ogawa et al found that the naturally occurring ferromagnetic properties of haemoglobin (Hb), could be harnessed to visualize neuronal tissue function (Ogawa et al., 1990). This was in contrast to standard magnetic resonance imaging (MRI), already used clinically to gain structural diagnostic information about multiple tissue types. Like MRI, fMRI utilizes non-ionizing radiofrequency pulses to excite hydrogen ions in tissue. However over and above this fMRI incorporates the physiological changes in the proportions of oxygenation haemoglobin (OxyHb) compared to deoxygenated haemoglobin (DeOxyHb), which reflect tissue metabolism and activity. This is known as the blood oxygen level dependent (BOLD) effect or signal. This signal served as the key component of functional neuroimaging development. More recently, in addition to the BOLD signal, alterations in blood volume and tissue perfusion have also been measured to gain a functional signal in fMRI studies. The BOLD signal however remains the most popular method for human neuroimaging (Logothetis, 2008).

1.1.3.1. FMRI Physics

Human tissue is composed of around 75% water (H_2O). Each water molecule contains two hydrogen atoms; each containing a simple nucleus made up of a single proton. The hydrogen proton possesses a natural charge and spin frequency referred to as a magnetic moment (fig 1.5). In tissue outside a magnetic field, each proton will have magnetic moments occurring in random directions (fig 1.5). Once placed into a magnetic field each individual proton's magnetic moment will line up either with or against the external magnetic field, creating an average measurable magnetization within the tissue (fig 1.5).

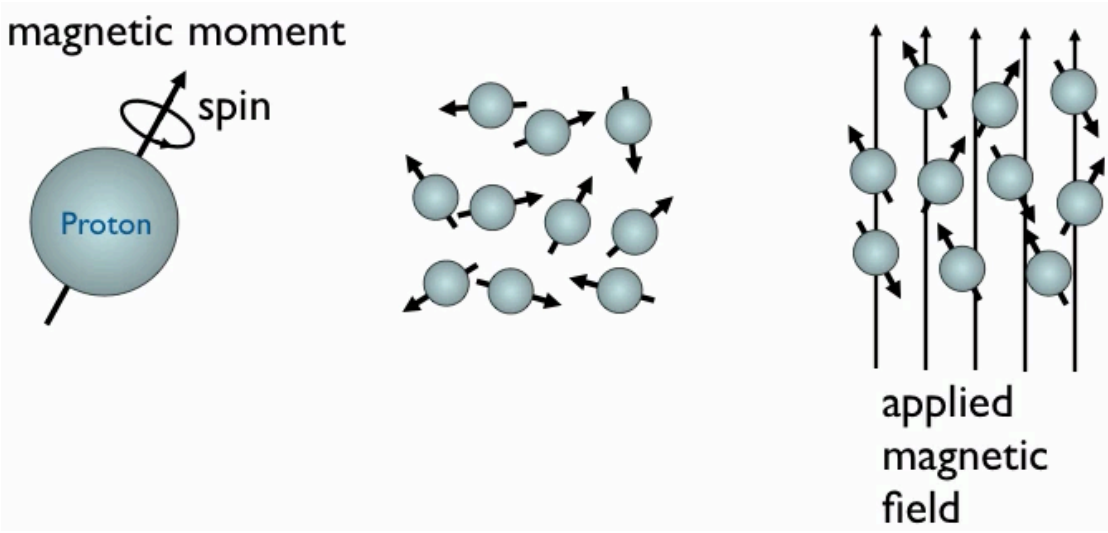


Figure 1.5: Magnetic Moment of Hydrogen Protons in Tissue. Far left the natural spin or magnetic moment of an individual hydrogen proton. Middle image shows the random direction of magnetic moments in tissue outside of a magnetic field. On the far right the alignment of proton magnetic moments is shown when a magnetic field is applied to the tissue (z plane). Figure adapted from (Jezzard et al., 2009) and (Clare, 2013).

If an excitation radiofrequency (RF) pulse, at 90degrees to the plane of the magnetic field is applied to the tissue, the protons will flip out of alignment perpendicular to their original plane (ie out of the z plane). If the RF pulse is then turned off and the protons are allowed to return to alignment within the magnetic field, they will emit energy. This energy is recorded as a signal by a recorder coil within the fMRI scanner. The time between successive pulse sequences is known as the repetition time or TR.

Following an RF pulse, protons in the 'excited' tissue undergo three processes, which are harnessed to record signal in MRI and fMRI. The first is restoration of the magnetization. This is referred to as the T1 (recovery time). The second is the loss of energy emitted by the excited protons, known as T2 (signal decay time). The third is known as the T2* and is related to the varied rate of signal decay caused by local inhomogenities in the magnetic field affecting each proton. The amount and impact of each of these processes on the MRI signal, is determined by the location and properties of the tissue under investigation (e.g. how densely packed the protons are). Although T1, T2 and T2* are all crucial to

MRI image generation, it is the T2* process, which by exploiting the presence of paramagnetic Hb in blood vessels, serves as the basis for the BOLD signal utilized in fMRI of the brain.

1.1.3.2. The BOLD effect & fMRI image acquisition

Physiologically, when neurons become active blood flow to these cells increases disproportionately, to accommodate increased metabolic demands (increased use of oxygen). This results in the proportions of oxygenated to deoxygenated Hb changing. The magnetic properties of these two types of Hb differ. OxyHb is diamagnetic while DeOxyHb is paramagnetic. Consequently, the alterations in ratio between the two types of Hb (increased OxyHb:DeOxyHb), following increased neuronal activity result in amplified MR signal, termed the BOLD signal (Jezzard et al., 2009). The BOLD signal or effect, serves as a stable, naturally occurring contrast, enabling visualization of active brain regions. Although the BOLD response is only a proxy for neuronal activity, work investigating the interplay between this effect and actual neuronal activity suggests that it is a stable albeit imperfect surrogate, for nerve cell firing (Logothetis, 2008).

MR signal generated by the BOLD effect is recorded by virtually dividing the brain into spatial sections or volumes. Typically signal is acquired in series of volumes. Within a volume, the brain is further partitioned into cuboidal sections called voxels, allowing signal location to be more precisely recorded. BOLD signal changes are measured in every physical plane (z, x, y), as well as across time (time-series) within each single voxel. The whole process of BOLD signal fluctuations, occurring at voxel level and summarising the physiological process of neuronal firing is known as the haemodynamic response function (HRF).

Acquired fMRI data is then analysed, typically using a simple statistical approach called general linear modelling (GLM), to determine which voxels show signal of interest and which has significantly greater intensity compared to the baseline noise level (Smith, 2004).

1.1.3.3. Limitations and challenges of fMRI

Although fMRI has become one of the most important tools for investigating the human brain *in vivo*, its use is subject to important limitations, which if not considered and accounted for may lead to grossly spurious, misleading results (Bennett and Miller, 2010). A concept that overarches all fMRI limitations is that of signal to noise ratio (SNR). Scientific reliability and reproducibility is inherently linked to the ability to accurately detect the signal of interest being measured, and differentiate this from a sea of irrelevant noise (Bennett and Miller, 2010). Therefore the higher the SNR in an experiment, the more likely that signal of interest will be accurately detected. In fMRI experiments sources of noise are linked to a number of influences. These can be divided into: data acquisition and equipment, participant, and data analysis and interpretation related factors; all are discussed in turn below.

1.1.3.3.1 Data acquisition & equipment related noise

A key influence on SNR is the main magnetic field strength (B0). In general terms, doubling the magnet strength from 1.5T to 3T can double the SNR (Haller and Bartsch, 2009, Bennett and Miller, 2010). Related caveats however include the increased influence of physiological and susceptibility noise at higher magnet strengths (see 1.1.3.3.2). This will slightly decrease maximum achievable SNR (Bennett and Miller, 2010). Nonetheless the general accepted move in fMRI experimentation is toward higher magnetic strength.

In parallel with increasing magnet strength head coil design is recognized as another key source of SNR optimization. Newer head coils tend to have multiple channels allowing higher sensitivity for more superficial brain structures (Haller and Bartsch, 2009).

Importantly, how the experimenter interacts with available hardware, through their choice of image acquisition parameters, also impacts SNR (Bennett and Miller, 2010). For example doubling voxel size from 1.5 to 3.0mm^3 can improve SNR by up to eight fold. This however will decrease spatial resolution (Bennett and Miller, 2010). Similarly, optimization of other parameters, for example

repetition time (TR), echo time (TE), slice gap, and flip angles all help optimize SNR and ultimately data quality (Haller and Bartsch, 2009). Consideration of other noise sources, perhaps more challenging to modify, such as MRI acoustic noise influence on auditory activation also, allows for improved data quality.

1.1.3.3.2 Participant related noise

Participant related influences on SNR can be broadly divided into two groups: cognitive factors and factors related to body physiology. Cognitive factors reflect the influence of study participants attention, arousal, and emotional status on the signal being measured (Bennett and Miller, 2010). For instance, a participant who received upsetting news the day before a pain fMRI experiment and spends time in the scanner reflecting on the impact of this news on their lives, will have varied responses to experimental stimuli in comparison to if they had for example received some very good news or indeed no news at all. Additionally, the influence of learning during a longer cognitive fMRI task may be a source of noise in an experiment uninterested in this process(Bennett and Miller, 2010).

The influence of body physiology can be further subdivided into the impact that this has on the magnetic field and thus SNR and the impact it has on the BOLD effect itself. Physiological functions such as breathing and heart rate, as well as anatomical variability such as intracranial sinus size have an important impact on local magnetic field and SNR (Brooks et al., 2008). Monitoring these parameters during an experiment and adjusting for them in the subsequent data analysis has been shown to improve SNR (Kongn et al., 2012) (fig 1.5). More recently a useful technique for removal of these sources of noise has been achieved through the combination of independent component analysis and computer based algorithms (ICA)(Salimi-Khorshidi et al., 2014). Similarly, to heart rate and breathing, head motion or other movement in the scanner alters the local magnetic field, SNR and data quality. Explaining this to participants beforehand often helps minimize this problem.

Finally, the impact of individual physiological parameters such as age, comorbidity, smoking status, brain pathology and associated pharmacotherapy

all influence the BOLD effect and may alter SNR in a given experiment (Iannetti and Wise, 2007). This is particularly true for fMRI studies investigating patient populations. These factors are often outside the experimenter's control and may have an unquantifiable impact on the signal of interest. It is therefore important to attempt to adjust for these variables during the experimental design and analysis phase of the studies in order to minimize the possible impact of bias on results (fig 1.6).

Figure 1.6 Physiological Noise Monitoring Setup. Showing respiratory bellows around participant diaphragm. These extend through the waveguide to the BIOPAC (a system of amplifiers and transducers used to acquire physiological signal), where respiratory rate is recorded for later use in analysis. Also a pulse oximetry probe attached to the participant's finger extends through a connecting cable to a filter installed in the penetration panel. From here, a secondary cable connects the pulse oximetry to the BIOPAC allowing recording of signal as detailed above.

1.1.3.3.3 Data analysis & interpretation related noise

FMRI data analysis can broadly be subdivided into pre-processing, first level (single subject) and second level (between subject) analysis (see 3.3). Each stage is susceptible to influences of SNR. Pre-processing stages, during which adjustment for known sources of measurement error can take place, are particularly important to optimize the impact of SNR variability. Specifically, spatial realignment, temporal filtering, use of high pass filter and spatial smoothing are all known to decrease the amounts of noise carried into first and

second level analysis stages (Bennett and Miller, 2010). Differences in choices regarding preprocessing stages will influence results, most notably in areas of lower signal. Additionally, due to variations in location, shape and size, the impact of preprocessing steps, such as for example spatial smoothing, may not be uniform across brain areas (Haller and Bartsch, 2009). Standardization of preprocessing approaches in fMRI data analysis is therefore considered a cornerstone of minimizing bias in fMRI experiments (Jenkinson et al., 2002).

Information impacting signal detection at the first and second level stages of analysis, centre on the use of corrections for multiple comparisons as well as the adjustment for between subject and between session variance (Haller and Bartsch, 2009). At first level, inadequate correction for the problem of multiple comparison in fMRI data (where the same general linear model is often fitted and tested at every voxel) can lead to extreme false positive results, in the order of 25,000 spuriously 'active' voxels per acquisition (Haller and Bartsch, 2009). Equally, excessively conservative corrections may lead to false negatives, a situation undesirable for both experimental and clinical fMRI. Similarly, choice made during the second level analysis may lead to altered sensitivity of signal detection within a group. Therefore, careful knowledge of the impact of analysis decisions, as well as a clear understanding of what a specific change in BOLD signal actually means, can limit the influences of spurious noise on fMRI data analysis and interpretation.

1.1.3.4. FMRI and Pain Research

Despite the limitations detailed above, carefully designed fMRI experiments have provided an objective measure of the subjective pain experience. This has allowed for a greatly improved understanding of nociceptive processing in humans and a description of the neuronal mechanisms underlying various pain phenotypes (Tracey, 2005, Tracey, 2011). Phenomena such as central sensitization, neuropathic pain and the differences between chronic and acute pain have, thanks to fMRI, been translated from animal experiments to humans (Wartolowska and Tracey, 2009, Lee et al., 2008, Vincent et al., 2011). Moreover, fMRI work has shown key regions of the reward and descending pain network to

be associated with conversion from acute to chronic pain states (Baliki et al., 2012). Consequently, fMRI as a research tool greatly lends itself to probing hitherto unresolved clinical problems such as CIPN.

1.2. Thesis Aims and Research Questions

1.2.1 Aims

The aim of this thesis is to use fMRI to explore the development of CIPN, by prospectively assessing whether there are baseline differences in pain processing between patients who develop CIPN and those who do not, and to investigate baseline variations in resting state networks and subcortical structures, between CIPN and non CIPN patients. Secondly this thesis aims to outline the development of an fMRI study exploring the effect of menthol gel versus placebo in the treatment of chronic CIPN. For the purposes of this thesis the clinical syndrome of CIPN that where pain is one of the presenting features (see 1.1.2.1).

1.2.2 Research Questions

1. Is the brain different structurally between cancer patients who develop CIPN and those who do not prior to peripheral nerve damage with chemotherapy?
2. Are there differences in resting state networks between cancer patients who go on to develop CIPN and those who do not prior to chemotherapy onset?
3. Are there differences in descending modulatory pathways between cancer patients who develop CIPN and those who do not? Are these influenced by positive emotional distraction?
4. Is it possible to develop an fMRI study investigating the effect of topical menthol gel, versus placebo in chronic CIPN?

1.3. Thesis overview

At the onset of this thesis a literature review pertinent to the research questions described above will be undertaken (chp.2). The literature review will explore two parallel themes. Firstly, a summary of CIPN – its epidemiology,

developmental predictors and treatment methods will be presented. This is a systematic review and meta-analysis of CIPN prevalence. Secondly, a description of pain fMRI research detailing neuropathic pain, resting state networks related to neuropathic pain, changes in subcortical structures and analgesic trials utilizing fMRI, will be presented. Methodological details of the literature search strategy including websites and databases used, along with the related publication can be found in the appendix (see Appendix A and B).

A detailed methodology section will follow the literature summary (chp.3). This chapter will describe CIPN study design and setting, inclusion and exclusion criteria, trouble-shooting data acquisition difficulties, data collection, and relevant definitions. An overview of statistical methods utilized for fMRI analysis will follow. Ethical considerations and data protection issues will also be addressed in this chapter.

The thesis will then be divided into three sections, each associated with one or more of the research questions detailed above (chp.4: exploration of subcortical structures and resting state networks, chp.5: functional MRI analysis assessing the descending pain modulatory system, and affective image processing during punctate stimuli, chp.6: description of development of an fMRI treatment study for CIPN). Each section will contain methods, results and discussion subsections, applicable to the research question under study. The strengths and limitations of the entire thesis, its links to existing knowledge in the field, as well as its implications for future research, will be considered in the overall discussion and conclusion section of this thesis (chp.7).

2. Systematic Literature Review

A review of the literature related to this thesis is presented in this chapter. The literature review details two themes. Firstly, the clinical problem of CIPN is explored, describing the epidemiology, risk factors and treatments for CIPN. This review was undertaken as part of a now published systematic review and meta-analysis investigating CIPN prevalence. Secondly, the pain fMRI literature is reviewed, with a focus on functional and resting state studies of neuropathic pain as well as assessment of subcortical volume changes in chronic neuropathic pain conditions. Finally, an overview of fMRI use in analgesic trials will be presented. Details of medical subject headings (MeSH) and free text terms, databases and websites used are presented in Appendix A.

2.1 Epidemiology of CIPN

2.1.1 Background

Chemotherapy treatments for cancer have an array of side effects. These are balanced against the benefits of cancer treatment and prolonged survival. As survival has increased long lasting side effects such as CIPN have become more clinically relevant. Moreover, acutely, treatments for other chemotherapy side effects such as haemopoetic stimulating drugs, which limit bone marrow suppression, and anti emetic drugs that mitigate severe nausea have become available (Miltenburg and Boogerd, 2014). Consequently, the profile of CIPN as a clinical problem potentially limiting chemotherapy dose or duration has risen (Hershman et al., 2014). The need for understanding CIPN risk factors and frequency has therefore also increased.

Investigating CIPN development and risk factors is limited to observational studies and more recently genome wide association studies (GWAS). This is predominantly because altering a patient's chemotherapy regimen in order to research a potential side effect is unethical. Observational work however has both strengths and limitations; these are discussed in detail in Appendix C.

2.1.2 Difficulties with epidemiological measures of CIPN

Epidemiological understanding of a disease is only as accurate as the definitions used to identify affected cases (Bhopal, 2008). CIPN, as discussed above (see 1.1.2.1), is difficult to uniformly define because of its variable clinical course, chemotherapy specific clinical presentations, and previously non-standardised approach to diagnosis (Cavaletti et al., 2013). Consequently, summarising CIPN epidemiology across cancer and chemotherapy types is limited by the inevitable pooling of non-uniform clinical entities; such as for example acute oxaliplatin induced CIPN and chronic bortezomib induced CIPN. Conversely, the benefit of comparing across all cancer and chemotherapy types, is that the resulting data summaries are useful to a wider group of clinicians and can be consulted when planning CIPN related health care costs. Below a summary of all CIPN related literature is presented. This is based on a systematic review of the literature. Detailed methods are available in the systematic review protocol at: http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42013005524. The publication detailing this work is also included in Appendix B.

2.1.3 Incidence and Prevalence of CIPN

Incidence is defined as new cases of a disease divided by the population at risk (Bhopal, 2008). This measure is useful for acute disease states (e.g. appendicitis in a defined population of primary school children). Prevalence consists of all cases of a disease in a population, divided by the population at risk (Bhopal, 2008). Prevalence measures are appropriate for long lasting chronic diseases such as hypertension in a defined population (e.g. coal miners living in a specific town). Prevalence measures require knowledge of new cases arising during the period of interest, along with existing cases.

In the case of CIPN it can be argued that both incidence and prevalence measures are appropriate. If the point of the measure is to establishing how many patients experience CIPN acutely during their chemotherapy treatment an incidence count would be worthwhile. If, however, a summary of patients limited by CIPN two years following cancer treatment was needed than a prevalence count would be more appropriate. The CIPN literature reports both measures, although

incidence counts are reported more frequently. These are discussed in turn below and conclude with a section detailing a meta-analysis carried out to obtain an overall summary measure of CIPN prevalence (Seretny et al., 2014).

2.1.3.1 Studies reporting CIPN Incidence

Since the mid 1970's, the majority of cancer types have shown improved survival rates (Cancer Research UK, 2014). Consequently, understanding the incidence of side effects such as CIPN has become important for chemotherapy planning, patient information and also development of preventive measures (Park et al., 2013).

The earliest incidence counts for CIPN can be derived from RCTs completed in the 1990's. Control arms of RCTs seeking to assess preventive measures for CIPN give standard care with or without placebo, whilst monitoring occurrence of CIPN prospectively. These allow the number of patients developing CIPN to be calculated thereby producing incidence counts.

Five seminal RCTs from the 1990's assessed the effectiveness of four novel chemo-protective agents in patients receiving cisplatin (Vanderhoop et al., 1990, Cascinu et al., 1995, Gandara et al., 1995, Kemp et al., 1996, Planting et al., 1999). Each study had development of CIPN as an outcome measure. Incidence of CIPN can be derived from the control arms of the studies. Calculated incidence ranges from 12% to 67.5% (see table 2.1). This wide variation in documented occurrence of CIPN, despite use of the same chemotherapeutic agent, highlights the discrepancies in classification and therefore epidemiological counts discussed above (see 2.1.2). Each study used different criteria for CIPN diagnosis making between study incidence comparisons difficult. Additionally studies used cancer specific chemotherapy doses, a factor which also influences CIPN occurrence. Study quality was also variable.

In the subsequent decade prospective cohort studies followed patients receiving various chemotherapy types in order to accurately track CIPN development. All of these studies had small sample sizes (range: 14 to 34 patients), were carried

out in single centres and compared a range of chemotherapeutic agents using a variety of CIPN diagnostic criteria. Consequently, the biases affecting observational studies (see appendix C) and CIPN epidemiological measures likely influence all the counts presented here.

The highest incidence of CIPN reported by Chaudhary *et al* was 96.3% in 27 patients receiving a combination regimen of bortezomib and paclitaxel (Chaudhry et al., 2008). This is comparable to the 96% incidence reported by Plasmati *et al* in 25 patients receiving thalidomide (Plasmati et al., 2007). Importantly, both studies assessed cohorts of multiple myeloma patients, up to 83% of whom have been shown to suffer a cancer related neuropathy before any chemotherapy treatment is given (Richardson et al., 2006). Pace et al proposed a comparatively high CIPN incidence of 92.8%, in 14 breast cancer patients receiving paclitaxel (Pace et al., 2007). In contrast, two studies investigating CIPN resulting from paclitaxel plus cisplatin, or paclitaxel reported a lower CIPN incidence of 69.2% and 61.5% respectively (Argyriou et al., 2006, Argyriou et al., 2007b). Cohort studies from the same period in patients receiving oxaliplatin or cisplatin chemotherapy, also suggested variability (42.8% to 66.7%) (Krishnan et al., 2005, Argyriou et al., 2007a, Antonacopoulou et al., 2009, Attal et al., 2009). It is therefore difficult to decipher whether differences in reported incidence are a result of inherent bias in the studies or a result of variable mechanistic susceptibility to the individual cancer and chemotherapeutic regimens.

The control arms of seven controlled trials investigating chemotherapy regimens and CIPN prevention from the same decade show similar variability in CIPN incidence (41.6% to 93.7%) to the cohort studies described above. The majority of these studies were also single centre and had small sample sizes. Three of the seven did not randomise group allocations (see 2.1) possibly biasing findings. Various cancer types, CIPN assessment methods and chemotherapeutics were used, likely causing at least some of the reported variability in incidence. A notable exception regarding sample size and randomisation is the sub-analysis of a phase III multiple myeloma treatment study carried out by Dimopoulos *et al* which assessed CIPN incidence in 340 patients receiving bortezomib

(Dimopoulos et al., 2011). The authors reported a CIPN incidence of 46.7% a more conservative estimate compared to the myeloma CIPN cohort studies discussed above. This is likely due to robust methodological approaches where in addition to a large sample size and randomisation, application of careful exclusion criteria meant patients with baseline neuropathy were not included. This example suggests that methodological precision gives greater accuracy in CIPN incidence estimations.

Both observational studies and controlled trials carried out in the last five years have attempted to improve methodology by optimising sample sizes and using randomised group allocation where appropriate. Observational cohort studies have included no less then 50 patients (Kawakami et al., 2012) and some have recruited up to 855 (Baldwin et al., 2012) for genetic association studies. The range of reported CIPN incidence has decreased; 83.3% to 40% for observational studies and 70% to 32% for control arms of RCTs, with differences in counts being more easily attributable to variations in chemotherapy, cancer types and assessment methods rather than methodological study design inconsistencies.

In summary conservative estimates of CIPN incidence are agreed as being around 38% by expert consensus, although this number is acknowledged to increase with use of high risk agents such as taxanes, platinum compounds, vinka alkaloids and or bortezomib (Hershman et al., 2014). It is important to note that to date the only statistically derived epidemiological summary measure weighing various study types in meta- regression, has been undertaken as part of this thesis and is described below (see 2.1.3.3).

2.1.3.2 Studies reporting CIPN Prevalence

In contrast to the many studies reporting CIPN incidence, CIPN prevalence is a less frequently reported measure. Studies reporting CIPN prevalence published in the last six years have employed a cross sectional design to assess long term CIPN in testicular, mixed and colorectal cancer survivors. Rossen et al contacted testicular cancer survivors who had completed chemotherapy three years earlier

or more. The authors found that 24% of the 150 eligible patients reported symptoms of CIPN. Strength of this study included a large sample size and inclusion of a matched surveillance cohort. Comparison of the rate of neuropathy was statistically tested and found to be significantly more burdensome in the chemotherapy group (Rossen et al., 2009). Limitations centre on the usual limitations affecting observational studies (see appendix C). Similarly, the findings of Brydoy *et al's* cross sectional study are strengthened by large sample size and robust statistical methods used by the authors (Brydoy et al., 2009). The authors assessed 528 cancer survivors and found 29% showed symptoms of CIPN. They used both self-reported measures of CIPN as well as physical examination. Additionally the authors calculated the odds of developing CIPN related to chemotherapy dose, confirming previous findings that higher doses cause more CIPN.

The averaged prevalence counts from the above two studies have been confirmed by Glendenning *et al*'s robust study investigating the long-term side effects suffered, by testicular cancer survivors (Glendenning et al., 2010). This study assessed 293 men of whom 59 had symptoms of CIPN, resulting in a CIPN prevalence of 20% at least 5 years after treatment cessation. Importantly, the study assessed only the more severe grades of CIPN based on an *a priori* definition of what was deemed clinically significant. Consequently a milder form of the condition would have gone unreported ultimately pointing to prevalence more in line with that reported by Brydoy and colleagues.

Similar to the testicular survivors studies, Mols *et al* have recently reported a long-term CIPN prevalence of 29% in colorectal cancer survivors. The authors assessed 500 individuals who had completed chemotherapy in the preceding two to eleven years. Interestingly, the study reported the individual symptoms specified by patients in the CIPN20 questionnaire (see 3.1.2). Symptoms most bothering respondents included: erectile dysfunction (42% men), hearing loss (11%), problems opening jars (11%), tingling in fingers and toes (10%) and challenges climbing stairs (9%) (Mols et al., 2013). Both Mols *et al's* and Glendenning *et al's* studies were well designed and had high methodological

quality, statistically adjusting for known confounding factors where possible (see appendix C).

In contrast to the above studies Kautio *et al* derived prevalence counts by grouping a cohort exposed to chemotherapeutics known to cause high CIPN rates, but used to treat different cancer types within the patient group (Kautio et al., 2011). The authors assessed patients who had received vinka alkaloids, platinum compounds or taxanes for chemotherapy, using postal questionnaires and a subsequent screening visit for eligible respondents. Of the 336 patients who responded to questionnaires 76% reported CIPN symptoms. However, of these only 193 came to the screening visit and only 152 were eligible for further CIPN assessments. When assessing CIPN symptoms in this group of 152, using the same questionnaire but administered in clinic CIPN prevalence decreased to 59% and showed a marked cancer related variability (74% prevalence in lymphoma survivors and 69% in colorectal cancer survivors). Kautio *et al's* study design was of a lesser methodological quality than the two studies reporting prevalence discussed above. Consequently, the differing prevalence counts in comparison to Mols *et al's* work may be a consequence of bias rather than a true measure of CIPN prevalence.

In summary, the prevalence of CIPN beyond a year following chemotherapy cessation appears to range from 20 to 59%. In view of the fact that 4 studies suggest counts averaging around 30% and only one suggests higher prevalence the likelihood is that long term CIPN affects around a third of cancer survivors. Cancer, chemotherapy type and dose impact the prevalence counts in a similar fashion to incidence counts.

2.1.3.3 Pooled Prevalence of CIPN across all studies

Most published reviews concerned with the epidemiology of CIPN are narrative in nature (Weimer, 2013, Miltenburg and Boogerd, 2014). These types of review seek out relevant studies and synthesise the data to provide clinically useful summaries. Authors usually have extensive experience in the field. Although

beneficial, especially to clinical readers, these types of summaries are inherently affected by bias (Sena et al., 2014). Specifically, the process by which studies are identified and data summaries pooled is not transparent. Authors may not be able to describe the process by which they reach the conclusions presented (Sena et al., 2014).

In contrast summaries based on systematic review and meta- analysis are driven by *a priori* protocols. Methods used in these reviews should be reproducible and transparent clearly describing how the impact of bias was mitigated. In view of this a systematic review and meta-analysis of CIPN epidemiology was carried out as part of this thesis. Details of the methods, strengths and limitations of the review can be found in the published manuscript (Seretny et al., 2014) (see Appendix B).

The findings of the review confirmed the CIPN prevalence trends outlined above. At one month after chemotherapy cessation CIPN prevalence was 68.1% (95% CI: 57.7 to 78.4). Three months after chemotherapy cessation it decreased to 60.0% (95% CI: 36.4 to 81.6) and was 30.0% (95% CI: 6.4 to 53.5) at six months or more after chemotherapy completion.

The usefulness of this type of summary measure is related to both healthcare resource allocation as well as cost estimation. A recent study investigating CIPN related healthcare costs in the US estimated CIPN related healthcare costs from insurance company data which suggested an acute CIPN incidence of 11.3% and a prevalence of up to a year from chemotherapy onset of around 46% (Pike et al., 2012). Based on this data the authors estimated that CIPN patients had $21,739 more annual healthcare costs compared to non-CIPN cancer controls. CIPN patients also used 18% more outpatient appointments and had 24% more hospitalisations. The weakness of this study lay in the lack of availability of clear codes for CIPN diagnosis and a baseline assumption that CIPN would be coded as peripheral neuropathy within 9 months of chemotherapy cessation. Use of a measure derived from meta-analysis such as the one calculated here would have made these costs estimated more reliable.

Finally, the pooled summary of CIPN literature presented here enables a calculation of the influence of specific chemotherapy types on CIPN occurrence. Although it is known that different chemotherapy types and doses alter the risks of CIPN a statistically derived numerical measure for all chemotherapeutics commonly associated with CIPN had not previously been available. This data is summarised in table 2.1 and is likely to be useful to individual clinicians discussing the risks of CIPN with their patients.

	Study Type	**Main Cancer Class**	**CIPN Severity Report** (Count by grade if given)	**CIPN Assessment Time Points**	**CIPN Assessment Method(s)**
OXALIPLATIN: 72·3% (95%CI 59·7 to 86·8)					
Antonacopoulou& (2009)	Prospective Cohort	Colorectal	NR	Unclear	TNSc
Argyriou (2007a)	Prospective Cohort	Colorectal	Grade I (6/16) Grade II (8/16) Grade III (2/16)	Baseline Cycles 4,8,12	TNSc NPS NCI-CTC
Argyriou (2012)	Prospective Cohort	Colorectal	Grade I (38/125) Grade II (46/125) Grade III (41/125)	Baseline Cycles 3,6 (FOLFOX) Cycles 4,8 (XELOX)	TNSc NPS NCI-CTC
Argyriou** (2013)	Prospective Cohort	Colorectal	Grade I (62/169) Grade II (46/169) Grade III (61/169)	Baseline Cycle 6, 12 (FOLFOX) Cycles 4, 8 (XELOX)	TNSc NCI-CTC
Attal (2009)	Prospective Cohort	Colorectal	Sensory symptom counts described as means/individual	Baseline Cycle 3,6,9 12 +/- 2 months post chemo end	NCI-CTC NPS (EORTC) QLQ-C30
Cascinu (2002)	RCT	Colorectal	Grade I (4/15) Grade II (6/15) Grade III (4/15) Grade IV (1/15)	Baseline Cycles 4,8,12 Within 2 weeks of chemo end	NCI-CTC NPS
Gobran (2013)	RCT	Colorectal	Grade I (7/21) Grade II (0/21) Grade III (14/21) Grade IV (0/21)	Unclear if at baseline At each chemo cycle until end of chemo (variable no of cycles) Longer follow up for those with CIPN (but denominator unclear)	NCI-CTC
Ishibashi (2010)	RCT	Colorectal	Grade I (15/15) Grade II (1/15) Grade III (0/15) Grade IV (0/15)	Baseline At each chemo cycle until end of chemo	NCI-CTC
Krishnan (2005)	Prospective Cohort	Colorectal	NR	No baseline Within one month of	NCI-CTC NPS

				chemo end only reported assessment	TNSc
Lin (2006)	Controlled Trial	Colorectal	Grade I (1/9) Grade II (5/9) Grade III (3/9) Grade IV (0/9)	Baseline Cycles 4, 8, 12 Within 2 weeks of end of chemo	NCI-CTC NPS
Milla (2009)	Controlled Trial	Colorectal	Grade I (0/13) Grade II (9/13) Grade III (4/13)	Baseline Cycles 5, 9, 12 (some followed longer but denominator unclear)	NCI-CTC NES
Won (2012)	Prospective Cohort	Colorectal	NR	Unclear if at baseline At each chemo cycle until end of chemo (variable no of cycles)	NCI-CTC NES
CISPLATIN: 42·2% (95%CI 21·3 to 63·1)					
Argyriou[$] (2006)	Prospective Cohort	Lung	Reported by age group only	Baseline Cycles 3, 6 3 months post chemo end	PNS NPS
Cascinu (1995)	RCT	Gastro-intestinal	Grade I (3/16) Grade II (10/16) Grade III (2/16) Grade IV (1/16)	Baseline After 9 and 15 weeks of therapy Within a week post end of chemo	NCI-CTC NPS
Gandara (1995)	RCT	Ovarian & Lung	Only Grade ≥3 reported	Unclear if at baseline At each cycle until chemo end (variable no of cycles) Study stopped early after interim analysis due to high toxicity in intervention group	NCI-CTC
Kemp (1996)	RCT	Gynaecological	Grade I (31/81) Grade II (35/81) Grade III (15/81)	Baseline Cycles 4, 5,6 Monthly post chemo for 3 months	NCI-CTC NES
Pace (2003)	Controlled Trial	Multiple Solid	Grade I (6/12) Grade II (4/12) Grade III&IV (2/12)	Baseline After 6 cycles	TNSc NES
Pace (2010)	RCT	Multiple Solid	Only Grade ≥3 reported	Baseline Every cycle for 3 cycles A month post chemo end	TNSc NPS
Planting (1999)	Controlled Trial	Multiple Solid	Grade I (5/5)	Baseline Cycle 3, 6 3month post end chemo (longer follow up but no denominator	NCI-CTC NES

				info)	
Van der Hoop (1999)	Controlled Trial	Gynaecological	Mean vibration threshold	Baseline Cycles 2,4,6 End of chemo	NES
Von Schlippe (2001)	Prospective Cohort	Testicular	Grade I (4/5) Grade II (1/5)	Unclear if at baseline Every 6 weeks for first 6 months post chemo Thereafter 2 monthly for median 4 years (range 2 to 8 years)	NPS
CISPLATIN or Carboplatin & PACLITAXEL: 73% (95%CI 36·2 to 109·7)					
Argyriou (2007)	Prospective Cohort	Multiple Solid	Mild (2/9) Moderate (6/9) Severe (1/9)	Baseline Cycle 3,6 3 months post chemo end	PNS NPS
Kawakami* (2012)	Prospective Cohort	Lung	% severity with cumulative dose	Baseline Daily during cycle 1 Cycle 2,3,4 Chemo end	NCI-CTC
CISPLATIN & VINCRISTINE: 20.1% (95%CI -26.2 to 66.5)					
Glendenning* (2010)	Cross Sectional Cohort	Testicular	Only Grade ≥3 reported	Recruited patients at least 5 years post treatment Assessed once for this prevalence study	(EORTC) QLQ-C30 NES
PACLITAXEL: 70·8% (95%CI 43·5 to 98·1)					
Argyriou$ (2006)	Prospective Cohort	Breast	Reported by age group only	Baseline Cycle 3,6 3 months post chemo end	PNS NPS
Baldwin (2012)	Prospective Cohort	Breast	Only Grade ≥2 reported	Unclear if at baseline Cycles 4, 6 Within 1 month of chemo end	NCI-CTC
Ghoreishi (2012)	RCT	Breast	Mild (10/16) Moderate (5/16) Severe (1/16)	Baseline 1 month after end of chemo	TNSc NPS
Pace (2007)	Prospective Cohort	Breast	Mean neurotoxicity scores reported	Baseline After 12 weeks of chemo After 24 weeks of chemo	TNSc NPS
VINCRISTINE: 19·6% (95%CI -26·6 to 65·9)					
Johnson$ (2011)	RCT	Multiple Myeloma	Grade ≥ I 31.8% Grade ≥ II 11% Grade ≥ III 3.6%	Unclear if at baseline At each cycle For 6 months post end of chemo for induction (ie 36 weeks from start of induction therapy)	NCI-CTC
THALIDOMIDE: 63·5% (95%CI 29·3 to 97·8)					
Johnson$	RCT	Multiple	Grade details not	Unclear if at	NCI-CTC

(2011)		Myeloma	reported	baseline At each cycle For 6 months post end of chemo for induction (ie 36 weeks from start of induction therapy)	
Plasmati (2002)	Prospective Cohort	Multiple Myeloma	Grade I (12/24) Grade II (6/24) Subclincial (6/24)	Baseline After 4 months of chemo 3 months after stem cell transplant	NCI-CTC NPS
BORTEZOMIB: 46·7% (95%CI 0·3 to 93·1)					
Dimopoulos (2011)	RCT	Multiple Myeloma	Grade I NR Grade II (64/159) Grade III (45/159) Grade IV (1/159)	Unclear if at baseline Every 3 weeks until 1 month post last chemo dose Longer follow up but no denominator data	NCI-CTC
BORTEZOMIB & THALIDOMIDE: 96·2% (95%CI 49·7 to 143)					
Chaudhary (2008)	Prospective Cohort	Multiple Myeloma	Grade ≥2 reported	Baseline Cycles 2,4,6,8 End of chemo Note skin biopsy at baseline and end of chemo only	TNSc NPS Skin Biopsy

Table 2.1 CIPN Incidence according to chemotherapy type based on meta-regression. NR= not reported. &Abstract only available. $Raw data obtained from author or reported in paper, allowing counts reported in single study to be split by chemotherapy type. **Authors report both acute and chronic CIPN grade counts, only acute given here. TNSc: Total Neuropathy Score, NCT-CTC: National Cancer Institute – Common Toxicity Criteria, NES: Neurological examination, NPS: Neurophysiological examination (quantitative sensory testing and/or nerve conduction studies), PNS: Modified peripheral neuropathy score, (EORTC) QLQ-C30: The European Organization for Research and Treatment of Cancer.

2.2 Predictors of CIPN Development

Understanding predictors or risk factors associated with CIPN development can lead to effective preventive strategies and possibly eventual improvements in chemotherapy regimens. As discussed above (see 2.1.1) insight into risk factors associated with CIPN development is derived from observational and GWAS studies only. These are affected by the biases alluded to previously (appendix C). Nonetheless they currently form the clearest insight available into predictors of CIPN development. Overall although a myriad of risk factors has been related to CIPN the majority can be subdivided into the two categories of clinical or genetic

factors. Clinical predictors are those related to clinically measurable variables such as patients age, comorbidity status or pre chemotherapy nerve conduction. Genetic risk factors are associated with the identification of SNPs associated with CIPN. The literature will be summarised in the context of these two categories below.

2.2.1 Clinical Risk Factors

Attal and colleagues published one of the early seminal papers investigating predictors of CIPN development (Attal et al., 2009). This prospective cohort study followed 48 patients receiving oxaliplatin or cisplatin chemotherapy. The strength of this study lies in the consistent follow up, involving detailed questionnaires and nerve testing, which occurred not only during chemotherapy administration but also a year later. However as with many studies investigating CIPN prospectively recruitment was difficult and numbers lost to follow up large (only 18 patients were assessed at a year). The authors described cold hyperalgesia as a predictor of chronic CIPN development.

A year later Glendenning and colleagues published their use of the large testicular cancer register to assess both the long-term prevalence of CIPN (see 2.1.3.2) as well as the risk factors associated with its development (Glendenning et al., 2010). The authors employed multivariate analysis to statistically determine predictors of CIPN and identified chemotherapy dose, and patient age as independent predictors of neuropathy. The analysis was well adjusted and used a reasonably sized cohort. Data were however retrospective and in need of a validation cohort to confirm the identified predictors (Altman et al., 2009).

A similarly well-conducted predictive multivariate analysis based on a large cohort was published in 2011 (Dimopoulos et al., 2011). Like Glendenning's study this work was strengthened by a robust sample size but limited by a lack of a validation cohort for the predictors identified. Any additional strength however lay in it being based on a prospective phase three trial (the VISTA study) investigating the effects of bortezomib in patients with multiple myeloma. This lends credibility to the identification of baseline neuropathy as the only

predictor of subsequent CIPN development. It also fits with the early sensory abnormalities described by Attal and colleagues as predictive of CIPN development.

Shortly after Kawakami and colleagues published a novel set of CIPN predictors based on statistical modelling, following paclitaxel and cisplatin therapy (Kawakami et al., 2012). The authors recruited a sample of fifty patients and found that smoking history and low creatinine clearance increased the hazard ratio of developing CIPN. However due to the small sample size in this study the influence of bias is likely to be significant and limits the usefulness of these results.

Similarly to Kawakami's study, Alejandro and colleagues prospectively recruited 50 patients due to receive neurotoxic chemotherapy and followed them up to determine predictors of CIPN (Alejandro et al., 2013). The authors also used multivariate modelling to determine CIPN risk factors. In line with larger studies they found that cumulative dose of chemotherapy and prior neuropathy predicted development of CIPN (Dimopoulos et al., 2011, Glendenning et al., 2010). They additionally suggested that body surface area and weight were related to persistence of neuropathy during chemotherapy. These variables are however collinear with chemotherapy dose (which is calculated according to patients height and weight, BMI) and may highlight the confounding influencing statistical modelling in this study. Nonetheless, the careful prospective follow up of patients and the confirmation of identified risk factors in other larger studies, strengthen the proposed predictors of CIPN.

Using a similar prospective cohort methodology to Alejandro and colleagues, a recent multicentre study recruited 200 patients due to receive oxaliplatin and followed these patients in order to identify risk factors for CIPN (Velasco et al., 2014). The authors used the standard multivariate statistical modelling approach to determine risk factors. The large cohort and detailed prospective data collection methods strengthened their analysis. They reported that a larger number of CIPN like symptoms during early cycles of chemotherapy and

decreased peripheral nerve action potentials (tested during the study) were predictive of CIPN development. These findings fit with papers by Attal and colleagues, Dimopoulos and colleagues and Alejandro and colleagues, which suggested that altered sensory nerve function during chemotherapy is predictive of CIPN development.

In summary, clinical risk factors for CIPN have to date been derived from predictive statistical models. The reliability of these models is dependent on the size of the cohort they were based on and the details of the data collected from patients. Based on this a number of the published studies should rather be considered hypothesis generating only. However other risk factors, which are reported by multiple authors and are based on at least one large cohort may be considered as likely associated with CIPN development. These would include baseline neuropathy or sensory nerve abnormalities and cumulative dose of chemotherapy given. Indeed a recent study has shown that nerve fibre density is a viable and clinically measurable predictor of CIPN risk (Kosturakis et al., 2014).

2.2.2 Genetic Risk Factors

Genetic risk factors have been postulated to underpin CIPN development. A number of large genome wide association studies (GWAS), have reported single nucleotide polymorphisms (SNPs) associated with CIPN (Baldwin et al., 2012, Johnson et al., 2011, Pachman et al., 2011, Won et al., 2012, Argyriou et al., 2013). Many studies, but not all, used validation datasets and blinding to clinical status during the assessment. Reported polymorphisms encode a wide variety of proteins important in neuronal signal transmission, apoptosis and metabolisms. These include Schwann cell function related proteins, voltage gated sodium channels, receptors involved in neuronal apoptosis and enzymes involved in pyruvate metabolism.

The majority of CIPN related GWAS studies are limited by problems known to influence these kinds of studies (Cavaletti et al., 2011b). These include inadequate sample size, lack of or underpowered replication cohort and insensitive CIPN assessment tools. Consequently, many reported SNPs have not

been reproducible outside of the original study, which described their association with CIPN. Notable exceptions, recently published in relation to paclitaxel induced CIPN, include: rs7349683 in the EPHA5 and rs3213619 in ABCB1 genes. These were found to be associated with an odds ratio for CIPN of 2.07 and 0.12 respectively (Boora et al., 2016).

Although, as discussed above, there are some limitations related to the published genetic risk factors for CIPN, the usefulness of these studies to advancing CIPN prevention and diagnosis has been postulated (Postma et al., 2013). In particular, if the approach to these studies can be standardised, their findings will likely advance personalised oncology and possibly in future help prevent CIPN decreasing its prevalence.

2.3 CIPN Treatment

2.3.1 Current Treatment Approaches

The management of acute CIPN centres on chemotherapy dose reduction or complete chemotherapy cessation. Current treatment options for chronic CIPN are limited. The only evidence-based treatment is duloxetine, a serotonin noradrenaline reuptake inhibitor. Duloxetine's effectiveness in chronic CIPN was however only assessed over a period of 2 month in patients with oxaliplatin induced peripheral neuropathy (Smith et al., 2013). Moreover recent, post hoc analysis of the original duloxetine trial data showed that the best response to the treatment could be predicted in individuals with high emotional functioning on the EORTC-30 quality of life measure (Smith et al., 2015). Arguably this is the subgroup likely to respond to any treatment or cope well with limited treatment options.

Evidence related to the effectiveness of topical menthol gel in chronic CIPN patients also exists (Fallon et al., 2015). A single centre trial showed that 82% of patients using menthol gel had an improvement in pain score, mood, catastrophising, walking ability and sensation. However, this trial was exploratory only and no placebo treatment was given to a control group. The

findings therefore need to be confirmed with a larger randomised controlled trial. No other positive treatment trials exist for chronic CIPN.

In view of the limited treatment options, recent guidelines based on literature review and expert consensus, has endorsed the used of tricyclic antidepressants (such as nortriptyline) and gabapentin in patients with chronic CIPN (Hershman et al., 2014). This advice is based on evidence that these medications are useful in the management of neuropathies other than CIPN. Similarly, the use of topical baclofen, amitriptyline and ketamine was endorsed by these guidelines, as experimental treatment options in chronic CIPN.

In view of the increasing cancer survivorship and concomitant rising CIPN prevalence, it is obvious that novel treatments for chronic CIPN are urgently needed. Importantly, the approach used to assess any new medications needs to take into account the difficulties related to analgesic trials (see 1.1.1.2.2). Thorough screening of agents prior to progression to phase three trials with the use of fMRI has recently been described and should be employed in the assessment of novel CIPN treatments (Wanigasekera et al., 2016).

2.3.2 Experimental Approaches and Possible Drug Targets

The limited options in treatments for chronic CIPN have prompted investigation of multiple compounds, in the hope of identifying novel drug targets. However, many potential agents have failed to survive translation into human studies. An example of a promising agent under investigation was Acetyl-L-Carnatine known to limit mitochondrial dysfunction, showed encouraging results in early CIPN treatment as well as CIPN prevention trials (Lin et al., 2006). Despite this a recent review has shown that it was associated with worse outcome in randomised controlled trials (Hershman et al., 2014).

Other targets currently under investigation include glutamate receptors (Palazzo et al., 2014) and topical applications of small molecules activating GRF/Alpha RET receptors (Hedstrom et al., 2014). More recently, pifithrin a small molecule inhibitor, limiting mitochondrial p53 accumulation, has been shown to prevent

CIPN in mice models (Krukowski et al., 2015). Translation of these targets to successful human trials is still some way off. An exception in terms of successful translation is the TRP receptors family, also under continued investigated as a pain relief target (Moran et al., 2011). Menthol gel, a TRPM8 agonist, has as discussed above, been easily translated into clinical use, and is showing promising results in chronic CIPN (Fallon et al., 2015).

Alternative experimental approaches being employed in chronic CIPN treatment are complimentary therapies such as acupuncture (Garcia et al., 2014). These remain under investigation but are turned to by patients with chronic CIPN when faced with limited analgesic options. More generally in terms of chronic neuropathic pain, use of brain stimulation has been proposed as a viable treatment (Russo and Sheth, 2015). This may perhaps also be applicable as a management option for chronic CIPN in the future.

2.4 Functional Magnetic Resonance Imaging Research in Pain

2.4.1 FMRI Studies Investigating Neuropathic Pain

Multiple fMRI studies have been conducted in neuropathic pain patients. This body of literature has given insight into the central mechanism underpinning these pain states. A summary highlighting the main structural, functional and resting state changes reported by these studies follows. Experimental neuropathic pain models in both animals and humans have also been utilised in fMRI studies. The later provides a rich body of literature, which will briefly be reviewed below.

2.4.1.1 FMRI neuropathic pain studies in patients

Neuropathic pain has been shown to alter brain structure, function and also resting state connectivity. White matter structural brain changes have been demonstrated in chronic back pain, predicting transition from acute to chronic pain states (Mansour et al., 2013). Patients with neuropathic pain following spinal cord injury have shown reduced grey matter volume in their somatosensory and dorsolateral prefrontal cortex (Yoon et al., 2013, Mole et al.,

2014). Similarly, patients with complex regional pain syndrome (CPRS) show decreased grey matter volume in the insula amongst other regions (Barad et al., 2014). Indeed a recent meta-analysis of studies assessing grey matter changes in neuropathic pain showed that decreased volume in multiple structures is common (Pan et al., 2015). Regions most commonly altered include the insula, thalamus, superior frontal gyrus and post central gyrus. Abnormalities in the structure of the thalamus and somatosensory cortex have also been confirmed in a meta-analysis of brain changes in trigeminal neuralgia (Lin, 2014). It should be noted that the underlying cellular basis for these voxel based morphometry (VBM), related changes is still unknown. Comparative animal studies and histology have not confirmed that a decrease in VBM equates to neuronal loss. This is supported by human studies in osteoarthritis and chronic low back pain that showed normalisation of aberrant VBM changes with recovery of pain (Gwilym et al., 2010, Ceko et al., 2015).

Functional changes in neuropathic pain have been reported in regions associated with pain processing and cognition. Spontaneous pain, known to be a key feature of neuropathic pain has mainly been explored using positron emission tomography (PET) studies as opposed to fMRI studies. This is because PET enables a constant baseline measurement of cerebral blood flow. These studies associate increased baseline activity in the insula, anterior cingulate (ACC) and posterior cingulate cortices (PCC) with spontaneous pain in neuropathic pain patients (Seifert and Maihofner, 2009). FMRI specific investigation of spontaneous pain in patients with chronic back pain has shown activation in the similar regions (Baliki et al., 2006). Evoked pain, including thermal allodynia, and punctate hyperalgesia in patients with CRPS, trigeminal neuralgia, neuropathic and central neuropathic pain has shown consistent activation in the insula, ACC, PCC, thalamus, somatosensory cortex and brainstem (Maihofner et al., 2005, Becerra et al., 2006, Schweinhardt et al., 2006, Baliki et al., 2012, Gustin et al., 2014).

Resting state networks, which underpin tonic cortical connectivity, have also been shown to undergo changes in neuropathic pain conditions. Baliki et al

reported alterations in the default mode network (DMN), a network active during rest, in patients with chronic back pain (Baliki et al., 2008). These aberrations in the DMN in chronic back pain, were more recently confirmed using arterial spin labelling (a novel approach to resting state network analysis) (Loggia et al., 2013). Additionally, patients with CRPS have been shown to have changes in not only the DMN but also the sensorimotor network (Bolwerk et al., 2013). Changes in resting state networks, have been reported in children with CRPS (Becerra et al., 2014). Interestingly, these changes reverted following intensive physical and psychological therapy. Becerra *et al's* work highlights the potential clinical applicability of resting state network analysis in chronic neuropathic pain.

In terms of CIPN, only two brain-imaging studies have investigated CIPN development and chronic CIPN (Boland et al., 2014, Nudelman et al., 2015). These have shown altered function in the superior frontal gyrus in patients displaying symptoms of CIPN. Additionally, Nudelman and colleagues have reported decreased grey matter volume in patients with CIPN a year after chemotherapy. Both studies show that investigation of the brain to better understand CIPN is an important paradigm shift in the CIPN research field.

2.4.1.2 FMRI studies of experimental neuropathic pain models

Experimental models of neuropathic pain used in fMRI studies, can be subdivided into those utilised in humans and those used in animals. Review of the animal neuropathic pain model literature is beyond the scope of this thesis. It is however, important to note that multiple animal neuropathic studies have utilised fMRI to investigate changes in animal brains before, during and after induction of neuropathic pain (Chang et al., 2014, Hubbard et al., 2015, Baliki et al., 2014). These have upheld the changes reported in humans and have proven useful in probing causal mechanisms underpinning not only the development and maintenance of neuropathic pain but also its conversion from the acute to chronic state.

In humans, experimental models of neuropathic pain used in fMRI studies include heat and cold induced allodynia using an MRI safe thermode, punctate mechanical hyperalgesia, capsaicin (TRPV1 receptor agonist) induced allodynia and hyperalgesia, and menthol induced cold hyperalgesia(Brooks et al., 2005, Iannetti et al., 2005, Lee et al., 2008, Wanigasekera et al., 2011). fMRI studies in healthy volunteers has helped identify a 'neurologic signature' of noxious heat (Wager et al., 2013). Regions consistently activated when processing this type of stimulus include the anterior and posterior insulae, PAG, thalamus, secondary somatosensory cortex and anterior cingulate cortex. Similar regions are activated by noxious cold (Atlas et al., 2014, Tracey et al., 2000).

Indeed, brain processing related to all models of neuropathic pain reveals activation in these same consistently reproducible regions (Seifert and Maihofner, 2009). This highlights the fact that although there are no pain specific brain regions, key areas are consistently involved in pain processing (Lee and Tracey, 2013).

2.4.2 Use of FMRI in neuropathic pain treatment trials

To date fMRI has not been used as an adjunct in any trials related to CIPN treatment. Use of fMRI as an adjunct to CIPN treatment RCTs has been suggested following review of the literature presented here (Seretny et al., 2013). Implementation of an FMRI paradigm for further assessment of menthol gel in chronic CIPN is planned as discussed in chapter 6. More broadly in terms of general neuropathic analgesic trials, currently there is an on-going neuropathic pain trial investigating gabapentin in women with chronic pelvic pain, which is utilising fMRI as an assessment tool (Horne et al., 2012).

Evidence related to the usefulness of fMRI in neuropathic and more broadly all chronic pain trials to assess analgesic efficacy (e.g. target engagement, and anti-nociceptive or anti-hyperalgesic effect) has been documented (Wanigasekera et al., 2016). FMRI as an adjunct in pain trials is increasingly discussed in related forums such as that of the European Medicines Agency and the US Food and Drug

Administration (Turk et al., 2008). As fMRI studies become less expensive, and their applicability to neuropathic analgesic trials more evident, it is strongly argued that fMRI will become a tool regularly utilised in investigations of novel analgesic agents (Tracey, 2013).

3. Methodology

A comprehensive overview of methods used in this thesis is presented in this chapter. The chapter itself is split into two sections. The first describes the general aims, design and recruitment of the CIPN study. A detailed description of the embedded fMRI study, which is the focus of this thesis, is presented. Secondly, the chapter deals with fMRI data analysis methods. Subsequent chapters detailing exploration of key study hypotheses will refer back to this methods section for clarity and in order to avoid repetition.

3.1 CIPN Study Overview

Clinical development of CIPN remains inadequately characterised (Delforge et al., 2010). Previous studies have utilised single assessment modalities such as neurophysiological testing and clinical reporting to diagnose CIPN (Argyriou et al., 2007a). The aim of the CIPN study was to prospectively characterise CIPN using multiple modalities; (QST), fMRI, CIPN20 questionnaire and confocal laser scanning microscopy (vivascope ®) techniques. The goal was to describe the natural history of CIPN, gain insight into underlying mechanisms and identify the sub-cohort of patients who may benefit from pre-emptive management. Specifically, this early preventive approach it would include closer monitoring, lower thresholds for regimen alterations or even different regimen if a choice exists. The author's contribution to this study was the embedded fMRI sub-study, focused on prospectively probing mechanisms of CIPN development.

3.1.1 Study Design & Objectives

The CIPN Study was a prospective, observational, multi-centre, cohort[1] study investigating CIPN development in cancer patients. The study had the following objectives:

Primary:

[1] For the fMRI study 16 healthy age, sex, matched controls were also recruited and scanned towards the end of the study in early 2015. This was done in anticipation of possible reviewer comments for future publications. The healthy volunteer data was not analysed for the purposes of this thesis due to time constraints and will only be mentioned in terms of recruitment eligibility.

To prospectively characterise the psychophysical properties of chemotherapy induced peripheral neuropathy according to: 1) Quantitative Sensory Testing (QST) thresholds and 2) modified QST (punctate stimuli only) during functional Magnetic Resonance Imaging (fMRI), to determine the proportion of patients who experience neuropathic symptoms at the end of chemotherapy treatment compared to baseline. Of the primary objectives the second point relates to work detailed in this thesis and conducted by the author.

Secondary:

- During and after chemotherapy to prospectively determine the pattern of change over time in: physical function (outcome measures: slotted grooved peg board to assess hand dexterity) and patient rated symptoms of peripheral neuropathy (outcome measures: Visual Analogue Scale (VAS), Brief Pain Inventory (BPI) and CIPN-20), Rydel-Seiffer Graduated Tuning Fork MD Anderson Symptom Inventory (MDASI)) and mood ((Hospital Anxiety and Depression Scale (HADS) and Pain Catastrophizing Scale (PCS)) and their relationship to QST findings.
- To explore baseline predictors of neuropathy at the end of chemotherapy treatment and 1 year after chemotherapy
- To collect blood and sputum samples for future analysis of potential pharmacogenomics correlates of CIPN and mitochondrial changes, as well as to correlate sex hormone (testosterone) level with pain development.

Of the secondary objectives point 2 and 3 relate to work conducted by the author and presented in this thesis.

3.1.2 Patient Selection & Recruitment

Possible study patients were identified from the Edinburgh Cancer Centre, NHS Fife Oncology Clinic and Forth Valley Royal Hospital Oncology Clinic by oncologists and research staff. They were approached for recruitment only after the clinical team mentioned the study to them and if their consultants felt they were suitable for the study. If interested patients were given study information sheets and had a minimum of 24hours to consider the study. Interested patients

gave written informed consent and a suitable time for assessment was organised to coincide with their hospital visit. If appropriate patients consenting to the main study were asked to participate in the fMRI part of the study. If patients expressed an interest in the study but only wish to take part in the MRI part of the study this was offered until the time when the fMRI part of the study closed in May 2015. A flow diagram adapted from the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines (von Elm et al., 2007), detailing eligibility and recruitment to the fMRI component of the study is shown in figure 3.1.

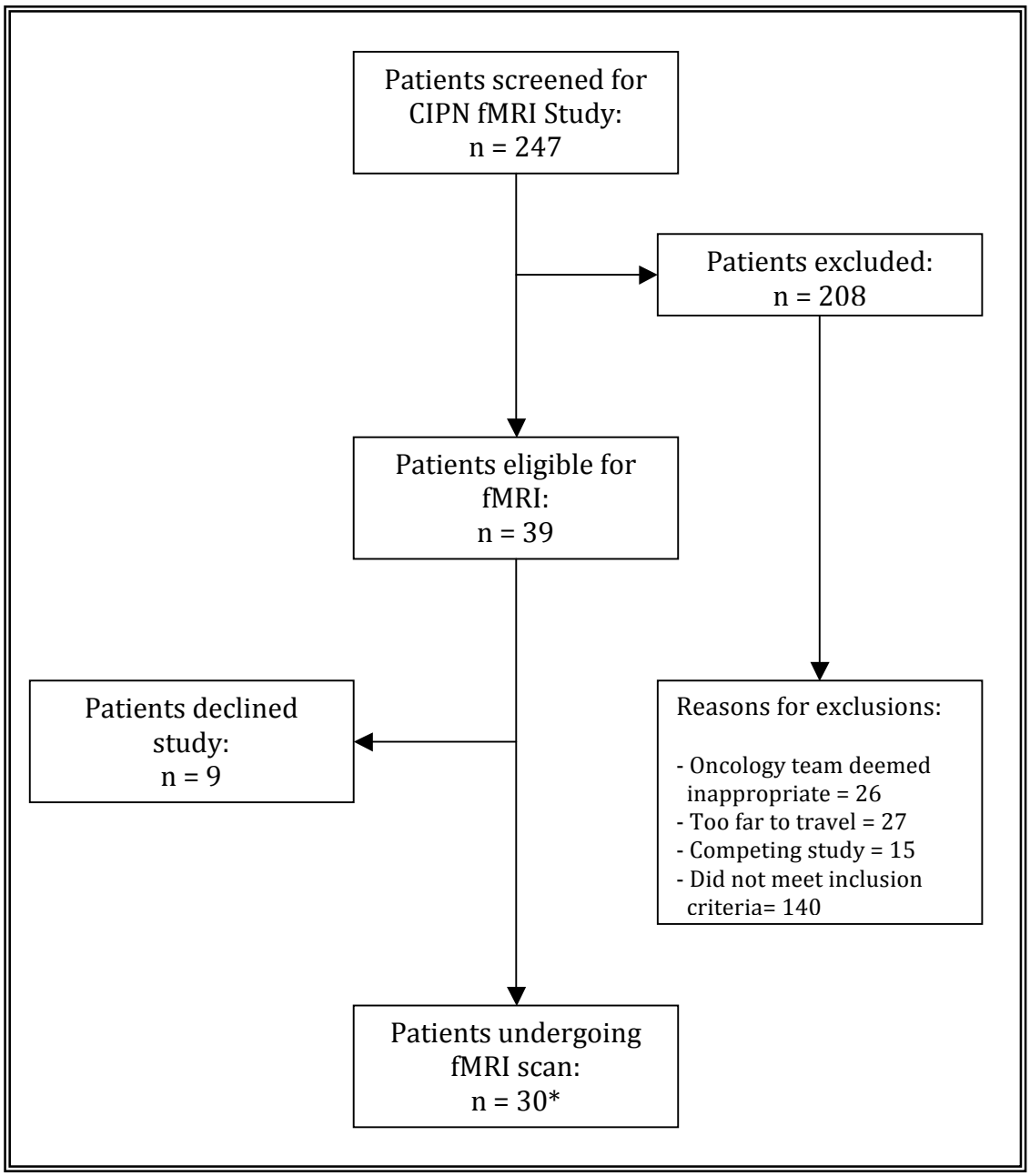


Figure 3.1 Flow diagram of patient eligibility and entry to the CIPN study. Adapted from the STROBE guidelines (von Elm et al., 2007). *The exact number included in each analysis is described in the results section of relevant chapters.

The following inclusion criteria were used for recruitment:

(a) Planned to receive bortezomib for the first time (for multiple myeloma) or oxaliplatin, paclitaxel, taxotere, cisplatin (for adjuvant treatment with curative intent of colorectal, testicular, uterine or ovarian cancer).
(b) Aged 18 years or over at study entry.
(c) Patient's usual medical team agree to their taking part in the study.
(d) Able to provide informed written consent to participation in the study after explanation of the study protocol.
(e) Have the ability to complete questionnaire assessments in English language.
(f) In the opinion of the investigator, the patient is able to complete the various assessments.

Patients were excluded if any of the following criteria were met:

(a) Neurological conditions which may influence findings (such as Multiple Sclerosis or residual signs/symptoms from a previous stroke).
(b) Patients with pre-existing neurological or chronic pain/neuropathic conditions.
(c) Patients with diabetes, a history of alcohol excess or pre-existing chemotherapy
(d) Skin conditions that prevent assessment of the relevant areas affected by peripheral neuropathy.
(e) Suffering from significant psychiatric illness, which would hinder their completion of the study.
(f) General medical condition is unstable or rapidly deteriorating, such that they are unlikely to be able to contribute to the study.
(g) In the opinion of the Research Team or their usual medical team, would be unable to complete the study protocol for any other reason.
(h) For the MRI component of the study: patients who have any contraindication to MRI (eg: metal implants)

Healthy participants (controls) were recruited from hospital and university staff family and friends. Volunteers were sought via posters. Interested persons were given study information sheets and had a minimum of 24hours to consider participation in the study. If interested, after written informed consent was obtained a suitable time for assessment for either QST or fMRI was scheduled.

Inclusion criteria for healthy volunteers was as follows:

(a) Similar age and same sex as the patients being matched to (anticipated to be age 50 and above)
(b) World Health Organisation performance status 0 – 1
(c) Able to provide informed written consent to participation in the study after explanation of the study protocol.
(d) Have the ability to complete questionnaire assessments in English language.

The following exclusion criteria applied to healthy volunteers in the study:

(a) A current cancer diagnosis (excluding basal cell skin cancer or early localised prostate cancer on 'watch and wait'/ active monitoring)
(b) Risk factors for peripheral neuropathy such as diabetes, chronic alcoholism and previous history or current use of drugs which cause peripheral neuropathy.
(c) Neurological conditions which may influence findings (such as remitted Multiple Sclerosis, residual signs/symptoms from a previous stroke or chronic on-going pain).
(d) Skin conditions which prevent assessment of the relevant areas affected by peripheral neuropathy
(e) Suffering from significant psychiatric illness, which would hinder their completion of the study.
(f) In the opinion of the Research Team would be unable to complete the study protocol for any other reason.
(g) Taking regular pain killers
(h) Contraindication to MRI (eg metal implants) for MRI part of the study.

3.1.3 FMRI Study

The rationale underpinning the introduction of fMRI into the CIPN study relates to the difficulties in studying neuropathic pain conditions. CIPN like other neuropathic conditions, is difficult to quantify clinically or using quantitative sensory testing (QST). As discussed above (see 1.1.3.4 and 2.4) fMRI has been used to understand pain processing in both health and illness. In particular fMRI allows for an objective quantification of pain states. To date the influence of brain pain processing on CIPN development has not been investigated in a prospective way in humans. This study is the first to utilise fMRI to prospectively explore associations between brain structure and function and CIPN.

3.1.3.1 FMRI Experimental Design

3.1.3.1.1 Study flow and fMRI scan timing

Cancer patients, prior to chemotherapy onset, were recruited and consented to undergo an fMRI scan at the clinical research-imaging centre (CRIC) at the University of Edinburgh. Prior to the scan patients completed the CIPN20 questionnaire and had basic demographic data collected. All patients underwent one scan only at this single time point.

Post scan follow up depended on whether the patients had consented for only the fMRI sub-study or had opted to also take part in the detailed quantitative sensory testing study arm. If patients consented to only the fMRI they were followed up with the CIPN20 questionnaire only. The CIPN20 questionnaire was chosen as this is the only questionnaire available, which is specific for CIPN development (see discussion in section 3.2). The questionnaire was administered over the phone or in clinic prior to each subsequent chemotherapy cycle and then at 3, 6, 9 and 12 months after chemotherapy completion. If patients opted for the QST arm of the study they were followed up at the time points detailed above with the following questionnaires: VAS, BPI, HADS, MDASI, PCS as well as detailed neurophysiological assessment. Patient flow through the CIPN fMRI study is shown in figure 3.2.

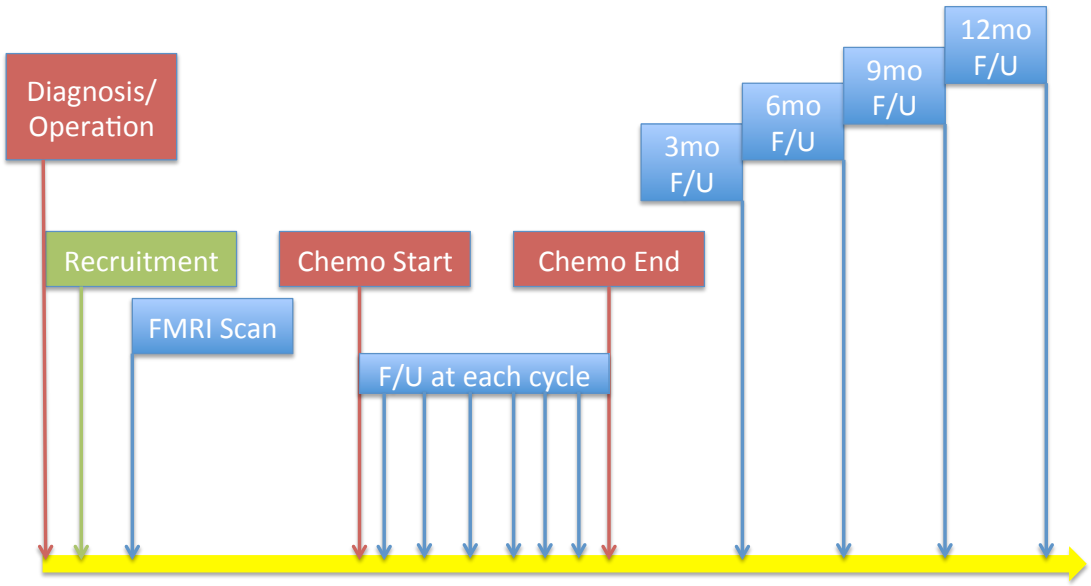


Figure 3.2. Summary of study and timing of scan. Yellow arrow represents time since diagnosis but is not to scale as timings varied. Standard time between diagnosis and recruitment was around 7 weeks; however, for the patients who did not need an operation this was shorter. Time between recruitment, fMRI scan and first chemotherapy cycle also varied for some patients, as it was within the same week for most, but for others up to 2 weeks. Number of follow up time points within chemotherapy depended on the number of chemotherapy cycles given.

3.1.3.1.2 Stimuli presented in the scanner

Design of the experimental paradigm for the CIPN fMRI sub study was guided by the research questions outlined above, clinical knowledge and literature detailing previous successful fMRI pain experiments. Past research and clinical experience suggested that both thermal (Attal et al., 2009) and mechanical (Park et al., 2009) nociceptors are involved in CIPN development. Consequently, assessment of the brain response to both punctate and thermal stimuli was planned in the experiment. However following study set up and several healthy volunteer scans over many months, thermal stimuli was removed from the experiment due to practical limitations related to equipment associated noise issues that despite considerable effort could not be resolved (see 3.1.3.2 and Appendix D).

The final experimental paradigm, shown in figure 3.3 included 64 punctate stimuli administered in a jittered sequence above the right medial malleolus in all patients. A 256mN von Frey filament was used for every participant.

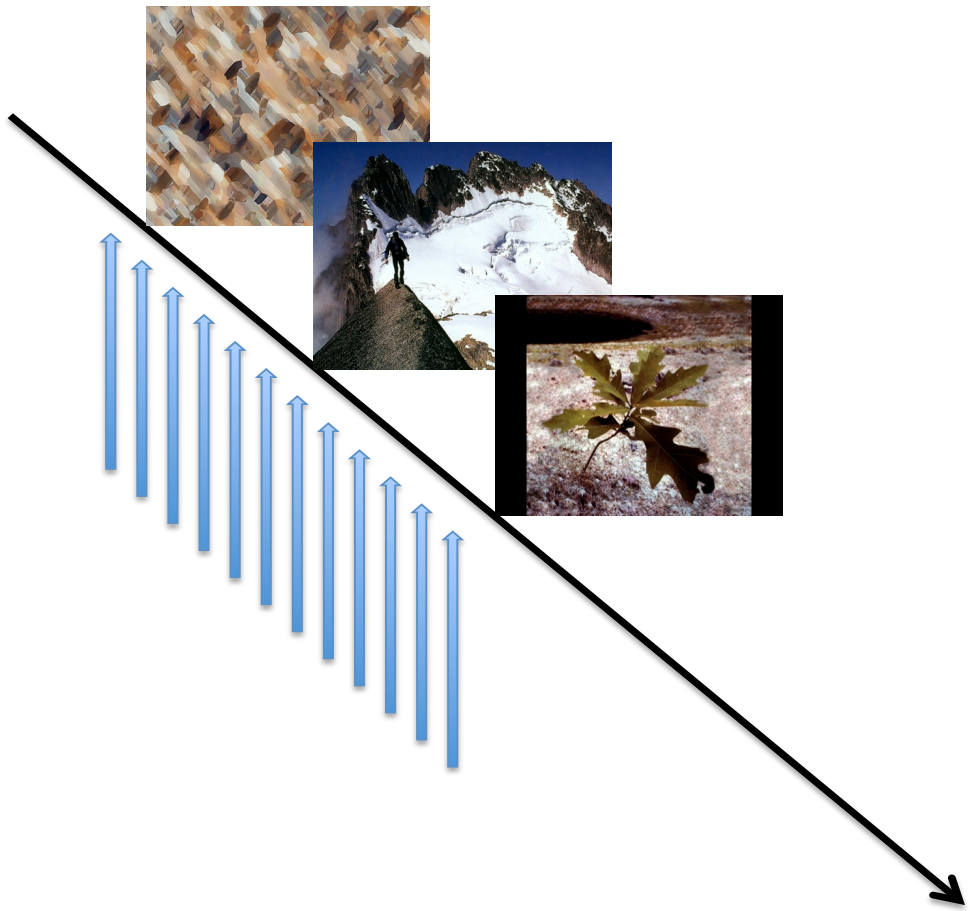


Figure 3.3: fMRI Paradigm. Black arrow indicating duration of fMRI sequence (16minutes), blue arrows representing jittered presentation of punctate stimuli. 64 punctate stimuli given in total (above right medial malleoulus using 256mN von Frey filament). An average of 4 stimuli was delivered during a block of images. Boxes from top left: top showing coloured jittered image with no content, termed 'snow'. Example positive (middle) and neutral (bottom) images from international affective picture system (IAPS). Images were presented in blocks containing ten images of each kind, with each image displayed for 10 seconds, throughout the whole duration of experiment. No image was repeated. Every participant was presented with the images in the same order. Snow images served as the baseline (ie patients never looked at a black screen to avoid confounding of visual cortex viewing images and then black screen).

The size of the filament and the standardised use of the same filament in all patients was chosen for pragmatic reasons. Specifically, lack of time and physical space in the imaging centre prevented identification of the individual sharpness

threshold for each participant individually prior to the scan. This approach was felt to be viable experimentally for two reasons. Firstly, previous fMRI pain studies have shown strong engagement of the descending pain modulatory system (DPMS) in healthy volunteers following stimulation with von Frey filaments (Lee et al., 2008, Iannetti et al., 2005, Zambreanu et al., 2005). Secondly, in CIPN specific research abnormalities in response to punctate stimuli have been demonstrated before and after chemotherapy (Kroigard et al., 2014, Wang et al., 2016).

In addition to the punctate stimuli, a clinically driven research question related to affective processing was included in the experiment. There is evidence demonstrating altered pain processing related to emotional states and diverse emotional input (de Wied and Verbaten, 2001, Ploghaus et al., 2001, Berna et al., 2010). It has been shown that affective stimuli processing differs between healthy volunteers and patients (Kamping et al., 2013). Whether this is also true of patients who develop CIPN is unknown. Therefore during the fMRI experiment in addition to being presented with punctate stimuli patients were also shown positive and neutral images from the international affective picture system (IAPS) dataset (Lang et al., 2008). Ekman Faces, another known affective dataset, was not chosen, as the emotional responses are less specific and lack variation in terms of content (i.e. human faces only).

IAPS are a well-validated dataset of images known to have emotional valence. For this experiment only positive and neutral images were selected (fig 3.4). All images in the positive category were selected in order to provide enough variation without the need to repeat images. Moreover, to avoid the confounding effect of engaging and disengaging the visual cortex when moving from positive to neutral images, a coloured snow image aimed to match the complexity of the IAPS without providing any context, was shown instead of a black screen. The snow image was generated using a random image generator freely available at http://rndimg.com/default.aspx. The snow images served as the baseline condition. Each image was shown for 10 seconds. No image was repeated. Figure 3.4 shows examples of each image type.

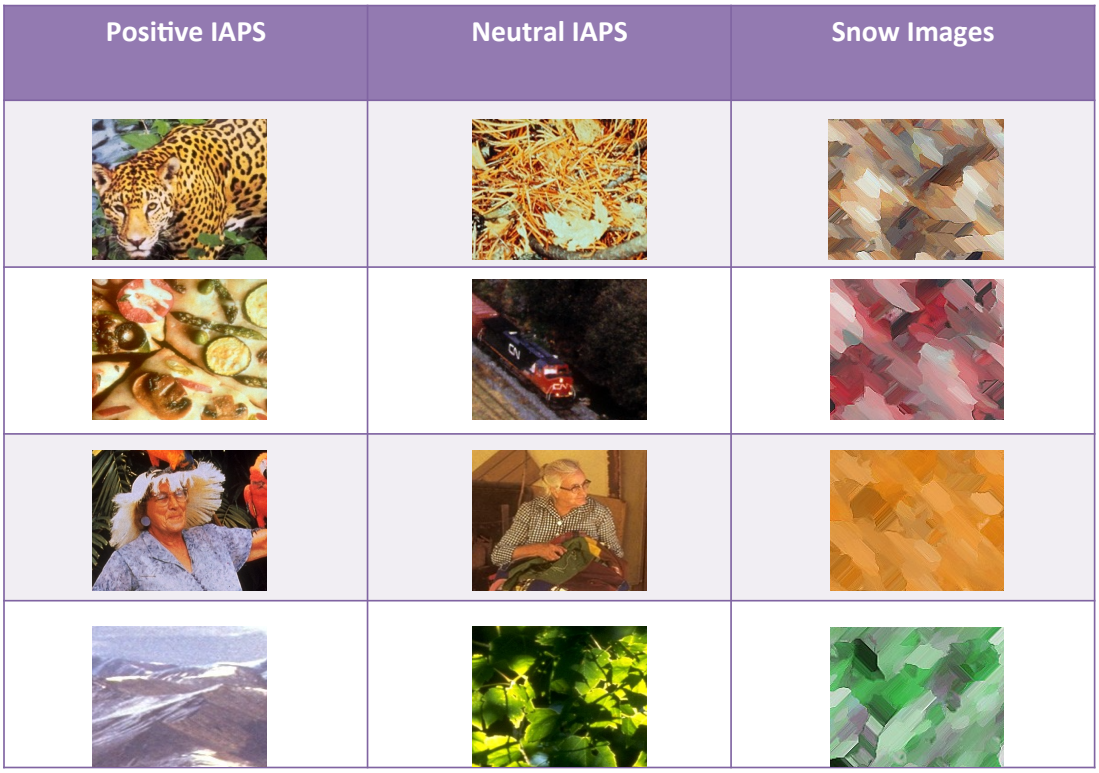


Figure 3.4. Examples of images shown to participants during the fMRI scan. Each image was displayed for 10seconds. No images were repeated. Images were presented in blocks of 3 neutral, 3 snow, and 3 positive images repeated in a pseudo randomised order (i.e. positive, snow, neutral, then neutral, snow, positive, etc.) throughout the whole 16 minutes of the sequence. During each block jittered punctate stimuli were delivered. On average 4 punctate stimuli delivered per block.

3.1.3.1.3 Sequences and Acquisition parameters

The following sequences were acquired for each participant in the order presented: structural T1 weighted MPRAGE sequence, a grey field map (see 3.2.1.3) for data preprocessing, a resting state arterial spin labelling (ASL) sequence (10 minutes), a resting state BOLD sequence (10minutes) and finally the BOLD functional sequence (16 minutes). For the functional acquisitions the TR (see 1.1.3.1) was 3.0 seconds, TE 30mseconds, slice thickness 3mm, voxel size 3x3x3mm, phase encoding direction anterior>posterior, and field of view (FOV) 192x192mm. This equated to a total of 204 volumes for the resting state sequences and 324 volumes for the fMRI sequences. The structural scan had a voxel size of 1x1x1mm and an anterior>posterior phase encoding direction.

3.1.3.2 fMRI Scan Set-Up for the CIPN Study

3.1.3.2.1 Experimental Equipment

Prior to the start of the CIPN study the University of Edinburgh had not engaged in pain research using fMRI. Consequently, although a Siemens 3T MRI scanner was available at the Clinical Imaging Research Centre (CRIC), no ancillary equipment necessary for pain fMRI studies was available. In particular, physiological noise monitoring and MRI safe heat stimulus presentation equipment had to be acquired and set up prior to commencing the CIPN fMRI study.

Physiological Noise Monitoring Equipment:
As detailed above (see 1.1.3.3.2), collecting data on heart and respiratory rate during pain fMRI experiments may help optimise data analysis down the line. Equipment needed for this recording needs to both be MRI safe and also compatible with software used to present any information to the participant in the scanner.

A standard set up using the BIOPAC system (www.biopac.com), a locally sourced optical to electrical signal transmitter box made following a design from Dr Brooks and colleagues at the University of Bristol, respiratory bellows and MRI safe connector cables was undertaken. Details are shown in figure 3.5.

It is worth noting that introducing new equipment into the MRI environment is not straightforward. Each piece of new equipment must be MRI compatible. Additionally, all equipment must be well insulated as to not act as a source of interference. Multiple pieces of equipment introduced together are more likely to cause noise as a result of interaction. Noise became a considerable problem after set up of the PNM equipment and heat stimulation equipment, as discussed below and in Appendix D.

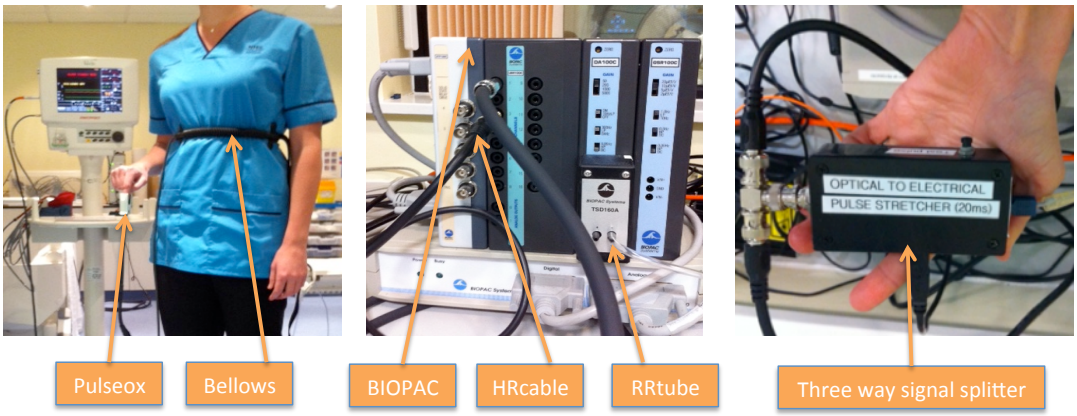


Figure 3.5. Set up of the PNM data recording equipment. The connection is in the following order: bellows around participant extend through the waveguide to the BIOPAC via a hollow plastic tube (RRtube). The BIOPAC is a system of amplifiers and transducers used to acquire physiological signal. The pulse oximetry probe (pulseox) is attached to patient and extends through a connecting cable to a filter installed in the penetration panel. From here, a secondary cable (HRcable) connects the pulse oximetry to the BIOPAC. The BIOPAC is connected via a three way optical to an electrical transmitter box (far right), to receive scanner pulses. This connection ensures that scanner triggers are identical between the BIOPAC and presentation script. The BIOPAC is then connected to a laptop with software able to display the relevant waveforms and scanner pulse triggers. Software on the laptop interacts with the BIOPAC to record the physiological data at a frequency of 20Hz.

Equipment for Thermal Stimulus

As with all laboratory environments, experimental conditions within the MRI scanner should be kept constant. As a result, any stimuli applied to the patient during an MRI experiment needs to be measurable, accurate and repeatable. To achieve this for thermal stimuli a computerised system is optimal.

A popular system used in other fMRI centres is the Medoc Pathway System (http://www.medoc-web.com/products/pathway). This was purchased and installed at CRIC in January 2013. Unfortunately, despite its MRI specifications the system did introduce some noise into the scanner environment. This was compounded by the simultaneous introduction of the PNM equipment. The impact on scan data quality was unacceptable. Therefore, after extensive noise diagnostics (see appendix D), the challenging decision was made to not utilise the Medoc thermal system in the CIPN fMRI study.

3.1.3.1.2 Additional Data Collected on Scanned Patients

Patients undergoing an fMRI scan were also asked to consent to giving blood samples for future genetic analysis. These samples were stored. Additionally, patients were asked to give samples of saliva to test testosterone levels at the time of the scan. All scans occurred during the day, but varied between afternoon and mornings sessions, and testosterone sampling also varied. Collection of testosterone was undertaken to explore the association of serum testosterone levels and CIPN development. There is a growing body of evidence suggesting that testosterone and other hormones influence the occurrence and maintenance of chronic pain conditions (Vincent et al., 2013, Vincent and Tracey, 2010). The impact of testosterone level on subsequent CIPN development is unknown. Exploration of these hormone data is presented in Appendix G.

During the scan patients were asked about their pain (scored from 0 to 10) on the day and in the preceding 2 weeks. Following punctate stimuli in the scanner patients were asked to give a sharpness rating (from 0 to 10 with 10 equating to a needle prick). Due to a protocol oversight sharpness ratings were only introduced during the second half of the study and therefore were unavailable for some of the scanned participants (see discussion on study limitations in chapter 7).

3.1.3.3 fMRI Data Completeness

FMRI data collection was mostly complete as follows. No participant withdrew from the study during the scan. However, a small number of sequences failed during acquisition and in one instance a field of view was set too small to enable data processing. These are detailed in table 3.1. Follow up data, including the CIPN20 questionnaire is still being acquired and the current status of patient follow up is summarised in table 3.2. Any missing sequences were excluded from the given analysis. Reasons are detailed in relevant chapters.

Patient ID	Sequence			
	Structural	ASL RS	BOLD* RS	BOLD* Functional (EPI)
CIPN0002	✓	✓	✓	✓
CIPN0003	✓	✓	✓	✓
CIPN0004	✓	✓	✓	✓
CIPN0005	✓	✓	✓	✓
CIPN0006	✓	✓	✓	✓
CIPN0007	✓	✓	✓	✓
CIPN0008	✓	✓	✓	✓
CIPN0012	✓	✓	✓	✓
CIPN0013	✓	✓	✓	✓
CIPN0015	✓	✓	✓	✓
CIPN0016	✓	✓	✓	Presentation software failure
CIPN0017	✓	✓	✓	✓
CIPN0018	↓ FOV	✓	✓	✓
CIPN0019	✓	✓	✓	✓
CIPN0020	✓	✓	✓	✓
CIPN0022	✓	✓	✓	✓
CIPN0023	✓	✓	✓	✓
CIPN0024	✓	✓	✓	✓
CIPN0025	✓	✓	✓	✓
CIPN0026	✓	✓	✓	✓
CIPN0027	✓	✓	✓	✓
CIPN0028	✓	✓	✓	✓
CIPNFV01	✓	✓	✓	✓
CIPNFV02	✓	✓	✓	✓
CIPNFV03	✓	✓	✓	✓
CIPNV001	✓	✓	✓	✓
CIPNV002	✓	✓	Scanner failure	✓
CIPNV003	✓	✓	✓	✓
CIPNV004	✓	✓	✓	✓
CIPNV005	✓	✓	✓	✓

Table 3.1. Summary table of MRI sequence acquisition, ticks indicate adequate data collection. FOV= field of view. Comments highlight issues with specific sequences. Bold* sequences refer to EPI T2* acquisitions optimised for the BOLD response (see 1.1.3.2).

3.2 Defining CIPN

CIPN may occur and resolve during chemotherapy cycles or within the first three months after chemotherapy completion. This is termed acute CIPN and is distinguished from chronic CIPN, occurring three months or more after

chemotherapy cessation (Ventzel et al., 2015). Insight into the evolution, and interrelatedness of acute and chronic CIPN is lacking. Clear clinical definitions are not uniformly available or used across centres. This has caused limitations for research purposes in terms of outcome measures, with recent attempts to standardise practice (Cavaletti et al., 2013).

One standardised tool currently in use for CIPN assessment in research is the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Chemotherapy-Induced Peripheral Neuropathy 20 (EORTC QLQ-CIPN20). This questionnaire contains 20 questions assessing the 3 components of CIPN- sensory, autonomic and motor (see Appendix H). It has been validated in a large international cohort (http://groups.eortc.be/qol/chemotherapy-induced-peripheral-neuropathy-eortc-qlq-cipn20) and is currently being assessed for sensitivity and specificity. CIPN20 is highly detailed yet quick to complete either in person or over the phone. Consequently, it was considered to be a good measure for defining chronic CIPN in the CIPN fMRI Study.

At the time of writing however, follow up of scanned patients was incomplete. Therefore, use of the CIPN20 questionnaire as a way of defining CIPN was not possible in terms of a standardised comparison of the same time point across all patients (see table 3.2). As a result, a decision was made to define CIPN in a clinically meaningful way for all analyses presented in this thesis. Consequently, the common toxicity score (see table 3.4) cut off was used. If a patient had any dose decrease or cessation due to CTS criteria being met they were defined as CIPN (see table 3.3). Criteria for reduction are pre specified for each individual chemotherapy regimen and specified locally according to latest evidence. Details of the criteria are shown in appendix K.

Chemotherapy dose reduction as a consequence of intolerable CIPN has likely implications not only for survival but also for patient morbidity. Arguably this clinical endpoint therefore has the most important patient centred implications, supporting its use here.

Patient ID	Time Point				
	Intra Chemo	3mo	6mo	9mo	12mo
CIPN0002	✓	✓	✓	✓	D
CIPN0003	✓	✓	✓	✓	✓
CIPN0004	✓	LTF	LTF	LTF	LTF
CIPN0005	✓	✓	D	D	D
CIPN0006	✓	✓	✓	✓	✓
CIPN0007	✓	✓	✓	✓	✓
CIPN0008	✓	✓	✓	✓	✓
CIPN0012	✓	✓	✓	✓	✓
CIPN0013	✓	✓	✓	✓	✓
CIPN0015	✓	✓	✓	✓	✓
CIPN0016	✓	✓	✓	✓	✓
CIPN0017	✓	✓	✓	✓	✓
CIPN0018	✓	✓	✓	✓	
CIPN0019	✓	✓	X	X	X
CIPN0020	✓				
CIPN0022	✓	X	X	X	X
CIPN0023	✓				
CIPN0024	✓				
CIPN0025	✓	✓			
CIPN0026	✓	✓			
CIPN0027	✓	✓			
CIPN0028	S	S	S	S	S
CIPNFV01	✓				
CIPNFV02	✓				
CIPNFV03	✓				
CIPNV001	✓				
CIPNV002	✓				
CIPNV003	✓				
CIPNV004	✓				
CIPNV005	✓				

Table 3.2. Summary of follow up status for CIPN fMRI study participants. D= deceased, LTF=Lost to follow up, X= restarted due to disease progression. S= chemotherapy stopped after one cycle. Tick indicates completed follow up.

Patient ID	Common Toxicity Score	
	Pre Chemo	Worst Score During Chemotherapy
CIPN0002	0	1
CIPN0003	0	0
CIPN0004	0	1
CIPN0005	0	1
CIPN0006*	0	2
CIPN0007*	0	2
CIPN0008*	0	2
CIPN0012*	0	2
CIPN0013	0	0
CIPN0015*	0	2
CIPN0016	0	2^
CIPN0017*	0	2
CIPN0018	0	2&
CIPN0019	0	0
CIPN0020*	0	2
CIPN0022	0	1
CIPN0023*	0	2
CIPN0024	0	1
CIPN0025	0	2$
CIPN0026*	0	2
CIPN0027*	0	2
CIPN0028*	0	3
CIPNFV01	0	1
CIPNFV02*	0	2
CIPNFV03*	0	2
CIPNV001*	0	3
CIPNV002	0	3@
CIPNV003*	0	3
CIPNV004*	0	3
CIPNV005*	0	2

Table 3.3: Common Toxicity Score for each patient. Patients classified as having acute CIPN are marked with an *. Please note this resulted in an acute CIPN incidence of 56% in the study cohort. ^=Gastrointestinal side effects/diarrhoea. &=Neutropenia. $=Thrombocytopenia. @= Non neuropathic complication.

Score	Definition
0	None
1	Mild paraesthesia or loss of reflexes
2	Moderate; limiting instrumental ADL
3	Severe symptoms; limiting self care ADL
4	Life-threatening consequences; urgent intervention required

Table 3.4 Common Toxicity Score. ADL= activities of daily life. Treatment dose was reduced if patients had grade 2 or 3 toxicity and stopped if grade 3 toxicity lasted for seven days or more.

3.3 Ethical Considerations and Data Protection for the CIPN Study

Ethical approval was obtained prior to commencement of this study (Appendix E). The study was carried out in full accordance with the current versions of the International Conference on Harmonisation Good Clinical Practice Guidelines (ICH GCP), the World Medical Association Declaration of Helsinki, as well as national and local regulations. All patients gave written informed consent at the start of the study and verbal consent was obtained at each follow up to confirm continued consent.

Data was handled in accordance with regulatory guidelines. All data including fMRI data was anonymised from the point of entry into the study with each subject being identified by a study number only. Paper copies of any study related documents (Case Report Forms (CRFs), source data, consent forms, and regulatory documents) were kept in a locked filing cabinet in a secure room with restricted access. Electronic data was anonymised and stored on password protected database, on a secure network with password protected and restricted access. All data and documentation was made available for monitoring, audit and regulatory inspection. Data will be stored for 5 years.

3.4 fMRI data analysis

3.4.1 fMRI Data Analysis Overview

The aim of acquiring and analysing fMRI in this study is to gain a quantitative summary of differences in both brain structure and function between patients who develop CIPN and those who do not. These quantitative summaries are derived from statistical inferences. For functional data these statistical comparisons are made between BOLD signal changes resulting from the experimental stimuli presented, and baseline BOLD signal. For structural data differences in segmented volumes of pre-specified structures are compared. All scan data for the CIPN study was analysed using FMRIBs Software Library (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) (Jenkinson et al., 2012). Chapter 6 detailing the development of a CIPN treatment FMRI study is the exception in terms of analysis. Data presented in this chapter was analysed using Statistical Parametric Mapping (SPM) software (http://www.fil.ion.ucl.ac.uk/spm/). SPM analysis follows the principles of FSL data analysis outlined below. The software packages are similar and usage depends on training and user preferences. Analysis of the single MINT3 pilot scan, summarised in chapter 6 was carried by a colleague (Dr Liana Romaniuk) who is trained in the use of SPM and not FSL software. In every analysis chapter key confounds are checked and detailed in table format to ensure no overt differences between the CIPN and non CIPN group exist, aside from the neuropathy.

3.4.2 fMRI Study Sample Size Calculation

Sample size calculation for fMRI studies is not straightforward (Friston et al., 1999). This is because specification of the alternative hypothesis, required for standard power and sample size calculations, cannot be made in quantitative terms, due to the convolution of the hemodynamic response function (Friston, 2011). Consequently, the standard approach used to derive sample size for fMRI studies is to base this estimate on the number of participants use in published studies of the same nature, able to reject the null hypothesis in one or more voxels. Estimates of 12 to 16 subjects per group have been used as a guide in fMRI studies (Desmond and Glover, 2002). There have however been recent calls

to make sample size calculations in fMRI studies more stringent (Guo et al., 2014).

For this study a pragmatic approach based on the above literature was used. Sample size was based on previous successful pain fMRI studies and an estimate of new chemotherapy cases known to present in to the Edinburgh oncology service. A sample size of 15 patients per group was planned, with 30 patients being recruited to the study.

3.4.3 fMRI Data Pre-processing

In order to compare the brain function and structure of individual patients a number of data preparation steps need to be undertaken prior to statistical analysis. An overview of these is given below.

3.4.3.1 Brain Extraction

Extracting the brain data from images of the whole head (see figure 3.6) is important in order to robustly assess both structural and functional changes in a standardised way. This is done because there is extensive variability in non-brain tissues (skull, subcutaneous fat, eyes), which may bias subsequent analysis steps. In FSL this preprocessing step is performed using a tool called Brain Extraction Tool (BET), which strips non brain tissue out of the image (Smith, 2002). For this analysis the central point of the brain, was set manually for each participant to enhance the quality of each BET run.

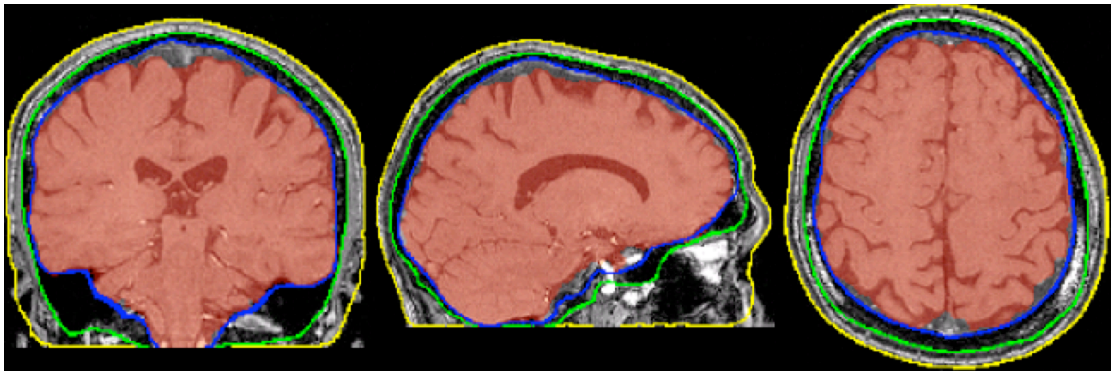


Figure 3.6. Example of brain extraction: in pink the brain after removal of other components, blue meninges and green and yellow the inner and outer surfaces of the skull respectively. Figure adapted from (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/BET#Research_Overview).

3.4.3.2 Motion Correction

Lying perfectly still for the duration of any fMRI experiment is impossible. Subjects always move in the scanner and because of the sensitivity of the BOLD signal to interference, motion is an important source of noise and decreased SNR in fMRI experiments. Consequently, correction for motion is a standard aspect of data preparation prior to analysis in order to ensure that each voxel in the image corresponds to a consistent anatomical point throughout the duration of the experiment. In FSL a tool called (MCFLIRT)(Jenkinson et al., 2002) is used to align each acquired volume to a single reference point (for this study the middle volume). This allows motion parameters to be extracted from the experimental time series. These can be included in later analysis as repressors of no interest and are also summarised by FSL to enable assessment of participants moving excessively during the scan. This was done for all functional analyses in this thesis.

3.4.3.3 Boundary Based Registration (BBR) & B0 unwarping

These two data cleaning steps are performed in one step in FSL data analysis and will therefore be described in one section here.

Boundary Based Registration

In order to identify anatomically understandable BOLD signal activation, the low resolution functional echo planar imaging (EPI) sequence must first be

registered or mapped onto the higher resolution structural T1 image of the same patient. By aligning the functional EPI sequence with the high quality T1 image, used clinically by radiologists to report structural findings in the brain, accurate identification of regions activated during an fMRI experiment can be achieved. Further to be able to quantify and compare activation not only within subjects but also between subjects, individual patients' T1 structural images need to be registered to a standard brain image. This enables certainty that in each of the voxels being compared the same part of the anatomy from each of the subjects' scans at different time points is present. The standard brain also referred to as a standard space, provides a coordinate reference system for each anatomical structure. The standard space used for registration in this thesis is the Montreal Neurological Institute (MNI) 152 2mm average brain (Grabner et al., 2006). This image is an average of 152 T1 weighted adult MRI images transformed to form a model in Talairach space[2].

The process used for registration of the subject EPI functional data to their high resolution structural scan is known as boundary based registration. This utilised white matter boundaries, sampling 2mm space around these boundaries to align the two image types in an accurate manner (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FLIRT_BBR). Prior to BBR completion, correction of magnetic field inhomogenities in the data is required. This is done using a field map (see below).

Grey Field Map Acquisition, Preparation and B0 unwarping

During fMRI scans of the brain inhomogeneity exists in the main magnetic field (B0) caused predominantly by areas of air, tissue interface such as sinus cavities. These inconsistencies in the field can bias the analysis of fMRI data and where possible should be corrected. This can be done by recording a map of the magnetic field and using this to adjust the acquired data prior to registration. For this analysis the field map was obtained from the scanner in 3 separate formats.

[2] Talairach Space is the original standard space, which supplied a coordinate system for the whole brain. This reference was derived from mapping a hemisphere of one post-mortem brain. This was used for many years but has now been superseded by the MNI space and other population applicable MRI derived standard spaces (eg for infants and patient groups).

These were reorganised into two folders (magnitude and phase images) prior field map preparation, which was done using a tool called Fsl_prepare_fieldmap (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FUGUE/Guide#Making_Fieldmap_Images_for_FEAT). Field maps were acquired, prepared and used for all subjects in all functional data analyses.

3.4.3.4 Spatial Smoothing

This processing step is utilised to improve the SNR by taking each volume of the data and convolving it with a Gaussian profile filter, which blurs the noise in the image out whilst leaving the desired signal intact. Smoothing using a Gaussian filter is also required for subsequent thresholding of data using *Gaussian Random Field Theory.* The default 5mm smoothing available in FSL was used throughout the analysis presented in this thesis. The mathematical theory underpinning spatial smoothing and subsequent thresholding is beyond the scope of this work and is further described in http://www.fil.ion.ucl.ac.uk/spm/doc/books/hbf2/pdfs/Ch14.pdf.

3.4.3.5 Temporal Filtering

In addition to optimising the signal to noise ratio at any given volume the consideration of time is also required for functional data, which was acquired over 16 minutes in the case of the CIPN study. The artefacts which impact the data over time include low frequency drifts which are scanner related (e.g. heating effects) and high frequency noise which may, for instance be physiological noise related (cardiac and respiratory cycles). Use of a high pass filter allows removal of unwanted low frequency noise whilst leaving neuronal activation signal intact. A high pass filter was used for all functional analyses in this thesis. Finally, it is important to note that depending on sampling frequency physiological noise may present as high or low frequency noise, use of a low pass filter very often eliminates signal, particularly in an event related experimental paradigm, such as the punctate stimuli used in this study. Therefore, other techniques to manage physiological noise are required and are described below.

3.4.3.6 Physiological Noise Correction

As discussed above (1.1.3.3.2 and 3.1.3.2.1) in order to correct for the interference of heart and respiratory rate on BOLD signal, data on these variables needs to be collected. This data is then prepared using an FSL tool called pnm_ prepare, which creates repressors which can be used in the set up of the individual subject statistical analysis model (Brooks et al., 2008). In doing so, the noise is regressed out whist the signal of interest -often co-linear in some way, particularly in pain experiments- remains. For the CIPN study these data were collected. However due to time constraints, PNM regressors were not prepared or used in the final model set up. In general physiological noise was instead removed using independent component analysis (ICA) and FMRIB's ICA-based Xnoiseifier (FIX) tool (see individual chapters for specific methodology). Use of FIX instead of PNM for noise correction, has not yet formally been compared in the literature. It is possible that FIX leads to a more stringent removal of signal from brainstem regions; this is discussed in further detail in relevant chapters. For the purposes of this thesis FIX was chosen for its greater capacity for automaticity and therefore time efficiency.

3.4.3.7 FMRIB's ICA-based Xnoiseifier (FIX)

An independent component analysis (ICA) decomposes functional MRI data into time series and spatial component maps (fig 3.7). FSL's Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) is the tool used for ICA in this thesis (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC). This analysis can be used for model free statistical inferences such as assessment of resting state networks (RSN). Additionally, ICA can be used to detect noise, which becomes distinguishable from signal because of to its spatial distribution and time series frequencies. Noise may either be detected by manual inspection of the components or using a new FSL tool called FIX (Griffanti et al., 2014, Salimi-Khorshidi et al., 2014).

Fix auto classifies components into; 'good' containing signal of potential interest, or 'bad' containing artefact or noise. Bad components can then be removed from

the data prior to analysis. It is possible to train FIX to detect specific 'bad' components whilst ignoring others for a given population. Training FIX is relatively time consuming when long experimental acquisitions, containing many independent components, have been undertaken Alternatively, a standardised classification dataset for use with FIX also exists. For this thesis a random set of classifications was compared between FIX's standardised classification and a manually reviewed ICA in 6 patients. Classifications were similar and therefore the standardised dataset was used to classify noise in the CIPN study data for the functional experimental analysis.

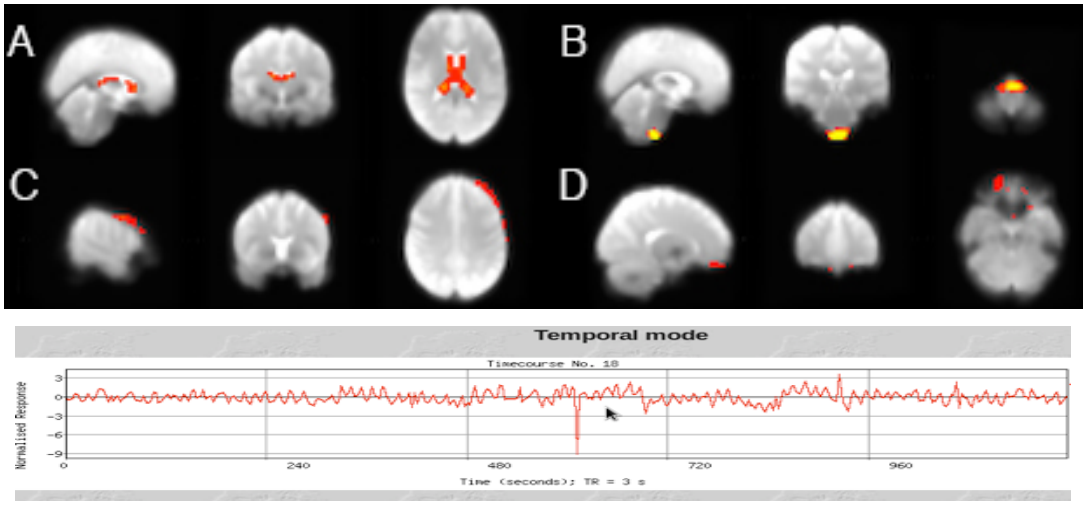


Figure 3.7. Spatial and time series components derived from ICA. The top panel demonstrates spatial maps showing noise-containing components. A=cerebrospinal fluid related artefact, B= physiological noise affecting brain stem regions, C= movement artefact, D= susceptibility artefact related to sinuses. Dr Chris Ng created the top panel for a final year medical school project supervised by me and using CIPN study data, which we analysed together. The bottom panel shows time series demonstrating high frequency noise.

3.4.4 First Level (Single Subject) Analysis

Following the data preprocessing steps described above, each subject's functional data was assessed using a general linear modelling (GLM) approach. This statistical approach aims to detect voxels or clusters of voxels activated in response to the punctate stimuli and IAPS images presented during the CIPN study experiment. The activation is compared to baseline. Within a voxel the HRF (see 1.1.3.2) is known to be slightly delayed in relation to actual neuronal activation following experimental stimuli. As a result, within FSL, the activation

curve (derived from the experimental events) is first convolved with the HRF before being compared to the actual functional data. The goodness of fit of this expected model to the actual data is summarised as a parameter estimate (PE). A PE can be converted to a t statistic by dividing it by its standard deviation. The t statistic can be further converted to a z statistic allowing for standardised comparison within normal distributions. These z-scores are displayed at voxel level as maps and thresholded using *Gaussian Random Field* theory to depict clusters of voxels activating together, thresholded at a $p<0.05$.

3.4.5 Second Level (Group) Analysis

In order to compare groups of patients the parameter estimates obtained from individual subject analysis are taken up to perform higher-level statistical comparisons. Depending on the question of interest, groups may be compared using mean group activations, t tests to assess differences between mean group activations or analysis of variance (ANOVA). In this thesis, comparison between groups was made using t-tests unless otherwise specified. Additionally at group level other factors possibly explaining variance between groups, including sex, age, cancer type and pain score at baseline were introduced into the statistical model.

3.5 Overview of Statistical Analyses of non fMRI data

All statistical analyses were performed using SPSS version 22. Statistical significance was taken as $p \leq 0.05$. Where appropriate, if distribution of a variable was not normal, the median was used instead of the mean. Group means were compared using a two-sided t test (for normally distributed variables) and a Kolmogorov-Smirnov test (for variables with a skewed distribution) as comparison groups always contained less then 25 patients per group. Categorical variables were compared using Pearson's chi square test or Fisher's exact test when group size did not fulfil expected cell counts. Where possible, 95% confidence intervals (CIs) were calculated to aid in interpretation of the results. Repeat measures ANOVA was used for subcortical structural volume analysis (see chp.4). In chp.5, % BOLD signal change in regions of interests (ROIs) was compared between the CIPN and non-CIPN groups using

independent sample t-tests and bootstrapping to correct for multiple comparisons in a small sample size.

4. Differences in subcortical structures and resting state networks prior to development of CIPN.

This chapter details analysis of structural and resting state data acquired during the CIPN study. Subcortical structures to be investigated were chosen a priori based on their known involvement in pain processing and pain vulnerability. Analysis was limited to four regions; the amygdala, brainstem, nucleus accumbens and thalamus in order to minimise statistical bias occurring as a result of multiple comparisons. Analysis of the function of participant's brains at rest, was undertaken to investigate whether there were any baseline differences in the resting brains of those who went onto develop CIPN and those who did not. Discussion of how the reported findings relate to existing literature as well as the strengths and limitations of these analyses is presented at the end of the chapter.

4.1 Background

Structural brain differences have been shown in both acute and chronic pain patients(Davis and Moayedi, 2012). Structural changes in brain areas known to be associated with pain processing including the anterior cingulate cortex; insula and somatosensory cortex have been reported. However, perhaps the regions most frequently implicated as altered in pain states include components of the subcortical structures, such as the thalamus, caudate nucleus, nucleus accumbens and brainstem (Barad et al., 2013, Kregel et al., 2015).

The subcortical structures comprise the most ancient of the pain processing brain regions in terms of evolutionary development. They have been shown to be involved in the survival fight or flight instinct and are key to the rapid removal of the body from acute pain associated with tissue damage (see 1.1.1.1). Although acute pain processing appears to be a key role of subcortical regions, differences in their shape and volume have been demonstrated in chronic pain states. These, amongst others, have included rheumatoid arthritis (Wartolowska et al., 2012), chronic back pain (Apkarian et al., 2004), chronic pelvic pain (As-Sanie et al.,

2012) and osteoarthritis of both the hip (Gwilym et al., 2010) and knee (Mao et al., 2016).

Postulated explanations for these differences in volume include neuronal reorganisation and central nervous system plasticity (Rodriguez-Raecke et al., 2013). Evidence shows that these regions, in particular the nucleus accumbens (NAc), are important in conversion from acute to chronic pain states and reflect individual vulnerability to chronic pain development (Denk et al., 2014). However, due to the difficulty in studying development of pain prospectively, little is known about any associations between subcortical structural brain regions and subsequent pain development. Currently only one longitudinal study in back pain proposes a baseline vulnerability for conversion from acute to chronic pain, based on white matter connectivity changes (Mansour et al., 2013). Whether this can be extrapolated to pre-pain changes remains unknown.

No previous prospective high-resolution neuroimaging work has been done in CIPN. It is therefore currently unclear if CIPN, in line with other pain conditions, is associated with any structural brain changes. Moreover, whether structural brain changes can be identified prior to any onset of peripheral nerve damage is likely of interest to not only the study of CIPN but also the study of pain in general.

In terms of brain function, traditionally fMRI analyses examine the brain engaged in specific functional tasks (chapter 5 in this thesis). Over the last two decades however, attention has been turned to understanding the brain's baseline neuronal activity (Zhang and Raichle, 2010). This has revealed that baseline BOLD signal fluctuations are organised into standardised resting state networks (RSNs) with reproducible temporal and spatial characteristics. RSNs have been shown to be consistent across individuals and across studies, and are known to underpin cognitive and perceptual processes (Cole et al., 2010). Some of the most frequently described networks include the default mode network, the executive control network, visual network and right frontopariatal attention

network. These govern our capacity for attention, planning, anticipation, rest and fear in the absence of direct external engagement with the outside world.

In relation to pain states, resting state networks have been demonstrated to be vital for pain perception (Borsook et al., 2013). Alterations in their architecture have been reported in chronic pain (Baliki et al., 2008, Kim et al., 2013, Colombo et al., 2015). Moreover, evidence of reversal of pain related RSN changes after appropriate analgesic treatment has also been described (Ceko et al., 2015).

In terms of cancer and chemotherapy, recent assessment of resting state networks has been used to investigate the effects of chemotherapy on cognitive function (Kesler, 2014). The mainstay of this work has been carried out in breast cancer patients after chemotherapy treatment. Investigations have focused on assessing the mechanisms underpinning a model of subtle brain changes following chemotherapy treatment, termed 'chemobrain'. These have suggested changes in RSN connectivity following treatment (Bruno et al., 2012, Hampson et al., 2015, Piccirillo et al., 2015). There is currently no data associating RSNs with subsequent CIPN development.

4.2 Hypothesis & Aims

The two hypotheses underpinning this chapter are as follows:

1. *Volumes of subcortical structures, specifically the thalamus, nucleus, accumbens, amygdala and brainstem, differ in patients who go on to develop CIPN as compared to those who do not develop CIPN, prior to peripheral nerve damage with chemotherapy.*
2. *Resting state networks differ in patients who go on to develop CIPN as compared to those who do not develop CIPN, prior to peripheral nerve damage with chemotherapy.*

The aim of this chapter is to address the following research questions:

1. Are the volumes of subcortical structures (thalamus, nucleus accumbens, amygdala, brainstem) different between cancer patients who develop

CIPN and those who do not prior to peripheral nerve damage with chemotherapy?

2. Are there differences in resting state networks between cancer patients who go on to develop CIPN and those who do not prior to chemotherapy onset?

The two research questions are addressed with different analyses and therefore the methods, results and discussion sections, which follow, are subdivided to reflect these two approaches.

4.3 Methods

4.3.1 Structural Analysis

T1 weighted images from the CIPN study were brain extracted using FSL's BET (see 3.4.1.1). Images were then registered and segmented using FSL's model based tool FIRST (Patenaude et al., 2011). FIRST registers the T1 brain optimising the registration for the subcortical structures. FIRST then segments all subcortical structures. Following segmentation the volumes of four structures chosen *a priori*: thalamus, nucleus accumbens, amygdala and brainstem were measured using fslstats. Structures were chosen based on the evidence from pain literature suggesting their altered shape in both acute and chronic pain states including chronic back pain, osteoarthritis and chronic regional pain syndrome (Baliki et al., 2010, Baliki et al., 2011).

In order to adjust subcortical structure volumes for grey matter volume (GMV) and whole brain volume (WBV), the T1 brain was segmented using FSL's tool, FAST. Volumes for these were calculated using fslstats. All volumes were reviewed prior to statistical analysis. One subject needed to be removed due to an inappropriately small field of view (FVO), which prevented appropriate segmentation of the brainstem, leaving a sample size of 29 patients for this analysis.

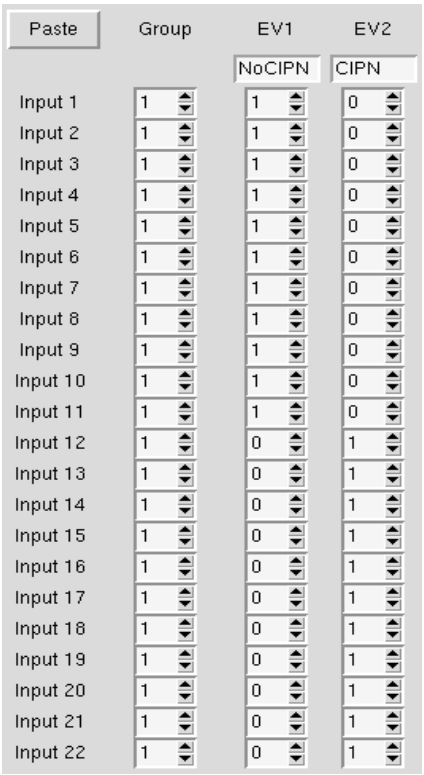
All volumes were then transferred into SPSS. Two models, one adjusted for WBV and the other unadjusted were calculated[3]. Repeat measures ANOVA was used for bilateral structures (e.g. right and left amygdala) and a univariate general linear model was used to analyse the brainstem. These approaches were decided upon after discussion with a statistician and were used to minimise multiple testing.

4.3.2 Resting State Analysis

Brian extraction was performed on the data as described above (3.4.1.1). Following this, independent component analysis (ICA) using FSL's Multivariate Exploratory Linear Optimized Decomposition into Independent Components (*MELODIC*) was carried out. Data was not denoised using FIX prior to concatenated ICA, as the stringent cleaning process used in FIX may have unwittingly removed signal of interest. Components were reviewed following the ICA run in order to a *priori* decipher which components contained only noise and which constituted signal of potential interest for further evaluation. Two ICA runs: one high dimensionality (60 component restriction) and one low dimensionality (30 component restriction) were carried out. This was done because restricting the data decomposition to fewer components is known to identify larger RSNs, while higher dimensionality decomposing more readily identifies smaller more functionally homogenous regions or nodes. In view of the fact that RSNs have not been explored in the context of CIPN before, a broad hypothesis related to difference was being tested. Consequently, without prior specification of whether the hypothesised difference was expected in large well-described RSNs or smaller more discrete regions of these networks the two approaches to ICA were undertaken in order to explore this hypothesis adequately.

[3] Although standard practice is to adjust structural volumes for whole brain volume, it can be argued that this potentially masks biologically important differences. This is because all measured volumes reflect glia and neuronal content. Adjusting for whole brain volume assumes that differences in WBV are unimportant in terms of global brain wide pathophysiological processes. This may not be an appropriate assumption and therefore both adjusted and unadjusted results are explored here.

A multi-subject design matrix and contrast file was created (fig 4.1) for use in the dual regression step of the analysis. Equal numbers of patients from the CIPN and non CIPN groups were chosen in order to avoid biases in the component maps generated. Eleven randomly chosen individuals per group were decided on to optimise sample size. Dual regression was carried out, using FSL's dual regression tool, in order to average the component maps from each group (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/DualRegression). Finally, between group comparison of RSN's was performed using FSL's *Randomise,* which enabled non parametric permutation testing (Winkler et al., 2014).



Paste	Group	EV1	EV2
		NoCIPN	CIPN
Input 1	1	1	0
Input 2	1	1	0
Input 3	1	1	0
Input 4	1	1	0
Input 5	1	1	0
Input 6	1	1	0
Input 7	1	1	0
Input 8	1	1	0
Input 9	1	1	0
Input 10	1	1	0
Input 11	1	1	0
Input 12	1	0	1
Input 13	1	0	1
Input 14	1	0	1
Input 15	1	0	1
Input 16	1	0	1
Input 17	1	0	1
Input 18	1	0	1
Input 19	1	0	1
Input 20	1	0	1
Input 21	1	0	1
Input 22	1	0	1

Fig 4.1 Design Matrix for group comparison. Showing the number of inputs and the group classification (CIPN = 1 and No CIPN =0) of each. Design for testing two group difference with two-sample unpaired t test. (as discussed in: https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/GLM#Two-Group_Difference_.28Two-Sample_Unpaired_T-Test.29)

4.4 Results

4.4.1 Structural Analysis

Demographic data for the 29 subjects included in this analysis is shown in table 4.1. 17 patients developed acute CIPN requiring a chemotherapy dose reduction. Patients who developed CIPN were matched to those who did not in terms of age, sex, cancer operation and cancer type (collinear with chemotherapy type). Difference in baseline pain score was marginally statistically significant.

However assessing the actual pain scores (maximum in the non CIPN group was 2 and in the CIPN group was 0.45 on a zero to 10 scale) suggests that this difference is not clinically important.

	Non CIPN 95%CI or % of 12	CIPN 95%CI or % of 17
Mean Age	57.6 (54.1-61)	62.3 (57.5-67.1)
Sex Female	5 (41%)	12 (70%)
Cancer Type •Lung •Gynae •Colorectal	 2 (16%) 5 (42%) 5 (42%)	 0 (0%) 4 (24%) 13 (76%)
Cancer Operation •No •Yes	 3 (25%) 9 (75%)	 0 (0%) 17 (100%)
Pain Score •Mean •Median (IQR)	 1.25 (0.3-2.3) 1 (0-2)	 0.18 (-0.1-0.45) 0 (0-0)

Table 4.1 Demographic data for Non CIPN and CIPN groups included in the structural analysis. Mean age shown in table, age range 50-79yo. For cancer type and operation % refers to within group proportion. Range of pain score shown in brackets. Chemotherapy type collinear with cancer type and therefore not specifically reported.

Standard masks of regions segmented for this analysis are shown in figure 4.2. The mean volumes for each group and the statistical comparison of these volumes are shown in table 4.2. In the unadjusted analysis the thalamus, nucleus accumbens and grey matter volume were different between the CIPN and non CIPN group. Following adjustment for whole brain volume the nucleus accumbens was the only structure, which significantly differed between the groups (fig 4.3).

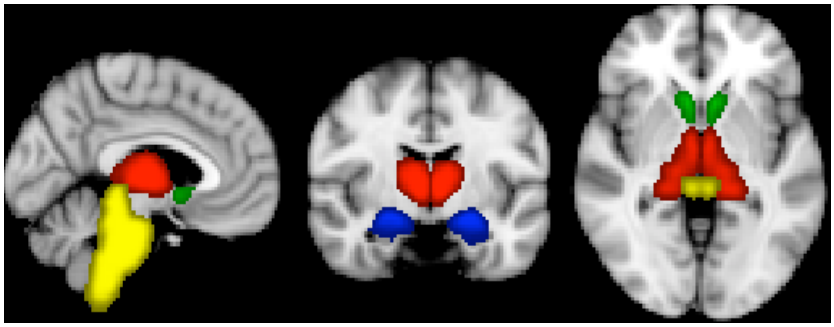


Figure 4.2 Structures segmented for subcortical analysis. All structures are derived from the Oxford Harvard Subcortical Atlas and are shown here in standard space (MNI152 2mm). Yellow=brainstem, red= thalamus right and left, blue=amygdala right and left, green =accumbens right and left. This atlas is based on T1-weighted images of 21 healthy male and 16 healthy female subjects (ages 18-50), individually segmented, transformed into standard space and combined. It is considered to have high accuracy. Applicability of the population used in this atlas to the CIPN cohort is discussed in 4.5.2.2

Assessment of the repeated measures ANOVA results presented below, suggested that there was variance in the model introduced by the two factors (right and left). This prompted post hoc exploration of their influence with a univariate analysis of each of the sides. The results of this are presented in table 4.3.

Structure	Mean vol (mm^3) 95%CI		Unadjusted sig	Adjusted sig for WBV
	NO CIPN	CIPN		
L Thalamus R Thalamus	7946 (7592-8301) 7734 (7367-8102)	7331(7089-7573) 7262(6991-7533)	p=0.01	p=0.08
L Accumbens R Accumbens	665(589-741) 530(482-577)	582(520-644) 416(345-487)	p=0.03	p=0.02
L Amygdala R Amygdala	1485(1315-1655) 1549(1446-1652)	1398(1289-1506) 1506(1336-1674)	p=0.38	p=0.87
Brainstem	22493 (21260-23725)	21044 (19863-22227)	p=0.08	p=0.48
Grey Matter	581582 (561574-601589)	542348 (520654-564043)	p=0.01	p=0.09

Table 4.2. Group differences in mean volumes. 95%CI = 95% confidence interval. L= left. R=right. Adjusted for multiple comparisons with Bonferroni correction.

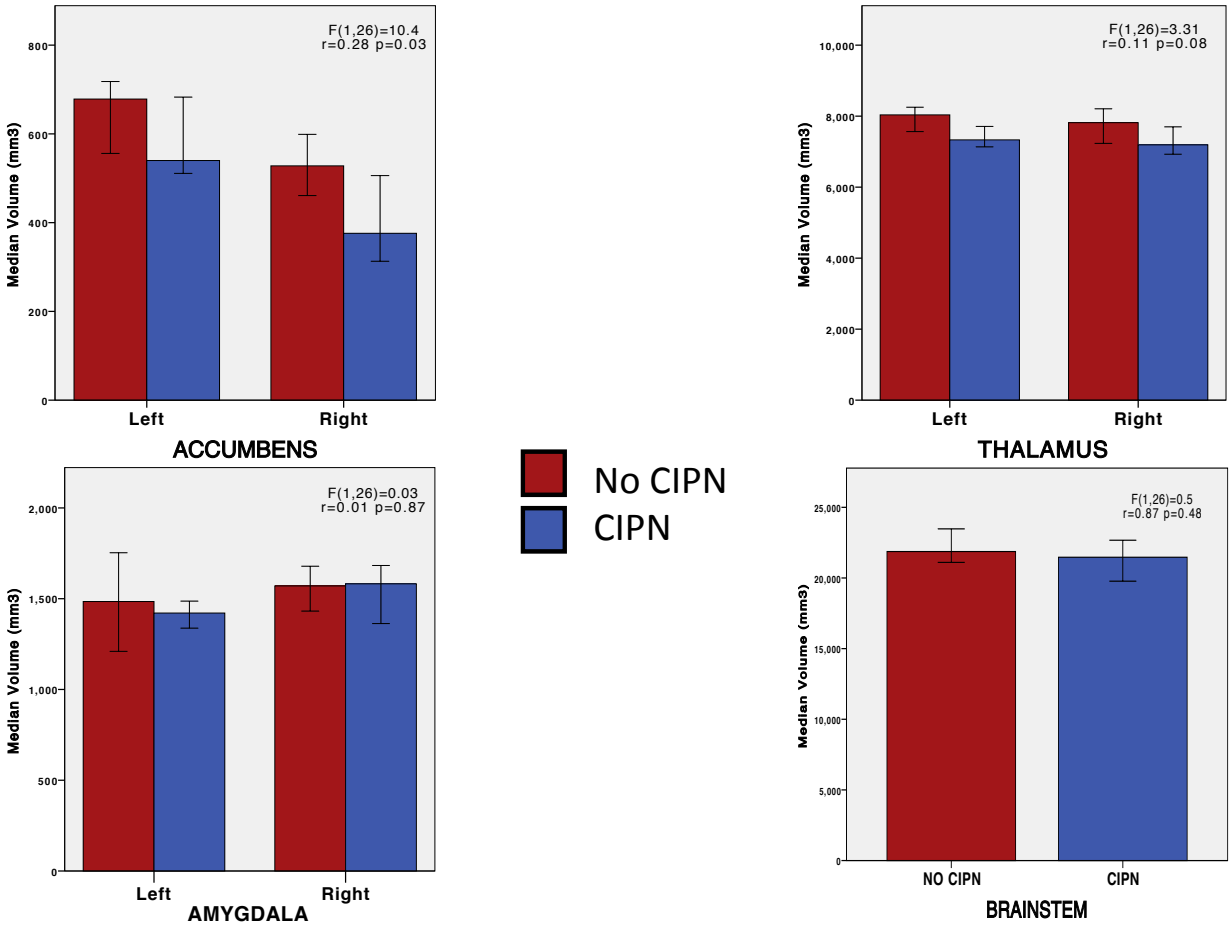


Figure 4.3 Comparison of median subcortical volumes in mm^3. Red = non CIPN. Blue = CIPN. A) thalamus, B) nucleus accumbens, C) amygdala, and D) brainstem after adjustment for whole brain volume. Horizontal axis showing median volume. Error bars represent 95% confidence intervals. In terms of size of effect, between group difference is small for all structures except for the nucleus accumbens, which shows a moderate effect size. The accumbens is the only structure that remained statistically significant after adjustment for whole brain volume.

Side	Unadjusted	Adjusted for WBV
Left Accumbens	p= 0.08	p= 0.08
Right Accumbens	p=0.01	p=0.01

Table 4.3 Exploration of the influence of laterality on the between group difference in volume of the nucleus accumbens. Analysis carried out using a univariate general linear model both adjusted and unadjusted for whole brain volume.

4.4.2 Resting State Analysis

Twenty-two subjects (11 Non CIPN and 11 CIPN) were included in the analysis. Groups were matched in terms of key confounding factors (table 4.4). Prior to dual regression, all component maps were reviewed for both the high and low dimensionality runs. Components clearly containing noise (fig 4.4a) or demonstrating RSNs frequently described in the literature (fig 4.4b) were noted.

	Non CIPN 95%CI or % of 11	**CIPN 95%CI or % of 11**
Mean Age	58.4 (54.8-62)	61.5 (54.5-68.5)
Sex Female	5 (45%)	7 (63%)
Cancer Type •Lung •Gynae •Colorectal	 1 (16%) 5 (42%) 5 (42%)	 0 (0%) 3 (24%) 8 (76%)
Cancer Operation •No •Yes	 2 (25%) 9 (75%)	 0 (0%) 11 (100%)
Pain Score •Mean •Median (IQR)	 0.82 (0.2-1.5) 1 (0-2)	 0.09 (-0.1-0.29) 0 (0-0)

Table 4.4 Demographic data for the No CIPN and CIPN groups included in the resting state analysis. Mean age shown in table, age range 50-79yo. For cancer type and operation % refers to within group proportion. Range of pain score shown in brackets. Chemotherapy type collinear with cancer type and therefore not specifically reported.

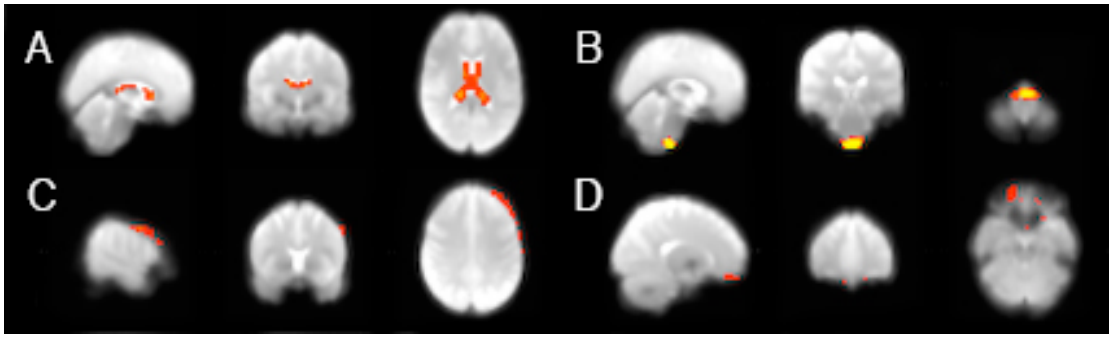


Figure 4.4a Examples of noise containing components. A= cerebrospinal fluid related noise. B= respiratory noise. C= movement artefact. D= susceptibility artefact related to air bone interface in areas of sinuses. Figure created by Dr Chris Ng for a final year medical school project supervised by me and using CIPN study data, which we analysed together.

For the low dimensionality ICA run, a single component showed a significant difference between those who developed CIPN and those who did not ($p = 0.04$). This was seen in a small region of the right somatosensory cortex of patients who did not develop CIPN, likely a part of the larger sensimotor network (fig 4.5).

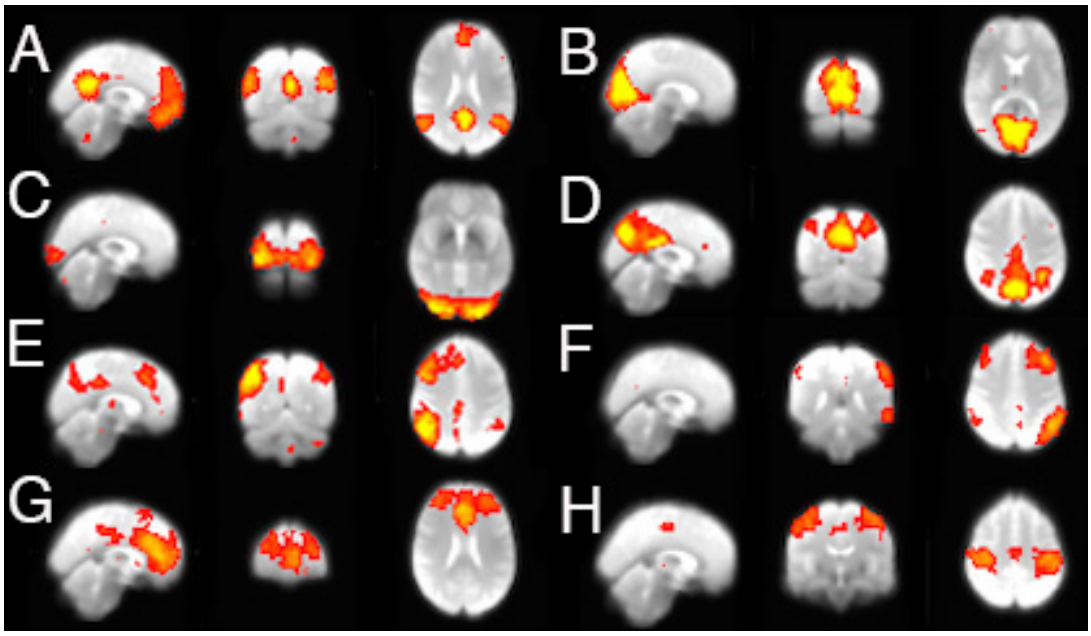


Figure 4.4b Previously described resting state networks observed in the CIPN study ICA. A= default mode, B= medial visual C=lateral visual, D= visual spatial, E= right frontparietal, F= left frontopartial, G= attention, H= sensorimotor. Figure created by Dr Chris Ng for a final year medical school project supervised by me and using CIPN study data, which we analysed together.

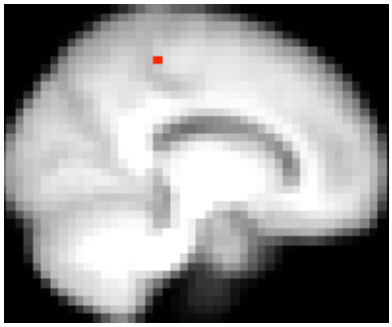


Figure 4.5 Low Dimensionality Dual Regression Results. Region of right somatosensory cortex showing significantly more activation during rest in patients who did not develop CIPN as compared to those who did. Likely part of the larger sensorimotor network.

As expected the high dimensionality ICA yielded a greater number of statistically significant differences. The differences were bidirectional and possibly spurious.

4.5 Discussion

4.5.1 Main Findings

The structural analysis showed that the volume of the nucleus accumbens was the only structure that differed significantly between the CIPN and non–CIPN group, after adjustment for whole brain volume; $F(1,26)=10.4$, $p=0.02$. The effect size of this result was moderate $r=0.28$. Exploration of the difference in influence of right and left showed the right accumbens to drive the variance between the groups. Assessment of other structures showed that the thalamus approached statistical significance ($p=0.08$), however the effect size of this difference remained small $r=0.11$. The volumes of the brainstem and amygdala did not differ between the groups either in terms of statistical tests or effect size.

The resting state analysis is difficult to interpret. High and low dimensionality analysis yielded varied results. Low dimensionality dual regression showed a single significant difference in the somatosensory cortex of patients who did not develop CIPN, likely a part of the larger sensorimotor network. The high dimensionality analysis, known to be more sensitive to differences in functionally homogenous regions or nodes, yielded a greater number of between group differences. These were bidirectional and impossible to interpret within the context of available data.

4.5.2.1 Strengths

The strengths of this experiment and analyses are as follows. Firstly, the prospective design of this study has enabled assessment of the association of baseline variance in subcortical structures and the subsequent development of acute CIPN. This is a unique approach in CIPN research where exploration of peripheral nervous system changes has been the norm (see 7.1.1). This approach may introduce a paradigm shift in the oncology and CIPN research communities; both in terms of introducing a focus on the brain and clinically a discussion of 'at risk individuals and preventative approaches' as compared to post damage treatment strategies (see 7.1.1). Finally, the general strengths of studying a clinical problem in a patient cohort as opposed to a nonclinical model (see 7.1.1) also translate to this analysis.

In terms of the analyses; the statistical approach used for between group comparisons of structural volumes (repeat measures ANOVA with introduction of whole brain as a covariate) enabled optimisation of power for group sample size, minimising the possibility of type 1 error. Regarding the ICA analysis; the decision to perform a high and a low dimensionality run optimised the investigation of the broad hypothesis being explored. This approach was also useful in determining future analysis steps for these data (see 4.5.4).

4.5.2.2 Limitations

The limitations of this chapter can also be subdivided into those related to the experimental design, and those related to the analyses. The possible impact of design limitations including unknown confounding factors, and type 1 error related to sample size. In terms of the structural analysis two main problems may have biased results. Firstly, the calculation of subcortical structural volume is heavily dependent on the quality of the registration of the individual T1 images to standard space (see 3.4.1.3). These were automated and quality checked but not optimised manually. The impact of imperfect registration would be a higher incidence of type 2 errors. Also although registration with FIRST

optimises for subcortical volumes, the standard space template it registers to is MNI152. This template is derived from a healthy subject population. The applicability of this population to the CIPN study group is unknown. Evidence regarding the need for population specific brain templates has been suggested in the neuroimaging literature (Mandal et al., 2012). Therefore, in terms of this study the registration to MNI152 may be an important source of bias in this analysis.

Secondly, in relation to the statistical analysis of calculated volumes the problem of multiple comparisons needs to be considered. In a cohort of 29 patients analysed in this chapter, four subcortical volumes, three of which had bilateral volumes were compared between groups. Despite optimising the analysis with the repeated measures ANOVA, spurious results at a significance level of $p<0.05$, are a possibility. This is a major limitation of this work.

In relation to the resting state network analysis, recent evidence in clinical populations suggests that de-noising data prior to ICA helps increase the reproducibility of clinical findings, by having a standardised approach to dealing with noise (Griffanti et al., 2016). This was not done here as the analysis was carried out prior to this publication, and may be a limitation of this approach. Further, because equal numbers of participants have to be introduced into the dual regression, the sample size for this analysis was limited to 22 (eleven per group), which may have resulted in the analysis being insufficiently powered to detect important differences. Moreover, this analysis only investigated differences and did not explore directionality in terms of increased or decreased connectivity between the groups. This approach may have yielded more results and given more insight into the clinical relevance of findings.

4.5.3 Comparison of results with other studies

4.5.3.1 Structural Analysis

The nucleus accumbens (NAc) finding corroborates multiple lines of evidence from both human and animal pain research. In healthy human volunteers NAc

involvement in processing noxious stimuli has been shown to reflect aversion and reward responses to pain (Becerra and Borsook, 2008). In patient populations the volume and function of the NAc is altered in both acute and chronic pain (Baliki et al., 2010). Baliki and colleagues also report changes in the connectivity of the NAc as predictive of conversion to chronic pain states (Baliki et al., 2012), a finding likely pertinent to future analysis related to CIPN chronicity (see 4.5.4). The actual circuitry of the NAc is complex (Baliki et al., 2013). It is therefore important to note that the association of acute CIPN with decreased NAc volume shown here is likely a crude representation of an intricate pathophysiological process.

Looking beyond human pain studies, animal models provide insight into altered circuitry of the NAc in neuropathic pain. Macro (fMRI) and micro (molecular) changes occur in the organisation of the NAc following peripheral nerve damage (Chang et al., 2014). These changes have been described as causative in terms of maintaining allodynia and can be reversed by injecting lidocaine into the NAc in experimental animals. Chang and colleagues' findings are further supported by recent work exploring the specific input of the outer (shell) and inner (core) aspects of the NAc in neuropathic pain maintenance in rodent pain models (Ren et al., 2016). These findings reemphasise the key role of the NAc in maintaining neuropathic pain states.

Finally, in terms of CIPN specific evidence from brain imaging studies, only two such studies exist (Boland et al., 2014, Nudelman et al., 2015). Boland and colleagues investigate function in a group of 12 chronic CIPN patients, with no mention of structural changes in this group. In this study it was demonstrated that patients with established CIPN had distinct changes in brain pain processing regions. In contrast, Nudelman and colleagues investigate structure and perfusion in a longitudinal study of breast cancer patients. However they do not assess individual structures and only comment on global grey mater changes following and not prior to CIPN development.

4.5.3.1 Resting State Analysis

Although no direct studies investigating RSNs in CIPN exist, a number of pain studies, show alterations in resting state connectivity in chronic and acute pain states. Specifically, recent work in fibromyalgia has reported varied default mode network connectivity between the precuneous and cingulate regions to the thalamus in acute pain (Ichesco et al., 2016). More akin clinically to CIPN, studies in diabetic neuropathy (DN) have found that compared to matched controls, patients with DN had altered resting state connectivity in areas of the default mode network including the precuneous, thalamus, brainstem regions, insula, pre and postcentral gyri as well as the superior frontal gyrus (Cauda et al., 2009). Another study investigating patients with acquired and hereditary neuropathy, found altered RSN connectivity of the sensorimotor network and precuneous (Rocca et al., 2014). Results presented here are difficult to interpret, however these studies will give insight into future analysis of these data.

4.5.4 Interpretation of findings and implication for future work

Despite the limitations of the structural analysis discussed above, the conformity of the results presented here, with existing animal and human work, gives credence to their likely importance. Specifically, the decreased volumes in the NAc in patients who go onto develop acute CIPN, suggests a vulnerable cohort of patients at risk of CIPN prior to nerve damage with chemotherapy. This evidence gives weight to a paradigm shift in terms of moving towards individualised approaches to chemotherapy treatment.

Future work related to the structural dataset will involve assessing the activity of the NAc as a region of interest, in response to the functional punctate task (see 5.3.1). Additionally, assessment of both volume and function in the NAc after the study follow up is complete and patients converting to chronic CIPN are identified, may yield insight into its role in chronic CIPN specifically.

Finally, use of NAc finding as a candidate biomarker for patient risk stratification is a possibility. For this to happen a longitudinal study with a larger cohort is required. This will yield data for a sensitivity and specificity analysis to determine the magnitude of change in the NAc prior to peripheral nerve damage, in order to devise cut off points for future post-test probability analysis.

The resting state analysis is difficult to interpret. What is however clear from the low and high dimensionality approach to this analysis is that the source of any between group differences, likely lies in the connectivity of functionally homogenous regions ('nodes') as opposed to large network.

Consequently, further analyses probing nodal connectivity specifically are warranted. One possibility is the use of the new FSL tool *FSLnets,* which enables individual subject network modelling and subsequent analysis of intergroup connectivity correlations. This is planned for future work related to this dataset.

Finally, these resting state data may also be useful for interrogating the effect of cancer on resting state brain networks in chemotherapy naïve patients. Future comparison of RSN from patients in the CIPN study, to a cohort of age, and sex matched healthy volunteers is planned. The aim of this will be to ascertain if there are any baseline differences between the two groups, which may be attributable to the presence of cancer. Data collection on a cohort of age, sex matched healthy volunteers has already been completed and will be used in this comparative analysis in the near future.

5. Descending Pain Modulatory System (DPMS) and influence of positive affective pictures in cancer patients prior to development of CIPN.

This chapter details the analysis of functional data acquired during the CIPN study. Processing of punctate stimuli alone and during viewing positive emotional images is compared between patients who subsequently go onto develop CIPN and those who do not. The strengths and limitations of this analysis as well as comparison of findings to existing evidence are detailed at the end of the chapter.

5.1 Background

Key regions known to modulate (inhibit and fascilitate) ascending nociception are cumulatively termed the descending pain modulatory system (DPMS). They centre on a network of cortical and brainstem areas which include, the rostral anterior cingulate, hypothalamus, amygdala, nucleus cuneiformis (NCF) mesencephalic pontine reticular formation (MPRF), rostro-ventral medulla (RVM) and periaqueductal grey (PAG). Function and interconnectivity between these regions, as well as their connectivity to areas of the spinal cord, is complex and an on-going area of research. RVM neurons directly project to the dorsal horn of the spinal cord. The RVM is in turn densely innervated by neurons arising from the PAG. Importantly, there is bidirectional processing of pain in this region, underpinned by the existence of 'off cells' and 'on cells' (Heinricher et al., 2009). Off cells inhibit ascending pain and enable pain tolerance in extreme conditions such as during sports injury or during physical combat. On cells in turn facilitate pain and have been implicated in conversion to pain chronicity (Bingel and Tracey, 2008, Fields, 2009).

Aberrance in structure, function and connectivity of these brainstem regions has been documented in various pain states. Specifically, studies investigating osteoarthritis (Gwilym et al., 2009), chronic pelvic pain (Vincent et al., 2013),

fibromyalgia (Fallon et al., 2013), and experimentally central sensitisation in healthy humans (Lee et al., 2008) have shown changes in DMPS. Moreover, changes in regions of the DPMS are thought to be of key importance in conferring vulnerability for developing chronic pain (Denk et al., 2014). Human studies investigating DPMS in pain, have been strengthened by animal models, which have associated maintenance of neuropathic pain, with reversible abnormalities in these pathways (De Felice et al., 2011) The impact of DPMS and in particular key areas of the brainstem, including the RVM, MPRF and PAG, on CIPN development in patients has not previously been assessed.

Other key regions involved in descending pain modulation(Tracey et al., 2002) include the anterior cingulate cortex (ACC), dorsolateral prefrontal cortex (dlPFC), amygdala and hypothalamus. Activity in these regions and their connections to the brainstem areas discussed above, underpins the known cognitive and emotional modulation of pain. The capacity to tolerate greater pain when distracted, in particular with positive emotional input, as well as increased pain sensitivity during periods of negative affective experiences is well documented (Kamping et al., 2013, Fox et al., 2013, Wiech et al., 2008). Specifically in neuropathic pain, positive emotional state has been shown to be important in pain experience and response to analgesic treatment (Petersen et al., 2014). Whether there is an association between this capacity to engage with positive emotion, as a source of distraction and actual development of pain states remains unknown. This is partly due to the difficulties in studying pain prospectively prior to its development.

Therefore assessing the integrity of the positive emotional state in patients with cancer, prior to neurotoxic chemotherapy and its relationship to the development of CIPN has the potential to provide insight into the impact of engaging with positive emotional distraction on painful neuropathy. In effect, establishing the patients' resilience or vulnerability in these key brain regions and networks.

5.2 Hypothesis & Aims

The hypotheses underpinning this chapter are as follows:

1. *Descending pain modulation prior to chemotherapy differs in patients who go on to develop CIPN as compared to those who do not develop CIPN.*
2. *Punctate stimuli during positive emotional input is processed differently in patients who go onto develop CIPN as compared to those who do not.*

The aim of this chapter is to address the following research questions:

1. Are there differences in descending pain modulatory system between cancer patients who develop CIPN and those who do not?
2. Does the processing of affective images differ between cancer patients who develop CIPN and those who do not and does this influence their processing of punctate stimuli?

5.3 Methods

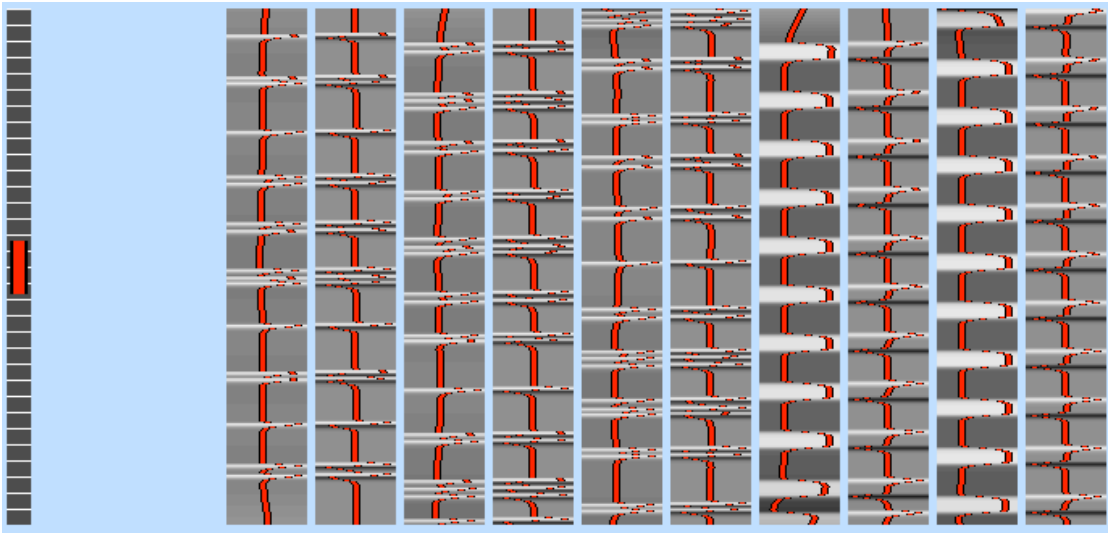
Standard data pre-processing (see 3.4.1) including brain extraction, B0 unwarping, motion correction, spatial smoothing, temporal filtering and registration to standard space was undertaken. FSL's Multivariate Exploratory Linear Optimized Decomposition into Independent Components (*MELODIC*) was used to perform ICA in order to assess noise in the data. Data de noising was carried out using FIX, with a standard training data set as discussed in 3.4.1.7. The two research questions described above were run as separate analysis and therefore the first and second level model set up is described separately for these below. From here on in, the analysis pertaining to question one, related to descending pain-modulating system, will be referred to as the DPMS analysis. The analyses addressing question two, detailed above, and related to the influence of international affective images will be referred to as the IAPS analysis.

5.3.1 Descending Pain Modulatory System (DPMS) analysis

The aim of this analysis was to assess how the brains of cancer patients processed punctate stimuli, if and how this processing was affected by viewing

images with emotional content, and relating these findings to subsequent CIPN development. Consequently, the following contrasts of interest where included in the first level model: punctate only stimulus (snow image viewed), punctate during positive images, punctate during neutral images, neutral and positive images greater than snow, snow greater than neutral and positive, positive greater than neutral, neutral greater than positive, snow greater than positive, positive greater than snow, snow greater than neutral and finally neutral greater than snow (figure 5.1). Each first level contrast was cluster corrected and considered significant at a z threshold of 2.3 and a p value less then 0.05.

At the second level, all eleven corrected parameter estimates (COPEs) were introduced and a mean difference between the group that developed CIPN and the group that did not was assessed. The group level analysis was undertaken using a mixed effects model and outlier de-weighting. The model was corrected for sex, age, cancer type and baseline pain score. The design matrix for the second level model is shown in figure 5.2. Results were explored at a z threshold equal to 2.3 and equal to 2. Cluster uncorrected z statistics were also explored. This was done due to the strong *a priori* hypothesis underpinning the research question 1.



		snow_p	snow_p	neut_p	neut_p	pos_p	pos_p	neut_b	neut_b	pos_b	pos_b
C1	punct_only	1	0	0	0	0	0	0	0	0	0
C2	punct_positive	0	0	0	0	1	0	0	0	0	0
C3	punct_neutral	0	0	1	0	0	0	0	0	0	0
C4	n+p>s	-1	0	0.500	0	0.500	0	0	0	0	0
C5	s>n+p	1	0	-0.500	0	-0.500	0	0	0	0	0
C6	p>n	0	0	-1	0	1	0	0	0	0	0
C7	n>p	0	0	1	0	-1	0	0	0	0	0
C8	s>p	1	0	0	0	-1	0	0	0	0	0
C9	p>s	-1	0	0	0	1	0	0	0	0	0
C10	s>n	1	0	-1	0	0	0	0	0	0	0
C11	n>s	-1	0	1	0	0	0	0	0	0	0

Figure 5.1 First Level Design Matrix DPMS Analysis. Columns from right to left show modelled events: snow punctate, temporal derivative of latter, neutral punctate, temporal derivative of latter, positive punctate, temporal derivative of latter, neutral image block, temporal derivative of latter, positive image block, temporal derivative of latter. Rows show the 11 contrasts of interest detailed above.

For the DPMS analysis, BOLD signal activation in regions of interest (ROIs) was decided on *a priori*. These were chosen based on their known involvement in descending pain inhibition and included: the RVM, MPRF, PAG, and Thalamus. ROIs were analysed using FSL's Featquery tool to extract percent BOLD signal change from each region. Regions were defined using masks drawn in standard space and made available from the FMRIB pain group. Masks were functionally defined and are detailed in the published literature, amongst others (Lee et al., 2008, Vincent et al., 2011). ROIs were compared using the whole dataset and also the dataset split by sex. The sex split was based on known differences in pain processing between males and females (Bartley and Fillingim, 2013). CIPN and non-CIPN groups were compared using an independent sample t test and considered significant at $p<0.05$. To correct for possible bias induced by low

sample size, bootstrapping to a sample of 1000 was performed for each ROI analysis.

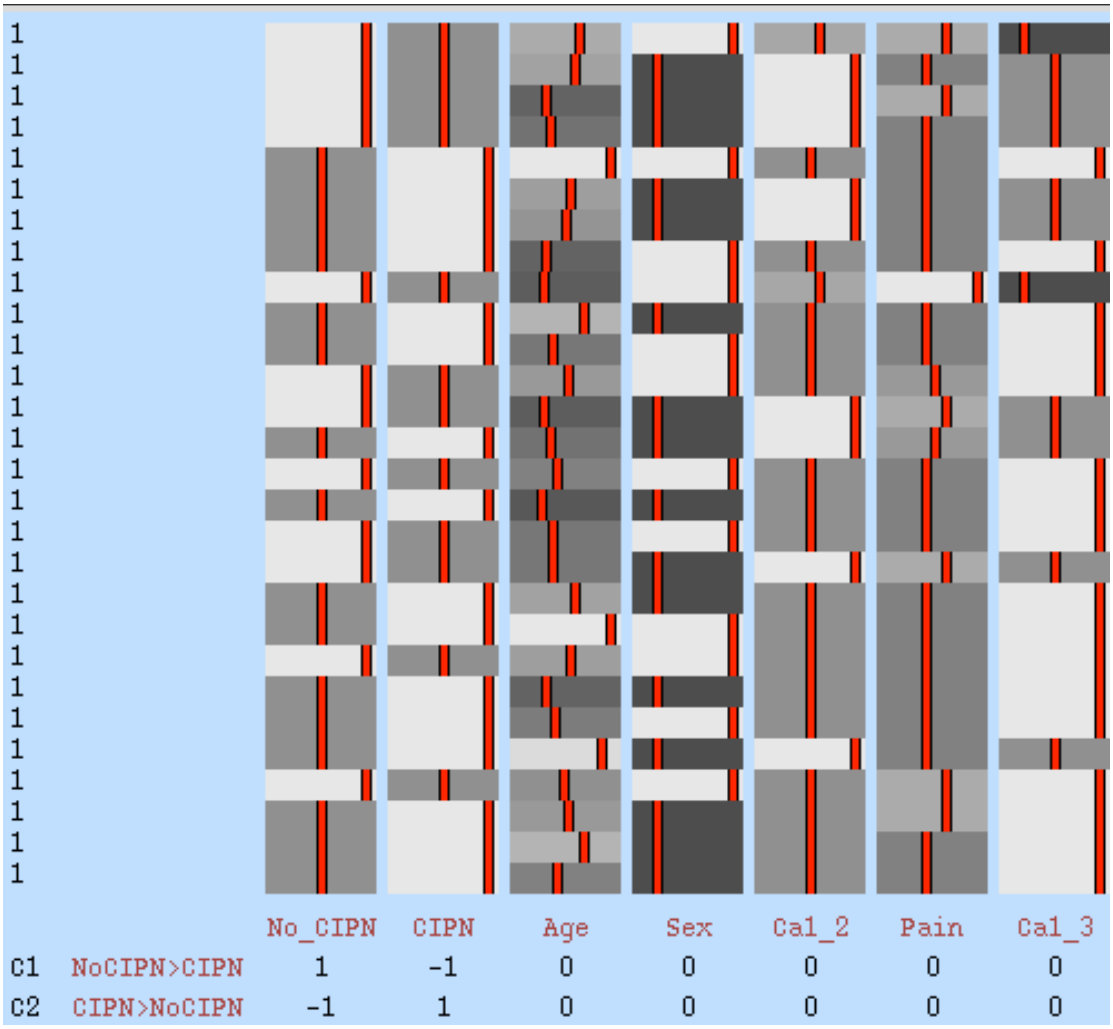
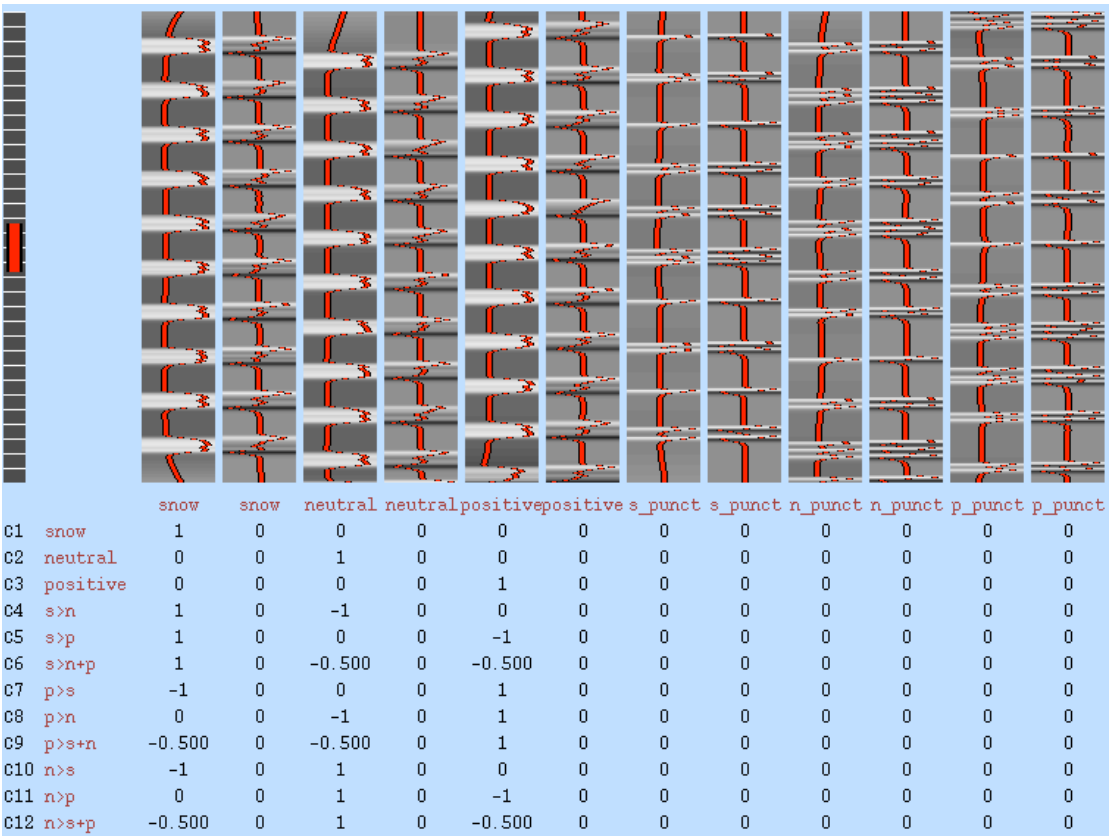


Figure 5.2 Second Level Design Matrix. Columns from right to left show the exploratory variables modelled: firstly, the two variables of interest: No CIPN and CIPN, and then known confounding variables included as contrasts of no interest, specifically: age, sex cancer type (split due to the specifications of FSL model set up) and baseline pain score.

5.3.2 International Affective Picture System (IAPS) Analysis

This analysis assessed the impact of the IAPS without the overlay of the punctate stimulus. The aim of this was two fold. Firstly, to aid in the interpretation of the impact of emotional processing on the DPMS when punctate stimuli were presented. Secondly, to assess if there were any differences in processing the emotional content between patients who developed CIPN and those who did not.

Therefore, the following contrasts of interest were included in the first level model: snow images, neutral images, positive images, snow greater than neutral, snow greater than positive, snow greater than neutral and positive, positive greater than snow, positive greater than neutral and positive greater than snow and neutral, neutral greater than snow, neutral greater than positive and neutral greater than snow and positive (figure 5.3).



		snow	snow	neutral	neutral	positive	positive	s_punct	s_punct	n_punct	n_punct	p_punct	p_punct
c1	snow	1	0	0	0	0	0	0	0	0	0	0	0
c2	neutral	0	0	1	0	0	0	0	0	0	0	0	0
c3	positive	0	0	0	0	1	0	0	0	0	0	0	0
c4	s>n	1	0	-1	0	0	0	0	0	0	0	0	0
c5	s>p	1	0	0	0	-1	0	0	0	0	0	0	0
c6	s>n+p	1	0	-0.500	0	-0.500	0	0	0	0	0	0	0
c7	p>s	-1	0	0	0	1	0	0	0	0	0	0	0
c8	p>n	0	0	-1	0	1	0	0	0	0	0	0	0
c9	p>s+n	-0.500	0	-0.500	0	1	0	0	0	0	0	0	0
c10	n>s	-1	0	1	0	0	0	0	0	0	0	0	0
c11	n>p	0	0	1	0	-1	0	0	0	0	0	0	0
c12	n>s+p	-0.500	0	1	0	-0.500	0	0	0	0	0	0	0

Figure 5.3 First Level Design Matric IAPS Analysis. Columns show the modelled events: snow images, temporal derivative of the latter, neutral images, temporal derivative of the latter, positive images, temporal derivative of the latter, timings of punctate stimuli occurring during snow images, temporal derivative of the latter, timing of punctate stimuli occurring during positive images, temporal derivative of the latter, and finally the timing of punctate stimuli occurring during positive images with the temporal derivative of the latter. Rows represent the contrasts of interest detailed above.

The second level model included all twelve COPEs from the first level for each subject. The statistical modelling approach and matrix design was identical to that used for the DPMS analysis (fig 5.2). Because the hypothesis underpinning

this analysis was not as strong as those for the DPMS analysis, only stringently thresholded (z = 2.3), cluster corrected results were reviewed.

5.4 Results

Data were collected for 30 patients. Two were excluded from the analysis. This was due to scanner failure during acquisition in one patient and non-progression to chemotherapy due to morbidity in another. This analysis therefore included 28 participants. The demographics of included patients were matched in terms of key confounders and are shown in table 5.1.

	Non CIPN 95%CI or % of 12	**CIPN 95%CI or % of 16**
Mean Age	57.5 (54.1-61)	62.2 (57.5-67.1)
Sex Female	5 (41%)	11 (68%)
Cancer Type •Lung •Gynae •Colorectal	 2 (16%) 5 (42%) 5 (42%)	 0 (0%) 4 (25%) 12 (75%)
Cancer Operation •No •Yes	 3 (25%) 9 (75%)	 0 (0%) 16 (100%)
Pain Score •Mean •Median (IQR)	 1.25 (0.3-2.3) 1 (0-2)	 0.2 (-0.1-0.5) 0 (0-0)

Table 5.1 Demographic data for the Non CIPN and CIPN groups. Mean age shown in table, age range 50-79yo. For cancer type and operation % refers to within group proportion. Range of pain score shown in brackets. Chemotherapy type collinear with cancer type and therefore not specifically reported.

5.4.1 DPMS Analysis

There were no significant results surviving cluster correction of z=2.3 for any contrast. When the z threshold was decreased to z=2, the contrast for punctate

stimuli presented during positive images (contrast 2) showed significantly greater activation in the posterior division of the right superior frontal gyrus in patients who did not develop CIPN (fig 5.4). Visual cortex activation was seen during punctate stimuli while viewing neutral and snow images (contrast 10 and 11) in CIPN and No CIPN group, respectively. There were no differences between the groups for any of the remaining contrasts.

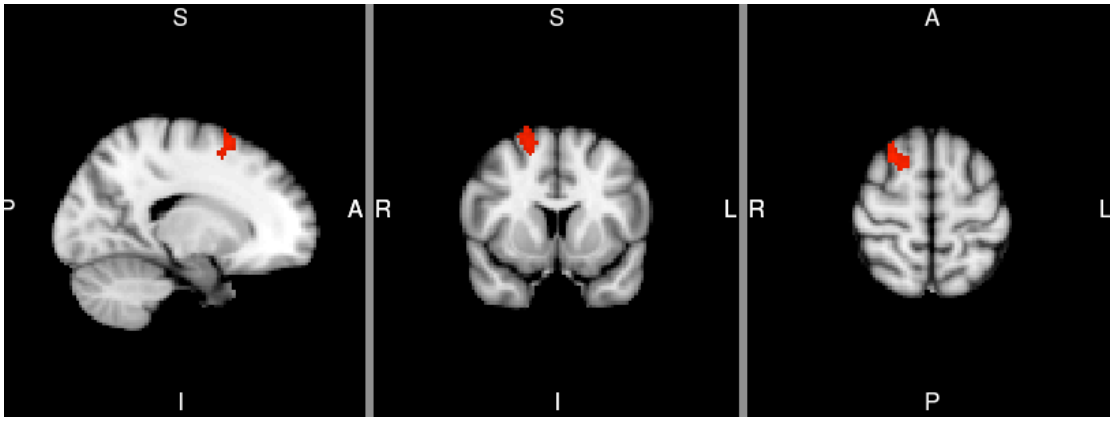


Figure 5.4 Activation for contrast no CIPN> CIPN during positive IAPS. Showing the posterior division of the superior frontal gyrus significantly more active in the no CIPN group. Image coordinates: x36, y68, z66. R = right, L= left, S= superior, I = inferior, A = anterior, P= posterior. Whole brain analysis, P<0.05 , cluster threshold z=2.

Exploration of non-cluster corrected z statistics, thresholded at z=2.3, showed DPMS brainstem activation in both the CIPN and non CIPN group, when presented with punctate stimuli alone (i.e. viewing snow images). Patients who did not develop CIPN had more activation in the thalamus. A summary of this exploration is detailed in figure 5.5.

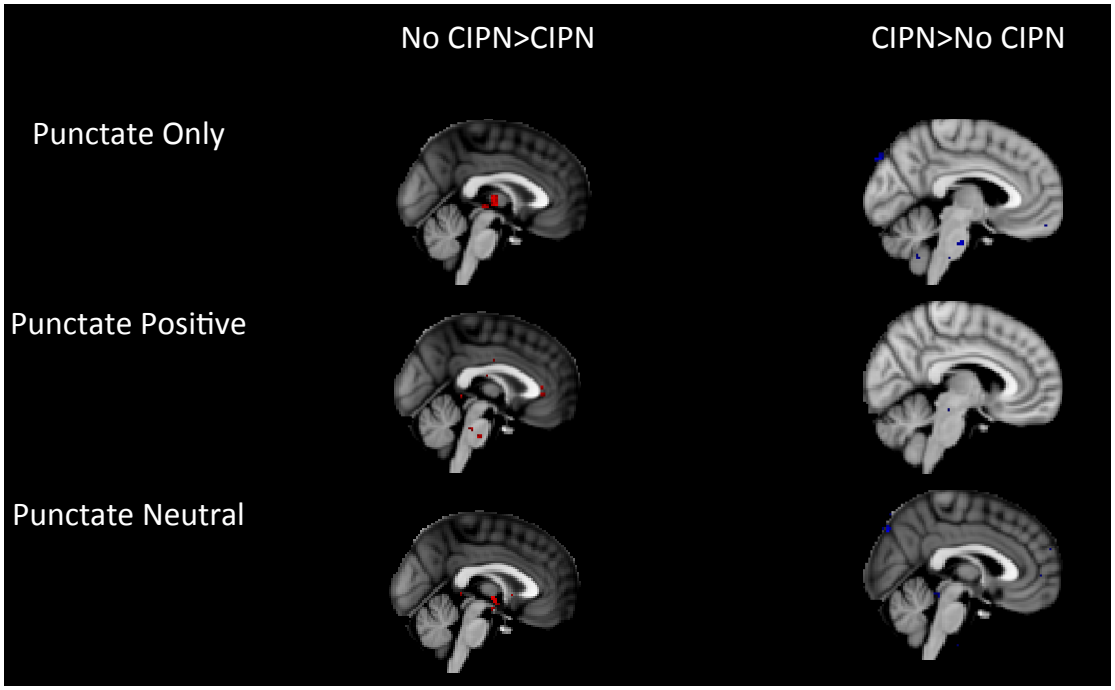


Figure 5.5 Cluster uncorrected z-statistic summary. P<0.05 Rows from top to bottom showing activation in response to punctate stimuli only, punctate during positive IAPS and punctate during neutral IAPS respectively. Patients who did not develop CIPN as compared to those who did develop CIPN had activation in: the thalamus during punctate only stimuli, nuclei of the MPRF when viewing emotionally positive images and receiving punctate stimuli, and right lingal gyrus, parahippocampus, insula cortex and precentral gyrus when viewing emotionally neutral images during punctate stimuli. Patients who developed CIPN as compared to those who did not, showed activation in the MPRF and cerebellum during punctate only stimulus, right hippocampus and left insula/operculum during positive images and punctate stimulus, and PAG, left putamen, left fontal operculum and right caudate nucleus during punctate stimuli when viewing emotionally neutral images.

5.4.2 DPMS Region of Interest Analysis

Whole group ROI comparison did not yield any statistically significant differences in BOLD signal change between patients who developed CIPN and those who did not. When the group was split by sex, females who developed CIPN had significantly more BOLD signal change in the MPRF. Males who developed CIPN had significantly less BOLD signal change in the thalamus (tables 5.2 and 5.3).

	FEMALE (mean % BOLD signal)		MEAN DIFFERENCE	SIG (2 tailed)
STRUCTURE	**NO CIPN (5/16)**	**CIPN (11/16)**		
MPRF	-0.03	0.04	-0.07	p=0.04*
L RVM	-0.04	0.03	-0.08	p=0.11
R RVM	-0.03	0.07	-0.10	p=0.17
PAG	0.04	0.06	-0.02	p=0.75
L Thalamus	-0.003	0.05	-0.05	p=0.13
R Thalamus	0.012	0.05	-0.03	p=0.19

Table 5.2 Summary of mean signal change in regions of interest in females. MPRF= mesencephalic pontine reticular formation, L =left, R= right, RVM= rostra ventromedial medulla, PAG= periaqueductal grey. *Denotes statistical significance. Mean difference and significance level denoted with a boostrap to a sample size of 1000.

	MALE (mean % BOLD signal)		MEAN DIFFERENCE	SIG (2 tailed)
STRUCTURE	**NO CIPN (7/12)**	**CIPN (5/12)**		
MPRF	0.02	-0.04	0.07	p=0.11
L RVM	0.03	-0.02	0.06	p=0.38
R RVM	0.02	-0.01	0.04	p=0.44
PAG	0.008	-0.08	0.09	p=0.18
L Thalamus	0.04	-0.07	0.11	p=0.03*
R Thalamus	0.03	-0.06	0.09	p=0.05

Table 5.3 Summary of mean signal change in regions of interest in males. MPRF= mesencephalic pontine reticular formation, L =left, R= right, RVM= rostra ventromedial medulla, PAG= periaqueductal grey. *Denotes statistical significance. Mean difference and significance level denoted with a boostrap to a sample size of 1000.

5.4.3 IAPS Analysis

BOLD signal activation following both neutral and positive IAPS (contrast 2 and 3), was greater in the region of the right frontal operculum adjacent to the insula,

in patients who did not develop CIPN (fig 5.6). This group also had more activation in the left inferior temporal lobe particularly the temporal fusiform cortex abutting the parahippocampus when viewing positive images over any other images (contrast 9) (fig 5.7). There were no differences between the groups for any of the remaining contrasts.

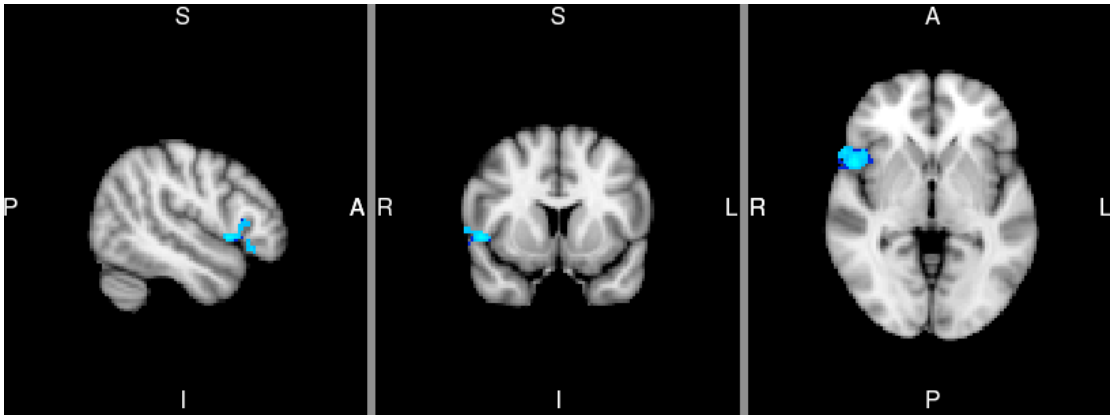


Figure 5.6. Statistically significant activity in response to viewing positive and neutral images in patients who did not develop CIPN. Activation to positive images is depicted in light blue, this overlays an almost identical area of activation for neutral images shown in dark blue. This image depicts the right frontal opercular region as significantly more active in patients who did not get CIPN as compared to those who did. Image coordinates: x20, y68, z35. R = right, L= left, S= superior, I = inferior, A = anterior, P= posterior.

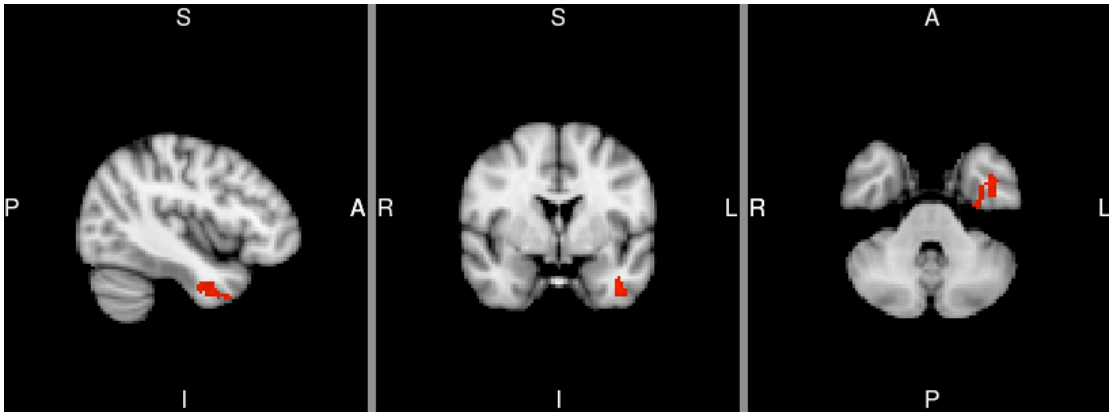


Figure 5.7. Statistically significant activity in response to viewing positive images over both neutral and snow images (p>s+n), in patients who do not develop CIPN. Activity seen in the left inferior temporal lobe. Image coordinates: x66, y63, z18. R = right, L= left, S= superior, I = inferior, A = anterior, P= posterior.

5.5 Discussion

5.5.1 Main Findings

The DPMS analysis assessed at a z statistic of 2 (equivalent to a p value =0.04), showed significantly more activation in the posterior division of the right superior frontal gyrus, in those who did not developed CIPN as compared to those who did. No other activation survived whole brain analysis with cluster correction. Review of raw z statistics showed some suggestion of differences in punctate processing between the CIPN and non-CIPN groups. A larger sample size in each group might therefore be postulated to reach statistical significance; however this is speculative and therefore not discussed further here.

ROI analysis for the whole group did not yield differences between CIPN and non- CIPN patients. However, when the group was split by sex, male patients who went onto develop CIPN had significantly less activity in their left thalami then males who did not get CIPN. Female patients who went onto develop CIPN had significantly more activity in their MPRF compared to those who did not get the neuropathy.

In terms of the IAPS analysis, processing of images with and without emotional content differed between those who developed CIPN and those who did not. As anticipated there was no difference in the way the snow images activated the brains of patients. Snow images served as the holding baseline in the experiment and were expected to have the same impact on both groups. Images that had content, be it emotionally positive or neutral, activated the frontal opercular region significantly more in patients who did not develop CIPN. When assessing the impact of positive emotional images over all other images (neutral or snow) the non CIPN group also had more activation in their inferior temporal regions.

5.5.2 Strengths and Limitations of Experimental Approach and Analysis

5.5.2.1 Strengths

A key strength of these findings, as discussed previously (see 4.5.2.1), relate to the prospective design of the CIPN study. This allowed patients to control for each other within the study cohort in terms of effects of cancer and chemotherapy. Moreover, in terms of this analysis the fMRI experimental paradigm was hypothesis driven and based on strong pre-existing animal and human literature, which has clear evidence of aberrance in DPMS in terms of pain vulnerability.

5.5.2.2 Limitations

The main limitations of this chapter relate to the stringent approach to data processing and the possibility of type II error. In particular the inclusion of age, sex and cancer types as repressors of no interest in the second level model may have masked important effects in the analysis. This is likely in view of the known impact of sex hormones on pain processing (Bartley and Fillingim, 2013)and the differences found in the ROI analysis when the group was split by gender. Additionally, the stringent approach to data cleaning with FIX may have removed important signal from the brainstem regions. Fix was used as the main hypothesis understudy in this chapter was brainstem related, an area known to be affected by noise (see 1.1.3.3).

In terms of experimental protocol a key oversight was the failure to take sharpness ratings following punctate stimuli from all participants during the scan. Also valence ratings for IAPS exist and these were not cross checked with patients recruited to this study. FMRI data should always be related to behavioural measures to aid interpretation and the lack of this possibility in relation to sharpness ratings and emotional valance may have limited this analysis. Finally, the possible impact of unknown confounding factors should always be considered in observational studies and is listed here as a possible limitation affecting the analyses presented in this chapter (see appendix C for further discussion).

5.5.3 Comparison of results with other studies

5.5.3.1 Descending Pain Modulatory System Analysis

The superior frontal gyrus (SFG) has recently been divided into three distinct functional regions (Li et al., 2013). The posterior division, activated here in patients who do not develop CIPN, has been shown to be involved in the reappraisal of negative emotional stimuli (Falquez et al., 2014) and shown to be connected to aspects of the descending pain modulation system (Li et al., 2013). More specifically in terms of pain, the SFG has been implicated in deciphering mismatch between expected and actual pain (Ploghaus et al., 2000). SFG activity has also been demonstrated in revaluation of painful stimuli, and related to emotional modulation of pain (Cheng et al., 2007). Kong and colleagues associated activation in the SFG, among other regions as important in the cognitive aspect of pain encoding (Kong et al., 2006). Moreover, experimental models of pain learning and adaptation, connectivity between the anterior insular cortex and SFG was shown to vary between direct and indirect pain experiences (Fan et al., 2016). Recent review of grey matter anomalies in neuropathic pain states, has reported the right SFG as one of the regions most frequently showing decreased volume in neuropathic pain (Pan et al., 2015).

The MPRF is composed of a number of brainstem nuclei including the nucleus cuneiformis and dorsal reticular nucleus. Similarly to the RVM these nuclei are known to contain both 'on cells' and 'off cells', responsible for the facilitation and inhibition of ascending nociception. The MPRF is known to be involved in the expression of opioid induced hyperalgesia in healthy humans with a role in propagating perception of central sensitisation (Wanigasekera et al., 2011). Although there is no previous evidence of gender based differences in MPRF activity, varied RVM activity, related to MPRF architecture in terms of presence of 'on' and 'off' cell function, has been demonstrated in women with low estrodiol states (Vincent et al., 2013). Although Vincent *et al's* findings were in a premenopausal cohort of women compared to those studied here. Mechanisms of gender differences in pain are complex, future studies in CIPN should take account of hormonal status (Bartley and Fillingim, 2013).

The thalamus is known to be involved in bidirectional relay of nociceptive input with brainstem DPMS, in particular the PAG (Wu et al., 2014). Moreover, the thalamus has strong resting state connectivity with all three divisions of the insula, a key area involved in pain perception (Wiech et al., 2014). Thalamic connectivity to premotor areas has been shown to be decreased in fibromyalgia patients (Flodin et al., 2014). More specifically in neuropathic pain, the thalamus, has been reported to have decreased function, as well as fewer N-acetylaspartate and GABA containing neurons (Gustin et al., 2014). Animal work in neuropathic pain models has also shown desensitisation of mu-opioid receptors in the thalamus (Hoot et al., 2011). Importantly, contralateral thalamic depression has been deemed as one of the most consistent findings in neuropathic pain studies (Garcia-Larrea and Peyron, 2013). Although there appears to be no previous evidence of gender specific decrease in thalamic activity in neuropathy, the evidence related to thalamic hypoactivity in neuropathy supports the finding presented here.

As previously discussed (see 4.5.3), there are limited brain imaging studies relating CIPN to brain function. Interestingly however, the two existing CIPN fMRI studies both report alterations in the right superior frontal gyrus. Boland *et al* investigating chronic CIPN, reported decreased BOLD signal activity during heat pain in the superior frontal gyrus in chronic CIPN patients as compared to healthy controls (Boland et al., 2014). This study did not report any altered activity in the thalamus or MPRF, but did note alterations in insula activity; known to be functionally connected to these regions. More recently, Nudelman and colleagues showed that increased perfusion in the right SFG was correlated with CIPN symptoms a month after chemotherapy administration (Nudelman et al., 2015).

5.5.3.2 International Affective Picture System Analysis

Brain processing of IAPS has been investigated in a number of healthy volunteer studies (Aldhafeeri et al., 2012, Britton et al., 2006). Positive IAPS have been shown to activate the prefrontal cortex, superior, medial and middle frontal

gyrus, the anterior cingulate cortex and the temporal lobe bilaterally. These support the regions identified here in patients who did not develop CIPN. Processing of positive emotional content has been associated with emotional regulation and cognition and gives some traction to the interpretation of the impact of these images in the CIPN study, discussed below.

Brain responses to emotionally neutral IAPS have not been clearly documented. Recently, questions regarding the true neutrality of IAPS rated as having neutral emotional valence have been raised (Schneider et al., 2016). The likely impact of this on the CIPN study is unknown, but any emotional bias resulting from emotional ambivalence as opposed to true neutrality would have affected both those who develop CIPN and those who did not, and so is likely inconsequential for the present analysis.

5.5.4 Interpretation of findings and implication for future work

As hypothesised, pain modulation as well as engagement with IAPS images, differed between patients who did not develop CIPN and those who did. The finding of greater functional activity in the posterior division of the SFG during positive images in patients who did not get CIPN may be tentatively interpreted as a capacity in this group to reappraise the negative punctate stimulus. Whether this reappraisal constitutes interaction of the SFG with brainstem inhibitory regions requires further investigation. Future connectivity analysis of these regions is planned, and was not undertaken for this thesis due to time constraints.

Further, the varied engagement with images between the non CIPN and CIPN group, which showed the same directionality as the SFG finding (No CIPN group> CIPN), strengthens the proposed interpretation that the non CIPN patients had a capacity to engage cognitive modulatory influences during the presented punctate stimuli. Based on previously cited literature, it would appear that this is an innate ability. It is proposed here that lack of engagement in those who develop CIPN can be considered as aberrant in this group. This line of thought will be further investigated in future. Specifically, in line with Boland et al's work

in chronic CIPN, future analysis of these data will investigate whether there was any decreased activity in the SFG and regions responding to the IAPS in patients who developed CIPN.

The region of interest analysis, showing decreased contralateral thalamic activity in male patients who develop CIPN, corroborates with published literature reporting decreased thalamic function and connectivity in neuropathic pain states. Similarly the increased MPRF BOLD signal in women who develop CIPN fits with the described permissive influence of this region on central sensitisation. The exact impact of 'on' and 'off' cells in this area needs further elucidation, as activity in both would result in the statistically significant increase in BOLD signal identified in the analysis. However, in view of the association of increased MPRF signal and subsequent CIPN development, it is possible to postulate that 'on' cell activity predominates, allowing a permissive central perception of subsequent chemotherapy induced peripheral nerve damage.

The sex difference suggested by the ROI analysis needs further exploration. In the first instance a whole brain analysis divided by sex is planned. Secondly, more detailed investigation of the possible impact of gender on CIPN development in general is likely warranted. In particular, exploration of the influence of sex hormones may be useful. In this study salivary testosterone levels were tested at the time of the scan. These yielded some moderate correlations with ROI activity, which needs further exploration (see appendix G for further discussion). Finally, how these findings relate to chronification of CIPN remains to be determines once long term follow up data is obtained.

In summary, taken together this analysis implies differences in the descending pain modulatory system between patients who develop CIPN and those who do not. Specifically, it appears that key regions associated with top down pain modulation, including the thalamus and MPRF have altered activity in those who progress to CIPN, at baseline prior to peripheral nerve damage and clinically significant pain. Additionally, response to positive emotional images and the

capacity to reappraise punctate stimuli while viewing these images also differs between those who develop CIPN and those who did not. Although further investigation is needed, this analysis provides evidence of aberrance in key pain-processing regions in the brain prior to peripheral nerve damage with chemotherapy. It is important that future studies are carried out in larger numbers of patients in order to improve statistical power and clinical confidence in findings.

6. Levo-menthol as a treatment for Chronic CIPN: pilot fMRI study development

Predicting CIPN vulnerability, which has been the focus of this thesis, contributes to possible future preventive strategies. However, there is already a significant cohort of cancer survivors who suffer because of chronic CIPN. Treatment strategies for these patients remain limited. Treatments currently in use are moderately efficient and often associated with unacceptable side effects. Novel approaches to treatment of chronic CIPN are needed. This chapter details the development of a randomised controlled trial investigating levo-menthol versus placebo in patients with chronic CIPN. A central point of the study is the use of functional magnetic resonance imaging to assess the efficacy of the drug.

6.1 Background

Clinically, the problem of CIPN is complicated by difficulty in early detection, no effective preventive strategies and limited treatment options (Albers James et al., 2011). Currently, treatment options for CIPN includes oral antidepressants and anticonvulsants, which often have intolerable side effects (Smith et al., 2013, Manji, 2011). The only effective long-term option in severe CIPN is chemotherapy cessation. These measures have obvious negative impact on patient morbidity and mortality. Moreover dose reduction may not improve established CIPN (Albers James et al., 2011).

Assessment of new analgesics for CIPN patients is marred with the difficulties that affect all analgesic randomised controlled trials (RCTs)(Dworkin et al., 2010). Specifically, the subjective nature of pain and the influence of an active placebo response, results in analgesic RCTs often describing small effect sizes, difficult to interpret clinically (Quessy and Rowbotham, 2008). In particular, in patients with CIPN, the varied individual experiences of neuropathic pain, not easy to standardise clinically or with QST, make the assessment of new analgesics particularly difficult (Maier et al., 2010, Attal et al., 2011).

The advent of fMRI as a research tool, with its ability to detect established behavioural changes, has greatly aided in unravelling neuropathic pain (Schweinhardt et al., 2006, Tracey and Mantyh, 2007). In particular the subjective non-standardised nature of pain has become more readily understood using fMRI (Tracey and Mantyh, 2007). Over the last decade fMRI has also been implemented in drug discovery and drug efficacy assessment (Duff et al., 2015). Most recently it has been shown to provide useful outcome measures in analgesic drug studies (Wanigasekera et al., 2016). Guidelines aiming to standardise the implementation of fMRI in drug trials have been published (Schwarz et al., 2011).

To date fMRI has not been utilised to assess CIPN treatment. In the context of CIPN where treatment options are lacking and new analgesic trials continue to report negative results, a novel approach to assessing potential analgesic treatment seems prudent (Hershman et al., 2014, Gewandter et al., 2014). In view of this the aim here was to explore the feasibility and usefulness of implementing fMRI in the assessment of a topical transient receptor potential melastatin (TRPM8) agonist 3% levomenthol in patients with chronic CIPN.

6.1.1 Levomenthol; a new treatment for CIPN

Cool sensitive transient receptor potential melastatin (TRPM8) channels were identified as a novel target for neuropathic pain/dysaesthesia relief some years ago(Proudfoot et al., 2006). Activation of these channels results in recruitment of a central inhibitory loop within the spinal cord, involving metabotropic glutamate receptors. Levomenthol, a topical cooling compound, used in dermatology, was known to selectively activate TRPM8 receptors, up regulated after sensory nerve injury (Patapoutian et al., 2009). It was therefore postulated that menthol would likely work in CIPN cases (Moran et al., 2011). This postulate was promptly translated into clinical practice and extended to a successful proof of concept (PoC) study using menthol 1%, which showed marked clinical improvement in 82% of participants (Colvin et al., 2008) (Fallon et al., 2015). Topical application of levomenthol can initially be associated with skin cooling.

Clinically this may be perceived as uncomfortable. The temperature range that application cools the skin to and at which point this becomes uncomfortable has not been elucidated.

This chapter details the development and pilot data collection for the next phase of assessing levomenthol as a treatment for CIPN. The name of the study is the Menthol IN Treatment (MINT3) of chemotherapy induced peripheral neuropathy fMRI study. The 3 denotes the 3rd stage of this work in terms of levomenthol assessment in CIPN patients.

6.1.2 Aims of this chapter

The aim of this chapter is:

1. To develop a protocol for a randomised controlled trial, using fMRI to investigating levo-menthol as a treatment for chronic CIPN?

6.2 Methods and Analysis Plan

6.2.1 Study Design, Objectives and Endpoints

The Menthol IN Treatment (MINT3) of chemotherapy induced peripheral neuropathy fMRI study, is a single centre randomised, double blind, controlled, exploratory study of menthol gel versus placebo gel. Treatment allocation is a 1:1 ratio. The study is sponsored by the Academic and Clinical Central Office for Research and Development (ACCORD) for NHS Lothian and the University of Edinburgh and co-ordinated by the Edinburgh Clinical Trials Unit (ECTU). The trial will be conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The study has been registered on the International Standardised Randomised Controlled Trials Registry (ISRCTN: 69917256) and the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT 2013-003968-31).

The primary objective of this trial is to determine the central pharmacodynamic efficacy (activity) of 3% menthol in patients with post treatment CIPN, using functional magnetic resonance imaging (fMRI).

The secondary objectives are to:
1) determine the cool temperature range causing discomfort to CIPN patients using a non invasive skin thermometer,
2) assess the degree to which 3% menthol gel cools the patient's skin on application,
3) evaluate the safety of menthol gel application (assessed by no worsening of pain and monitoring of unexpected symptoms/signs).

Therefore, the primary trial end point is to determine if there is an analgesic effect of menthol which is distinguished from placebo effects, using diminished activation of established pain brain networks (blood oxygen level dependent (BOLD) signal activation on fMRI) as a surrogate measure of efficacy.

The secondary endpoints are:
1) Thermal QST: to determine the cool temperature range causing discomfort to CIPN patients using a non invasive skin thermometer and standardized QST testing.
2) Skin temperature after gel application: to assess the degree to which 3% menthol gel cools the patient's skin on application.
3) To establish that there is no worsening of pain after menthol gel application.

6.2.2 Patient screening and selection for the MINT3 study

Patients with CIPN attending the palliative and supportive clinic will form the pool of potentially suitable study patients. Suitable patients have their medical records reviewed for eligibility by the research team following the clinic. Patients meeting the following criteria will be invited to take part in the study:

Inclusion Criteria

a) Patients have received any neurotoxic chemotherapy.
b) Patients have experienced post treatment Chemotherapy Induced Peripheral Neuropathy (CIPN) pain for a minimum of 3 months.
c) Patients reporting a distressing or uncomfortable neuropathic symptom (such as pain or tingling) with a score of ≥4 on a scale of 0-10 with 0 being none.
d) Aged 18 years or over at study entry.
e) Patient's Oncology team agrees to their taking part in the study.
f) Patients are able to provide written informed consent to participation in the study after explanation of the study protocol.
g) Patients have the ability to complete questionnaire assessments in the English language.
h) In the opinion of the investigator, the patient is able to complete the various assessments.
i) Neuropathy must be confined to the distal extremities (distal to elbows and/or knees).

Exclusion Criteria

a) Preexisting or history of peripheral neuropathy due to any cause other than chemotherapy (diabetes, alcohol, toxin, hereditary, etc.).
b) Patients with any contraindication to the use of topical therapy or menthol.
c) Neurological conditions which may influence findings (such as Multiple Sclerosis or residual signs/symptoms from a previous stroke).
d) Skin conditions which prevent assessment of the relevant areas affected by peripheral neuropathy.
e) Suffering from significant psychiatric illness, which would hinder their completion of the study.
f) General medical condition is unstable or rapidly deteriorating, such that they are unlikely to be able to contribute to the study.
g) In the opinion of the Research Team or their usual medical team, would be unable to complete the study protocol for any other reason.

h) Current treatment of ≤ 30 days duration with anticonvulsants, tricyclic antidepressants, MAO inhibitor, or other neuropathic pain medication agents such as carbamazepine, phenytoin, valproic acid, gabapentin, lamotrigine or amifostine. (If on a stable dose of any of these medications for >31 days, patients will be asked to continue these for the duration of the study. Analgesic agents such as acetaminophen, nonsteroidal antiinflammatory agents, or opioids, are allowed).
i) Application of topical lidocaine patch/gel or capsaicin cream or patch (to the limb extremities) currently or within the last 30 days (as this would interfere with application of the menthol cream and potentially study outcome).
j) Other medical conditions, which in the opinion of the treating physician/allied health professional would make this protocol unreasonably hazardous for the patient.
k) Contraindication to MRI: e.g. aneurysm clips, other metal work in body, claustrophobia.

6.2.3 Study setting, patient flow and assessment

The trial will be conducted in NHS Lothian palliative care/ oncology. The patient's direct clinical care team will approach the patient in the first instance. From this, potential patients will be identified. At this point further screening for full eligibility will take place. If patients are eligible and give written informed consent they will undergo the baseline fMRI scan at the Clinical Research Imaging Centre (CRIC) in Edinburgh. Following the scan (either on the same or next day) patients will be seen at the Edinburgh Cancer Research UK (CRUK) Centre, where they will undergo other baseline assessments, randomization and receive the study medication along with instructions regarding gel application. Patients will undergo safety assessments weekly. This will involve a study nurse calling the patient and completing the side effects questionnaire over the phone. Patients will also have a direct number to call researchers if they have any concerns. At six weeks, patients will attend CRIC for the second and final study fMRI scan. Following the scan patients will attend CRUK for behavioral measure

assessment. Study medication will stop at this stage. At 12 weeks, patients will be invited back to CRUK for a final assessment (see table 6.1).

6.2.4 Randomisation procedures & treatment allocation

Participants will be randomised with equal probability to placebo or 3% menthol gel, using random permuted blocks of length four[4]. Randomisation will be carried out at the Edinburgh Clinical Trials Unit (ECTU), allowing researchers and participants to remain blinded to treatment allocation. Treatment will be allocated following randomisation at the ECTU. Participants will be given a standardised number of gel filled tubes. Both levomenthol and placebo will be in identical packaging labelled with the trial name and the patient's trial number.

[4] Random permuted blocks are blocks of different sizes used to select which study arm the given participant will go into. Use of blocking for randomisation ensures that the resulting treatment groups are balanced within the study. The size of the next block is randomly chosen from the available block sizes. For example, here is a list of random permuted blocks of sizes 4: AABA, BBAB, ABBB etc.

	<30 days prior to registration	Baseline	6 weeks	12 weeks
Screening	x			
Consent		x		
Hx & Examination		x		
Weight		x		
Medication Hx		x		
Digit Symbol substitution Test (DSST)		x		
General Causality Orientation Scale (GCOS)		x		
National Adult Reading Test (NART)		x		
Hospital Anxiety and Depression Scale (HADS)		x	x	x
Brief Pain Inventory (BPI)		x	x	x
Side Effects Questionnaire (SEQ) (weekly)				
Quantitative Sensory Testing (QST)		x	x	x
SKIN TEMP		x		
CIPN20 Questionnaire		x	x	x
FMRI		x	x	

Table 6.1. Patient Assessment Schedule. X denotes time point of specified assessments. Hx= history.

6.2.5 Emergency unblinding procedures & withdrawal of study participants

In case of a need to unblind participants, the ECTU will provide a website with patient codes, this will enable the principal investigator to organise unblinding and inform the relevant parties (e.g. GP, Patient, hospital doctors). The researchers will remain blinded to the patient's treatment allocation. Should a participant wish to withdraw from the study they can do so at any time. They will then receive best practice standard care. They will be asked at consent if in case of withdrawal they consent to their data still being used by the research team.

6.2.6 Study Intervention

Patients will receive 6 weeks supply of gel (active or placebo), equating to 500g in metered tubes. Both active and placebo preparations will be in identical tubes, marked with the patient's trial number and name. If patients run out of the preparation they will be asked to contact the research team and a resupply will be given and noted for adjustment during data analysis. Participants will be advised to apply the gel twice daily over the affected area and will be provided with instructions on how to do this and what quantity of gel to use. Participants will be asked to return their empty tubes after six weeks. The tubes will be weighed. The patient will record start and end date of each tube in a trial diary. To mimic the characteristic aroma of menthol Carvone is contained in the placebo gel. Active Levomethol preparation as well as matching placebo gel is manufactured by Tayside Pharmaceuticals: Ninewells Hospital & Medical School Dundee. If for any reason participants needed to use another topical application on the areas being used for the trial medication the patient will be withdrawn from the study. If new analgesics are started within 30 days of recruitment to the study, patients will be excluded or withdrawn from the study.

6.2.7 Data Collection and Management

Case report forms (CRFs) have been developed in partnership with the ECTU (see appendix I). These forms will be completed in accordance with the CRF completion guidelines issued for the study. Queries should be handled as described in the study dataflow section of the CRF completion guidelines. Members of the research team will enter the data on to the database. CRFs for the study will be returned and stored in line with current regulatory requirements in as secure location. The trial sponsor will undertake regular audit following site initiation. The following data will be collected:

Standard demographic data:

• Age, sex, weight, height, co-morbidities, all regular medication, chemotherapy type and dosing will be recorded by the researcher recording baseline data following the fMRI scan and prior to randomisation.

Primary outcome data:

• Evidence of altered activity within the pain and placebo networks following standard pain provocation at baseline and after six weeks of treatment (menthol or placebo) will be identified using standardized MRI analysis. Collected sequences will include structural data, resting state data (ASL and BOLD), and functional data (punctate, thermal cold and reward/choice task). Anonymised fMRI data will be stored on secure university servers. Second level analysis will adjust for CIPN20 scores as a regressor of interest.

Secondary outcome data:

• Quantitative sensory testing (see appendix J for protocol), skin temperature after gel application, assessment of cognitive/affective components of pain perception (BPI and HADS), measures of side effects from treatment (SEQ), measures of higher cognitive function (NART, DSST, GCOS). These measures are included because of the relevance to mood and depression and will be explored in future collaborative work with the department of psychiatry.

6.2.8 Statistics and data analysis plan

Sample Size Calculation:

There is no principled power analysis applied in the context of classical inferences using the mass univariate approach, which underpins BOLD signal activation analysis. This is because specifying the alternative hypothesis is not possible in quantitative terms since the BOLD effect is underpinned by the hemodynamic response variable which is produced by a convolution of neuronal treatment effects. As a result for fMRI studies it has become standard practice to guide sample size selection by the size of similar studies which have been able to reject the null hypothesis in one or more voxels(Friston, 2011, Friston et al., 1999). Based on this approach, in our exploratory study 32 participants (16 per group) should be an adequate sample to detect significant fMRI changes, related to modulation of brain activity in the pain and placebo networks between the active and placebo arms. This study will allow calculation of parameters that can

be used in future sample size calculations, accessible through new neuroimaging sample size calculator tools(Joyce and Hayasaka, 2012, Guo et al., 2014).

fMRI Data Analysis

Blood Oxygenation Level Dependant (BOLD) imaging will be carried out at baseline prior to treatment commencement and at 6 weeks. During each scanning session pain will be evoked (punctate stimuli) and evidence of activity in the brain's pain and placebo networks will be reported. Resting state data will also be collected for subsequent independent component analysis (ICA), with a priori seeding in the placebo network. A standard approach to functional data analysis (i.e.: data acquired during painful stimulus presentation) will be used including pre-processing, first level and second level analysis (Smith, 2004). Region of interest analysis will be decided on *a priori* based on previously published evidence regarding pain and placebo networks. CIPN20 scores will be utilised as regressors of interest in the second level fMRI analysis. ICA will be used to clean data as well as to establish group average spatial maps. Dual regression will then be used to generate subject-specific versions of the spatial maps, and associated timeseries. Group differences will then be tested for using FSL's *randomise* permutation-testing tool (Beckmann and Smith, 2004).

Overall Analysis Plan (fMRI and non fMRI data)

Data concerning the primary outcome measure will be handled as described above. Secondary outcome measures pertaining to change scores for HADS and BPI will be compared using the Wilcoxon rank - sum test across the menthol and placebo arms of the trial. Baseline skin temperature changes associated with gel application will be treated as a continuous variable. A standard mean difference between the two groups will be analysed using an independent t test. Descriptive statistics will be used to report temperature ranges associated with pain and discomfort at baseline. Secondary outcome measures relating to occurrence of side effects and any other adverse events will be represented as binary outcomes. Proportions will be analysed and a relative risk ratio obtained.

Where there is missing data for an outcome variable, in the first instance, those records will be removed from any formal statistical analysis, unless otherwise specified. An intention to treat approach will be undertaken. Distributional assumptions underlying the statistical analyses will be assessed by visual inspection of residual plots. Normality will be examined by normal probability plots. If the distributional assumptions for the parametric approach are not satisfied, further data transformation (to alleviate substantial skewness (i.e. normalizing) or to stabilise the variance), or other suitable methods will be considered. If applied this will be documented in the statistical results report together with the reasoning supporting the action taken. fMRI data analyses will be carried out with outlier de-weighting. Where appropriate non-parametric analysis will be undertaken.

6.3 Ethical considerations & data protection

Approvals Obtained:

The trial protocol and information sheets have been reviewed and approved by the Scotland A Research Ethics Committee (13/SS/0201). Two substantial protocol inclusions have also been reviewed and approved by the same ethics committee since the original favourable opinion was granted (see appendix E). Approval has also been gained from the Medicines and Health Products Regulatory Authority (MHRA). Any subsequent amendments will be sent for approval as per standard Good Medical Practice requirements.

Written Informed Consent:

The research team will carry out consent once patients express interest to their direct clinical care team. Patients will be given verbal and written information and sufficient time (minimum of 24 hours) to review the information and to ask questions and have them answered before providing written informed consent. Following consent, fMRI scans will be carried out at the Edinburgh Clinical Research Imaging Centre (CRIC). Study baseline review and randomisation and follow up visits will take place at the CRUK after the baseline scan.

Pharmacovigilance:

Study investigators are responsible for the detection and documentation of adverse events (AE). Full details of contraindications and side effects for aqueous levomenthol are detailed in the British National Formulary. Topical menthol has been used in dermatology for over a decade and has an established safety profile. No severe adverse events related to the menthol gel are expected. If any adverse events occur, study investigators will make an assessment of severity for each and record this on the CRF. If the investigators become aware that a serious AE has occurred in a study participant, the information will be reported to the trial sponsor immediately or within 24 hours. The Academic and Clinical Central Office for Research and Development (ACCORD) the sponsor of the trial has appropriate insurance, which applies to this study.

Trial Monitoring and Oversight:

The trial will be coordinated by a Project Management Group, consisting of the grant holders- chief investigator (CI) and principal investigator (PI) in Edinburgh, a trial manager and coordinating nurse. The Trial Manager will oversee the study and will be accountable to the Chief Investigator/Principal Investigator. The Trial Manager will be responsible for checking the CRFs for completeness, plausibility and consistency. Any queries will be resolved by the Investigator or delegated member of the trial team. A Delegation Log will be prepared for the trial site, detailing the responsibilities of each member of staff working on the trial. A Trial Steering Committee (TSC) will be established to oversee the conduct and progress of the study. The TSC will be composed of clinicians and senior scientist supervising the chief investigator as part of her PhD (MF, LC, IT, SL) and a sponsor representative.

6.4 Plan for study completion and result dissemination

End of Trial:

The end of study is defined as the last participant's last visit. The end of the study will be reported to the research ethics committee and Regulatory Authority and a summary report of the study will be provided within 1 year of the end of the

study. If patients do respond to the treatment and feel a benefit, after the trial has completed patients will be prescribed the gel by their family doctor for continued use.

Dissemination of Findings:

Trial results will be published in peer-reviewed journals and presented at national and international conferences. Authorship will be determined by internationally agreed criteria for authorship. Patients who gave consent to be contacted with results at the start of the trial will be sent a lay summary at its conclusion. Anonymised trial data will be made available if interested researchers seek and are granted additional ethical approval. Future fMRI data pooling to investigate the interaction between depression and CIPN is anticipated through an on going collaboration with the Edinburgh University Division of Psychiatry.

6.5 Development of experimental paradigm

6.5.1 fMRI Paradigm

6.5.1.1 Punctate Task

The punctate task as used for the CIPN study (von Frey filament size 256mN) is planned for the MINT3 trial. This task (see 3.1.3.1.2) was validated in a patient cohort and shown here to be effective in terms of result generation. Clinically punctate allodynia is known to be an important abnormality in CIPN, and has been used in clinical trials of CIPN to test for degrees of neuropathic abnormality (Kosturakis et al., 2014).

6.5.1.2 Cold Task

Chronic CIPN patients are known to have aberrance in temperature sensation. In particular cold allodynia is an important feature of this neuropathy, which appears to be demonstrated over and above other forms of allodynia (Fallon, 2013). Consequently, it was decided that MINT3 trial participants should receive a cold stimulus, to assess this feature that is hypothesised to involve the TRP

family of receptors channels (Salat et al., 2013, Quartu et al., 2014) , which are known to be the site of action of menthol.

The 'cold task' described here, will be employed to test baseline allodynia and the effects of levomenthol versus placebo on its clinical and central (brain) manifestations in requited patients. The thermal stimuli will be presented as a cyclical block design, oscillating between a thermo neutral temperature of 34^0C, held for 70 seconds and a cold temperature of 5^0C, held for 10 seconds (fig 6.1). Optimally, this would be carried out using the Medoc pathway thermode. However, noise issues related to this equipment (see 3. 1.3.2) persist, so instead a simple model utilising blocks of ice cooled to a standard temperature will be used instead. The pilot data was acquired using this latter approach.

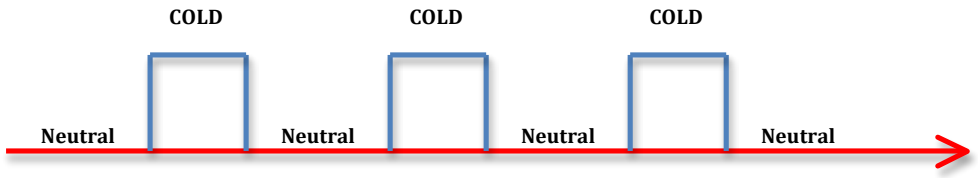


Figure 6.1 Cold Task Paradigm. Red arrow denoting scanning time. Neutral showing room baseline temperature held for 70 seconds. Cold denoting cold stimulus of 0C to 5C held for 5 seconds. Sequence acquired over 8minutes.

6.5.1.3 Choice and Reward Task

The choice and reward task was adapted from a task previously used by Leotti *et al* by Dr Liana Romaniuk. The main premise of the task is that having choice and capacity to exert control over ones environment has positive valance associated with it (Leotti et al., 2010). Leotti and colleagues' task focused purely on reward processing and motivational behaviour. The modified task used here additionally included a learning component by jittering the number of allocated reward

points, allowing assessment of reward and motivation during reinforced learning.

This task is planned for the MINT3 study for two reasons. Firstly, it is hypothesised that placebo responders to menthol will have a high response to the choice condition (Scott et al., 2007, Wanigasekera et al., 2012). Inclusion of this task will enable more detailed probing of the placebo response in this study. Secondly, future comparison of baseline data acquired for the MINT3 study, will be made to fMRI choice and reward task data acquired from a large cohort of depressed patients. This will allow investigation of pain and depression, known to be co-expressed clinically.

The task is implemented using *Presentation* software. There are two fundamental trial types: choice and no-choice, each presented 27 times. Each trial begins with a cue indicating which trial this would be (see figure 6.2). After a jittered period of choice/no-choice anticipation, the participant is shown a two colour stimuli: during choice trials, they are able to select their preferred coloured shape (see figure 6.2). During no-choice trials, a rectangle appears around the shape, which the computer has selected, and they are obliged to select before moving on. Selection is made via a button press. Following selection participants are presented with a reward outcome of the selection; 0, 50 or 100 points. Trial duration ranges between 13s and 22s, mean 17.5s. A total of 54 trials gave an experiment duration of 15m 45s. The reward element in the choice trial was jittered, enabling reinforced learning. The choice and no choice reward amounts were balanced.

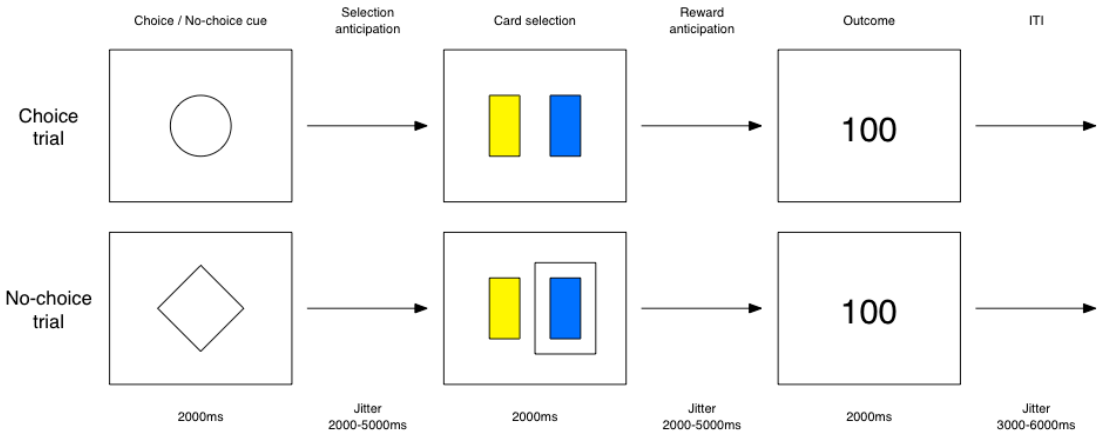


Figure 6.2. Summary of Choice and Reward Task. From left: circle indicates a choice trial and diamond a no choice trial. In the choice trial participants can choose a yellow or blue rectangle. Their choice is rewarded with points of 0, 50 or 100. In the no choice trial participants are obliged to choose the colour selected by the computer and denoted by a rectangle around it. The time to selection and also the time to revelation of reward is jittered. ITI = inter trial interval.

6.5.2 Behavioural and Physical Measures

6.5.2.1 Psychological and Cognitive Measures

A number of psychological measures will be used to assess the subject's cognitive processing capacity. These include the digit symbol substitution test (DSST) a subtest from the Wechsler collection of intelligence tests, known as the Bellvue Intelligence Scale (BIS). The General Causality and Orientation Scale (GCOS) assesses three aspects of motivational orientation: autonomy orientation, controlled orientation and impersonal orientation (Deci and Ryan, 1985) . It was chosen as a behavioural match for the choice and reward task in the scanner, and will also be administered in the on going depression study data collection to enable future data sharing. The National Adult Reading Test (NART) will further assess cognitive and intellectual capacity in the cohort.

For assessment of pain and affect the Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983) and the Brief Pain Inventory (BPI) will be implemented. A standardised side effects questionnaire will assess unwanted

effects of menthol gel. The CIPN20 ERCORT measure will be used to assess symptoms of CIPN specifically.

6.5.2.2 Physical Measures

Skin temperature testing before and after application of menthol gel will be used to determine the cooling capacity of the gel. Further painful thresholds of cold allodynia will be acquired from each patient as part of the Quantitative Sensory Testing (QST) protocol in order to correlate the range of the menthol gel cooling capacity and the actual level of painful cold allodynia in each study participant.

6.6 Pilot Scan Results

Due to time constraint the single subject pilot data was analysed using Statistical Parametric Mapping version 8: The Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, London running in Matlab (The MathWorks, Natick, MA) by Dr Liana Romaniuk based in the department of Psychiatry at the University of Edinburgh. Dr Romaniuk was involved in the paradigm design and pilot data analysis for the MINT3 pilot presented here. The approach in terms of data preprocessing and first level analysis was consistent with that used throughout the remainder of this thesis and described in 3.4.1 and 3.4.2. Please note that images are presented in direct view (i.e. right side of brain equals right side of image) as opposed to the radiological orientation presented in other parts of this thesis (right side of brain equals left side of image).

As part of the feasibility testing of the paradigm, a 53 year old healthy female underwent the pilot scan. She had no pain and was not taking any regular analgesic medication. She felt the duration of the scan was acceptable and that none of the experimental tasks were excessively difficult to take part in.

6.6.1 Punctate Task

Punctate stimuli were administered over the right medial malleolus. The right inferior parietal lobe, right temporal gyrus, the left insula and left somatosensory cortex were activated in response to these stimuli (fig 6.3). Brainstem activation during this whole brain analysis was not seen. Due to time constraints, region of

interest analysis, which is more sensitive to the small nuclei of the brainstem, and is planned for the MINT3 trial, were not carried out here. Contrast investigating the effects of punctate stimuli during positive images showed activation in left superior frontal gyrus, left post central gyrus and left temporal gyrus. Additionally, activation was also seen in the right caudate nucleus, right post central gyrus and right putamen.

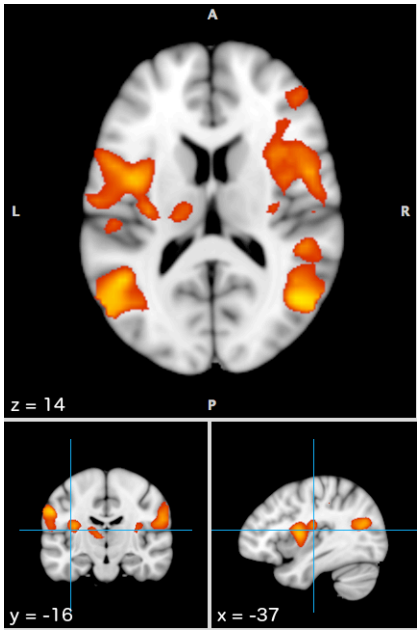


Figure 6.3 Activation following punctate stimulation. Activation is seen in regions known to be involved in pain processing including the insula and somatosensory cortex, contralateral to the administered stimulus.

6.6.2 Cold Task

Regions activating in response to cold stimulus (cold >neutral), administered above the left medial malleolus, included the right inferior gyrus, right central sulcus and right insula (fig 6.4). The reverse contrast investigating areas active when no cold stimulus was applied (neutral>cold) showed significant activity in the right mid occipital gyrus, right precentral gyrus and right putamen. Additionally activity was seen in the left angular and precentral gyri.

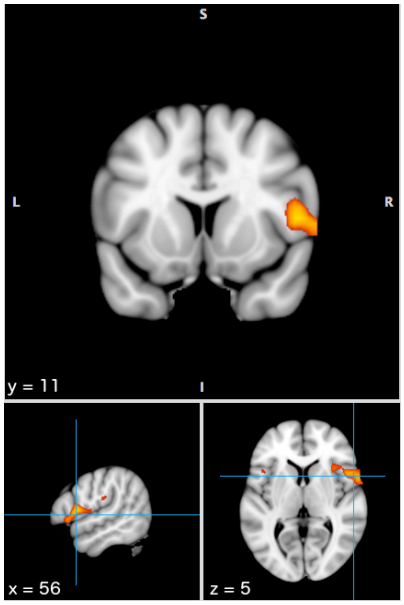


Figure 6.4 Activation following cold stimulation. The stimulus was applied above the left ankle. Contralateral activation in the right insula is seen.

6.6.3 Choice and Reward Task

In order to determine what impact the ability to choose had on activation associated with receiving a reward, the reward times choice interaction was examined (Choice reward 100 > Choice reward 0) > (No-choice reward 100 > No-choice reward 0). This revealed significantly greater activation of the left inferior frontal/orbitofrontal cortex (see fig 6.5). (Figure Z, MNI -36 29 -10, Z = 3.97, kE = 168, p = 0.047 FWE-corrected). The inverse contrast demonstrated very little activation. This suggests that the receipt of reward that has been personally earned rather than passively gifted elicited greater activation in regions which are known to play an important role in motivated behaviour and goal-seeking in this single participant.

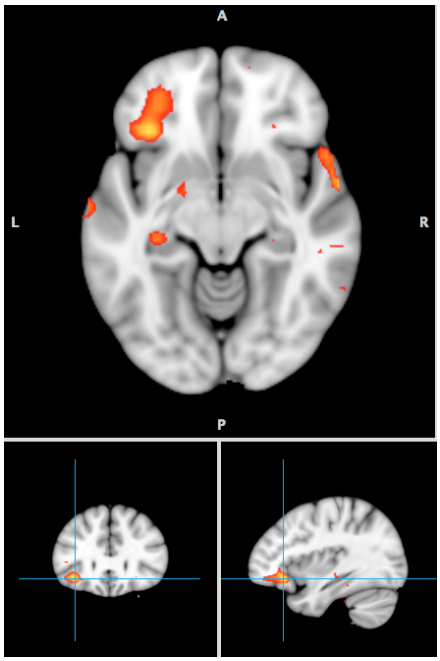


Figure 6.5 The reward times choice interaction during the outcome phase. Displayed at p=0,047, FEW-corrected, MNI coordinates: x-36, y29, z-10.

6.7 Moving the Study Forward

In order for the study to commence two issues need to be addressed. Firstly, two more pilot scans with concomitant inclusion of questionnaires are required to confirm the above fMRI results. Additionally, these are needed to aid timing of patient flow around the time of scan. Secondly, issues related to licensing agreements for study medication need to be resolved in order to source the placebo and active preparations in the format needed for the study. Once completed, study can commence as all other approvals are in place.

6.8 Addressing the aims of this chapter

The results presented here show that it is possible to develop and pilot a protocol for an fMRI study assessing the effects of menthol gel in patients with chronic CIPN. Key learning points from this process can be subdivided into those related to protocol development and those derived from the pilot fMRI scan. In relation to the pilot scan it is apparent that if ice is used instead of the thermode for the cold experimental task the experimenter requires a glove in the scanner to aid consistency of stimuli administration. Also related to the cold task, decisions regarding the duration and intensity of cold stimuli need to be reviewed, in particular as activation from the pilot scan seems to suggest that

latent haemodynamic response is being picked up in pain processing areas during the neutral rest condition, after the cold stimulus has been removed. Moreover individually thresholded noxious cold will likely need to be used in order to optimise test re-test accuracy (Upadhyay et al., 2015).

In relation to the choice and reward task, it is apparent from the feedback by the pilot scan participant that this task needs to be explained in more detail to patients prior to entering the fMRI scanner. This task proved slightly confusing for a very well educated healthy participant and is likely to be too complex for chronic pain patients. Finally, exclusion of the IAPS images from the punctate task maybe necessary in order to simplify the interpretation of the effects of menthol gel on punctate allodynia in chronic CIPN.

In terms of the protocol itself, the complexities of setting up a treatment trial using fMRI cannot be overstated. In particular, the coordination of the multiple approvals, with pharmacy production of trial medication and placebo, is imperative to study success. Planning is key to achieving success and a small pilot study is useful to help inform key decisions regarding study recruitment and flow.

7. Overall Discussion and Conclusion

This chapter outlines the unique contribution of this work and relates it to existing literature. The impact of work carried out and presented in this thesis can be divided into that relating to the field of CIPN and that effecting fMRI pain neuroimaging at the University of Edinburgh. These contributions will be discussed in turn below. Limitations of this work are also noted. The chapter will conclude with an overview of future research objectives and implications for clinical practice resulting from this thesis. An overall conclusion culminates this chapter and the thesis.

7.1 What is the novel contribution of this work?

7.1.1 Contribution to CIPN research

As shown in chapter 2, to date the mainstay of patient studies investigating CIPN development have focused on changes in the peripheral nervous system. From this rich body of literature, only two studies have investigated the impact of the brain on CIPN development and maintenance (Boland et al., 2014, Nudelman et al., 2015). Boland and colleagues reviewed brain function in response to noxious stimuli in chronic CIPN patients compared to healthy volunteers, while Nudelman and colleagues progressively looked at grey matter volume and general brain perfusion before and after chemotherapy. The study presented in this thesis investigated brain structure and function in cancer patients prior to chemotherapy onset. Consequently, both preparation for and execution of the CIPN fMRI study resulted in a number of unique contributions to the field of CIPN research. These include the following:

1. The systematic review and meta-analysis of CIPN literature, undertaken prior to recruitment to the CIPN study, enabled a coherent summary of CIPN prevalence across all cancer and chemotherapy types. A formal statistical calculation of CIPN prevalence had not previously been published. This work is useful for oncologists informing patients of CIPN

risks. It is also beneficial for clinicians planning service provisions and for researchers applying for funding to further understand CIPN.

2. Investigation of the structure of the brain in cancer patients prior to CIPN development has highlighted that pre-chemotherapy differences exist between patients who develop CIPN and those who do not. Specifically, the nucleus accumbens was found to be smaller in patients who went on to develop acute CIPN. This finding is postulated to serve as a future biomarker, useful in identifying individuals who have vulnerability for CIPN development.

3. Functional responses of the brain to punctate stimuli and positive emotional input, in cancer patients prior to chemotheray was shown to differ between patients who did not develop acute CIPN and those who did. This was the first study to prospectively show a difference in brain processing of noxious stimuli, prior to chemotherapy onset. These findings uphold the notion of 'at risk' individuals in terms of CIPN development.

4. Findings from resting state functional connectivity require greater exploration. The analysis undertaken here (low vs. high dimensionality) has enabled a clear plan for this to be undertaken in the future.

5. Utilisation of a number of cancer types requiring multiple neurotoxic chemotherapeutics, as opposed to restricting recruitment to a single malignancy make the results presented here generalizable to a wider number of patients. This is a unique approach in prospective CIPN studies investigating mechanisms of action, where the usual approach has been to focus on a single malignancy or single chemotherapeutic.

6. The set up of the MINT3 fMRI randomised controlled pilot trial, proposes a shift in the way that investigation of novel analgesic treatments for CIPN is undertaken. Specifically, use of fMRI is shown to be an acceptable

adjunct to traditional study design, in terms of ethical and research and development approvals. Moreover, successful completion of a pilot scan suggests that using a collaborative approach to data collection can propagate not only the research question at hand but also contribute to investigation of co-expressed disease entities, such as in this case depression.

7. Successful completion of the CIPN fMRI study shows that fMRI is an acceptable research tool for cancer patients. It also highlights that investigation of brain related mechanisms of CIPN is possible in a patient model.

7.1.2 Contribution to Pain fMRI at the University of Edinburgh

Prior to the CIPN fMRI study, pain related functional magnetic resonance imaging studies had not been carried out at the Clinical Research Imaging Centre (CRIC) at the University of Edinburgh. As this is currently the only scanner at the university with adequate resolution (3 Tesla) to conduct pain related studies of the brain, its use was necessary for this study and it therefore needed to be set up appropriately.

In collaboration with colleagues at CRIC and in the department of Psychiatry, auxiliary equipment was introduced into the scanner to enable collection of pain related fMRI data. Specifically, installation and assessment of the *Medoc* thermal stimuli experimental equipment as well as the *Biopac* physiological noise monitoring hardware and software was carried out, as described in chapter 3. The installation of this equipment required cross department organisation and subsequent scanner noise diagnostics.

These efforts enabled the CIPN study and two further unrelated pain fMRI studies to be conducted. One of the studies; a pilot investigating the use of gabapentin in women with chronic pelvic pain has since been progressed to a

large multicentre RCT, with an embedded fMRI component. Recruitment to this study is now underway.

7.2 Links between findings and existing knowledge

The findings of the CIPN study support animal and human evidence regarding aberrance in brain structure and function denoting vulnerability for development of neuropathic pain states (De Felice et al., 2011, Mansour et al., 2013). In particular the smaller NAc volume in patients who develop CIPN, corroborate with decreased NAc size and functional connectivity reported in patients who transition to chronic back pain by Mansour et al. Decreased thalamic activity in response to punctate stimuli in men who progress to CIPN and increased MPRF activity in women from this group also fits with previously described aberrant activity in these regions in neuropathic pain (Gustin et al., 2014). The identification of greater activity in the superior frontal gyrus in response to positive emotional input during punctate stimulation, in patients who did not get CIPN, is in line with reports from the only other two studies that have investigated the brain in CIPN (Boland et al., 2014, Nudelman et al., 2015).

7.3 Limitations of this work

Detailed limitations related to the analyses undertaken in this thesis are described in chapters 4 and 5. In terms of study conduct, a key limitation relates to the lack of punctate sharpness and IAPS valence ratings. These behavioural data would have enabled adjustment of functional fMRI findings and aided more extensive interpretation of observed changes. Further, due to limitations of time and resources a post-chemotherapy follow up scan was not acquired. This would have enabled direct intra-subject longitudinal comparison aiding interpretation of reported findings and enabling quantification of changes. Finally, a larger sample size to enable a minimum of 16 participants per group would be desirous. This would have increased the power of the study and strengthened conclusions.

7.4 Implications for future research and clinical practice

Notwithstanding the limitations presented above, there are a number of implications these results have for future research and clinical work. Related to the data presented here, future analyses will include connectivity analyses and exploration of the impact of manual de-noising to conserve brainstem signal for the functional data analyses. Moreover, once long term follow-up data and conversion to chronic CIPN is known, re-analyses of data is planned. In terms of future research, confirmation of findings in a new patient dataset is necessary. Additional validation of findings could also be achieved by translation to an animal model of CIPN. Specifically, manipulation of regions identified in these analyses including the NAc, MPRF, thalamus and superior frontal gyrus could aid assessment of any causal mechanisms. Further translation back to a patient model could enable relation of fMRI results to a bedside measure of CIPN risk.

This work has the capacity to introduce a paradigm shift into the field of clinical CIPN. To date CIPN severity has been deemed proportional to the type, duration and dose of chemotherapy and the damage treatment induces in peripheral nerves. However, results presented in this thesis suggest that a pre-chemotherapy brain centred vulnerability for CIPN exists. Viewed in this light, clinicians may need to consider certain patients as having a baseline risk for CIPN development alongside the specifics of chemotherapy dose and duration. Change in clinical practice will however only be possible after a sensitive and specific bedside measure of brain related CIPN vulnerability is identified. Moreover, development of chemotherapeutic regimens, which limit CIPN but retain tumorcidal activity, will be necessary to enable sustained change in clinical practice.

In relation to the set up of the MINT3 study, execution of this study is possible once study medication is sourced and two further fMRI pilot scans are completed to optimise the experimental paradigm. Implementation of fMRI in the assessment of topical menthol will aid the accuracy of efficacy findings and likely increase the speed at which any results are implemented in the clinic.

7.5 Overall Conclusions

The systematic review and meta- analysis presented in this thesis, confirms that CIPN is an important clinical problem affecting at least 75,000 cancer survivors annually in the UK alone. Pathophysiological understanding of CIPN development is limited. Therefore, assessment of this condition in a patient model, as presented here, is a useful contribution to the field. Taken together the findings described in this thesis denote that there are structural and functional differences in the brains of patients who develop CIPN. These aberrations are evident before peripheral nerve damage with chemotherapy ensues. This is a new proposition in terms of understanding CIPN development, where the mainstay of investigation has focused on the peripheral nervous system.

Although as with any novel scientific proposal, confirmation of these findings is required, a paradigm shift in the way CIPN development is viewed is warranted. Specifically, a move away from cause (chemotherapy) and effect (CIPN) paradigms, to a notion of brain centred pre-chemotherapy risk should be considered. This kind of change has the capacity to redirect research focus to the influence of the central nervous system in CIPN. It will likely also yield greater diversity in terms of exploring novel treatments, focused on brain related mechanisms of CIPN development.

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Appendix A

Search Strategy for 'Epidemiology of CIPN' Systematic Review

PREVALENCE & PREDICTORS OF CIPN:

DATABASE	TERMS
EMBASE	1) Chemotherapy Induced Peripheral Neuropathy 2) Chemotherapy Induced Neurotoxicity 3) Chemotherapy Induced Neurotoxicity Syndromes 4) CIPN 5) Oxaliplatin Induced Peripheral Neuropathy 6) Bortezomib Induced Peripheral Neuropathy 7) Paclitaxel Induced Peripheral Neuropathy 8) Taxane Induced Peripheral Neuropathy 9) Cisplatin Induced Peripheral Neuropathy 10) Vincristine Induced Peripheral Neuropathy 11) Thalidomide Induced Peripheral Neuropathy 12) Platinum Induced Peripheral Neuropathy 13) Carboplatin Induced Peripheral Neuropathy 14) Docetaxel Induced Peripheral Neuropathy 15) Proteasome Inhibitor Induced Peripheral Neuropathy 16) Neurotoxic Chemotherapy Induced Peripheral Neuropathy 17) Cancer Neuropathic Pain 18) Chemotherapy Induced Neuropathic Pain 19) Prevalence 20) Epidemiology 21) Occurrence 22) Burden 23) Predictors 24) Risk Factors 25) 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 26) 19 OR 20 OR 21 OR 22 27) 23 OR 24 28) 25 AND 26 29) 25 AND 27 30) 25 AND 26 AND 27
	1) Chemotherapy Induced Peripheral Neuropathy 2) Chemotherapy Induced Neurotoxicity 3) Chemotherapy Induced Neurotoxicity Syndromes 4) CIPN 5) Oxaliplatin Induced Peripheral Neuropathy 6) Bortezomib Induced Peripheral Neuropathy 7) Paclitaxel Induced Peripheral Neuropathy 8) Taxane Induced Peripheral Neuropathy

MEDLINE	9) Cisplatin Induced Peripheral Neuropathy 10) Vincristine Induced Peripheral Neuropathy 11) Thalidomide Induced Peripheral Neuropathy 12) Platinum Induced Peripheral Neuropathy 13) Carboplatin Induced Peripheral Neuropathy 14) Docetaxel Induced Peripheral Neuropathy 15) Proteasome Inhibitor Induced Peripheral Neuropathy 16) Neurotoxic Chemotherapy Induced Peripheral Neuropathy 17) Cancer Neuropathic Pain 18) Chemotherapy Induced Neuropathic Pain 19) Prevalence 20) Epidemiology 21) Occurrence 22) Burden 23) Predictors 24) Risk Factors 25) 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 26) 19 OR 20 OR 21 OR 22 27) 23 OR 24 28) 25 AND 26 29) 25 AND 27 30) 25 AND 26 AND 27
CAB ABSTRACTS	1) Chemotherapy Induced Peripheral Neuropathy 2) Chemotherapy Induced Neurotoxicity 3) Chemotherapy Induced Neurotoxicity Syndromes 4) CIPN 5) Oxaliplatin Induced Peripheral Neuropathy 6) Bortezomib Induced Peripheral Neuropathy 7) Paclitaxel Induced Peripheral Neuropathy 8) Taxane Induced Peripheral Neuropathy 9) Cisplatin Induced Peripheral Neuropathy 10) Vincristine Induced Peripheral Neuropathy 11) Thalidomide Induced Peripheral Neuropathy 12) Platinum Induced Peripheral Neuropathy 13) Carboplatin Induced Peripheral Neuropathy 14) Docetaxel Induced Peripheral Neuropathy 15) Proteasome Inhibitor Induced Peripheral Neuropathy 16) Neurotoxic Chemotherapy Induced Peripheral Neuropathy 17) Cancer Neuropathic Pain 18) Chemotherapy Induced Neuropathic Pain 19) Prevalence 20) Epidemiology 21) Occurrence

	22) Burden 23) Predictors 24) Risk Factors 25) 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 26) 19 OR 20 OR 21 OR 22 27) 23 OR 24 28) 25 AND 26 29) 25 AND 27 30) 25 AND 26 AND 27
GUIDELINES & ORGANISATIONS	National Institute of Clinical Excellence (NICE) http://www.nice.org.uk/ 1) Chemotherapy 2) Oncology 3) Pain Scottish Intercollegiate Guidelines Network (SIGN) http://www.sign.ac.uk/ Association of Anaesthetists of Great Britain and Ireland (AAGBI) http://www.aagbi.org/publications/guidelines.htm Department of Health World Health Organisation Cancer Research UK International Association for the Study of Pain (IASP) www.iasp-pain.org/
COCHRANE LIBRARY	SEARCH MANAGER: 1) "Chemotherapy Induced Peripheral Neuropathy" 2) "Chemotherapy Induced Peripheral Neurotoxicity" 3) "Oxalipatin Induced Peripheral Neuropathy" 4) "CIPN" 5) " Taxane Induced Peripheral Neuropathy" 6) "Bortezomib Induced Peripheral Neuropathy" 7) "Cancer Neuropathic Pain" 8) "Cisplatin Induced Peripheral Neuropathy" 9) "Docetaxel Induced Peripheral Neuropathy" 10) "Paclitaxel Induced Peripheral Neuropathy" 11) "Vincristine Induced Peripheral Neuropathy" 12) "Thalidomide Induced Peripheral Neuropathy" 13) "Carboplatin Induced Peripheral Neruopathy" 14) "Chemotherapy Induced Neuropathic Pain" 15) "Prevalence" 16) "Epidemiology" 17) "Occurrence" 18) "Burden" 19) "Predictors" 20) "Risk Factors" 21) 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10

	OR 11 OR 12 OR 13 OR 14 22) 15 OR 16 OR 17 OR 18 23) 19 OR 20 24) 21 OR 23 25) 21 AND 22 AND 23
PubMed CENTRAL	1) Chemotherapy Induced Peripheral Neuropathy 2) Chemotherapy Induced Neurotoxicity 3) Chemotherapy Induced Neurotoxicity Syndromes 4) CIPN 5) Oxaliplatin Induced Peripheral Neuropathy 6) Bortezomib Induced Peripheral Neuropathy 7) Paclitaxel Induced Peripheral Neuropathy 8) Taxane Induced Peripheral Neuropathy 9) Cisplatin Induced Peripheral Neuropathy 10)Vincristine Induced Peripheral Neuropathy 11)Thalidomide Induced Peripheral Neuropathy 12) Platinum Induced Peripheral Neuropathy 13)Carboplatin Induced Peripheral Neuropathy 14)Docetaxel Induced Peripheral Neuropathy 15) Proteasome Inhibitor Induced Peripheral Neuropathy 16) Neurotoxic Chemotherapy Induced Peripheral Neuropathy 17) Cancer Neuropathic Pain 18) Chemotherapy Induced Neuropathic Pain 19)Prevalence 20)Epidemiology 21)Occurrence 22)Burden 23)Predictors 24)Risk Factors 25) 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 26) 19 OR 20 OR 21 OR 22 27) 23 OR 24 28) 25 AND 26 29) 25 AND 27 30) 25 AND 26 AND 27
WEB OF KNOWELDGE	1) Chemotherapy Induced Peripheral Neuropathy 2) Chemotherapy Induced Neurotoxicity 3) Chemotherapy Induced Neurotoxicity Syndromes 4) CIPN 5) Oxalaplatin Induced Peripheral Neuropathy 6) Bortezomib Induced Peripheral Neuropathy 7) Paclitaxel Induced Peripheral Neuropathy 8) Taxane Induced Peripheral Neuropathy 9) Cisplatin Induced Peripheral Neuropathy 10)Vincristine Induced Peripheral Neuropathy

	11)Thalidomide Induced Peripheral Neuropathy 12) Platinum Induced Peripheral Neuropathy 13)Carboplatin Induced Peripheral Neuropathy 14)Docetaxel Induced Peripheral Neuropathy 15)Neurotoxic Chemotherapy Induced Peripheral Neuropathy 16) Cancer Neuropathic Pain 17) Chemotherapy Induced Neuropathic Pain 18)Prevalence 19)Epidemiology 20)Occurrence 21)Burden 22)Predictors 23)Risk Factors 24) 1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 (BY TITLE) 25) 18 OR 29 OR 20 OR 21 (BY TOPIC) 26) 22 OR 23 (BY TOPIC) 27) 24 AND 25 28) 24 AND 26 29) 24 AND 25 AND 26
CINAHL	1) Chemotherapy induced peripheral neuropathy 2) Prevalence 3) Risk Factors 4) Predictors 5) 1 AND 2 6) 1 AND 3 7) 1 AND 4
OTHERS	1) Hand searching through journal references 2) Review of CIPN related lectures 3) Review of Pain and Oncology textbook chapters 4) Review of chemotherapy drug trial data

Appendix B

Copy of published systematic review.

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Comprehensive review

Incidence, prevalence, and predictors of chemotherapy-induced peripheral neuropathy: A systematic review and meta-analysis

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ABSTRACT

Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling pain condition resulting from chemotherapy for cancer. Severe acute CIPN may require chemotherapy dose reduction or cessation. There is no effective CIPN prevention strategy; treatment of established chronic CIPN is limited, and the prevalence of CIPN is not known. Here we used a systematic review to identify studies reporting the prevalence of CIPN. We searched Embase, Medline, CAB Abstracts, CINAHL, PubMed central, Cochrane Library, and Web of Knowledge for relevant references and used random-effects meta-regression to estimate overall prevalence. We assessed study quality using the CONSORT and STROBE guidelines, and we report findings according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance. We provide a qualitative summary of factors reported to alter the risk of CIPN. We included 31 studies with data from 4179 patients in our analysis. CIPN prevalence was 68.1% (57.7–78.4) when measured in the first month after chemotherapy, 60.0% (36.4–81.6) at 3 months and 30.0% (6.4–53.5) at 6 months or more. Different chemotherapy drugs were associated with differences in CIPN prevalence, and there was some evidence of publication bias. Genetic risk factors were reported in 4 studies. Clinical risk factors, identified in 4 of 31 studies, included neuropathy at baseline, smoking, abnormal creatinine clearance, and specific sensory changes during chemotherapy. Although CIPN prevalence decreases with time, at 6 months 30% of patients continue to suffer from CIPN. Routine CIPN surveillance during post-chemotherapy follow-up is needed. A number of genetic and clinical risk factors were identified that require further study.

1. Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a disabling side effect of several commonly used antineoplastic agents. The development of CIPN may require chemotherapy dose reduction or cessation, which can increase cancer-related morbidity and mortality [17,31]. CIPN is a predominantly sensory neuropathy that may be accompanied by motor and autonomic changes [62]. Similar to other neuropathic pain conditions, pain in CIPN can be stimulus dependent or independent [66]. The pathophysiology of CIPN is poorly understood, and treatments to prevent CIPN are inadequate. Meta-analyses of clinical trials for CIPN prevention report inconclusive results [1,49]. Treatment options for established CIPN are also limited. Clinical trials of antiepileptic or antidepressant agents to treat other neuropathic pain conditions have generally been negative [30,41,54,55]. Only 1 recent, double-blind, randomized controlled trial showed improvement in CIPN symptoms after 5 weeks of treatment with duloxetine [57].

Understanding of the epidemiology of CIPN is also limited [37]. Previous studies have largely focussed on individual chemotherapeutic agents, with reported CIPN incidence rates ranging from 19% to more than 85% [23]. Annually 165,544 patients survive cancer in the United Kingdom, and more than 1 million in the United States [12,44]. It is therefore important to provide a more precise measure of the prevalence of CIPN to allow appropriate resource

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allocation and research planning, and to inform patient decisions about treatment. Understanding risk factors (including genetic risk factors) for CIPN may guide future research and treatment.

Previous reviews of CIPN have combined narrative review with expert opinion, with potential risk of bias [15,28,29]. Here we present what we believe to be the first systematic review and meta-analysis of the incidence and prevalence of CIPN. We also aimed to assess the influence of potential publication bias on our estimation of CIPN measures, and to seek empirical evidence of the impact of study design factors.

2. Methods

2.1. Search strategy

We searched Embase, Medline, CAB Abstracts, CINAHL, PubMed central, Cochrane Library and Web of Knowledge in July 2013 for English-language references. Searches were not limited by date restrictions. Search terms were free text and included; [“Chemotherapy Induced Peripheral Neuropathy” OR “Chemotherapy Induced Neurotoxicity” OR “Chemotherapy Induced Neurotoxicity Syndromes” OR “CIPN” OR “Oxaliplatin Induced Peripheral Neuropathy” OR “Bortezomib Induced Peripheral Neuropathy” OR “Paclitaxel Induced Peripheral Neuropathy” OR “Taxane Induced Peripheral Neuropathy” OR “Cisplatin Induced Peripheral Neuropathy” OR “Vincristine Induced Peripheral Neuropathy” OR “Thalidomide Induced Peripheral Neuropathy” OR “Platinum Induced Peripheral Neuropathy” OR “Carboplatin Induced Peripheral Neuropathy” OR “Docetaxel Induced Peripheral Neuropathy” OR “Proteasome Inhibitor Induced Peripheral Neuropathy” OR Neurotoxic Chemotherapy Induced Peripheral Neuropathy” OR “Cancer Neuropathic Pain” OR “Chemotherapy Induced Neuropathic Pain”] [Search 1] AND [“Prevalence” OR “Epidemiology” OR “Occurrence” OR “Burden”] [Search 2] AND [“Predictors” OR “Risk Factors”] [Search 3]. The search strategy was adapted for each database (see supplementary text A). We also hand searched reference lists of relevant studies and systematic reviews of CIPN prevention trials, and searched the databases of National Institute for Health and Care Excellence (NICE) and the Scottish Intercollegiate Guidelines Network (SIGN). Our review followed an a priori protocol according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [43]. The review protocol was registered on the PROSPERO website before data extraction (registration no. CRD42013005524) [11].

2.2. Inclusion and exclusion criteria and study selection

We included prospective observational studies of adult cancer patients receiving chemotherapy of any type. Our definition of observational studies included cohort studies in which patients were prospectively identified and followed up using relevant predefined outcomes of interests. We also included control group data from randomized controlled trials (RCTs) of CIPN prevention in which details of the patients who developed CIPN were reported.

Studies were excluded if they described animal models of CIPN, were investigating CIPN treatment or prevention, included pediatric populations, or investigated other causes of neuropathy in cancer patients (eg, pre-existing neuropathy such as diabetic neuropathy or other cancer related causes of neuropathy such as post-mastectomy).

Two investigators (M.S. and S.R.) independently read and selected from all the retrieved references and abstracts. Discrepancies between the reviewers’ selections were resolved by discussion. Full texts of potentially eligible studies were retrieved (Fig. 1).

2.3. Data extraction and quality assessment

We extracted data to a bespoke form, recording the prevalence or incidence of CIPN, and any reported risk factors or predictors of CIPN. We included all relevant outcomes determined after the end of chemotherapy, noting the time (in relation to the end of chemotherapy) at which these were assessed. Where information was incomplete we contacted authors by email. Two investigators (M.S. and S.R.) extracted data, which were then entered into the study database. Discrepancies were resolved by discussion and agreement with a third reviewer (M.F.).

We assessed study quality according to the PRISMA guidelines [43]. We evaluated risk of bias in individual studies using the following criteria: investigator blinding of any type, presence of a control group, use of externally validated instruments for CIPN assessment, clear description of statistical methods used to identify CIPN predictors, and description of longitudinal follow up. Adherence of each study to relevant reporting criteria (STROBE or CONSORT) was assessed [2,61]. We assessed the risk of bias for our summary estimate by seeking evidence of publication bias, selective outcome reporting bias (if a published protocol of the included study was available), reporting of a sample size calculation, and whether the study reported participants lost to follow-up.

2.4. Data synthesis and analysis

Our primary outcome was the prevalence of CIPN. We used random effects meta-regression to quantify heterogeneity and its potential sources. We hypothesized that chemotherapy type and the time of CIPN assessment would explain a large proportion of the observed heterogeneity. Therefore, we included chemotherapy type, last time point of CIPN assessment, and measures of study quality as independent variables in our regression model. We also planned for assessment of risk factors for CIPN across studies. We assessed publication bias using funnel plots, Egger’s test, and trim and fill [22]. We appraised studies using STROBE criteria for observational studies and CONSORT criteria for trials. Where a criterion was partially met, we considered, for the purposes of this analysis, that it was completely met, for ease of calculation. In open label studies (Table 1), we modified the CONSORT criteria by not considering the point for blinding, to account for the design of these studies. STATA 13.1 was used for statistical analyses.

3. Results

3.1. Studies included

We identified 4128 potentially relevant studies, and examined the full text of 138. A total of 31 studies (involving 4179 patients) [4–9,13,14,18,21,24–27,32–36,38,39,45–48,52,53,60,63–65] met our inclusion criteria. A total of 30 studies reported the incidence of CIPN (new CIPN cases divided by the population at risk). One study reported CIPN prevalence (all CIPN cases divided by population at risk) [26]. Because CIPN might have occurred, and resolved, between study assessments, we calculated the prevalence of CIPN at the time of each assessment [59].

3.2. Study characteristics

Of the 31 studies included, 15 were prospective cohort studies, 10 were RCTs, 5 were nonrandomized controlled trials, and 1 was a cross-sectional cohort study. All nonrandomized controlled trials were open labeled and not blinded. Eight of 10 RCTs (80%) reported investigator blinding of some type. Blinded assessment of outcome was reported in 3 of 14 prospective cohort studies. One prospective

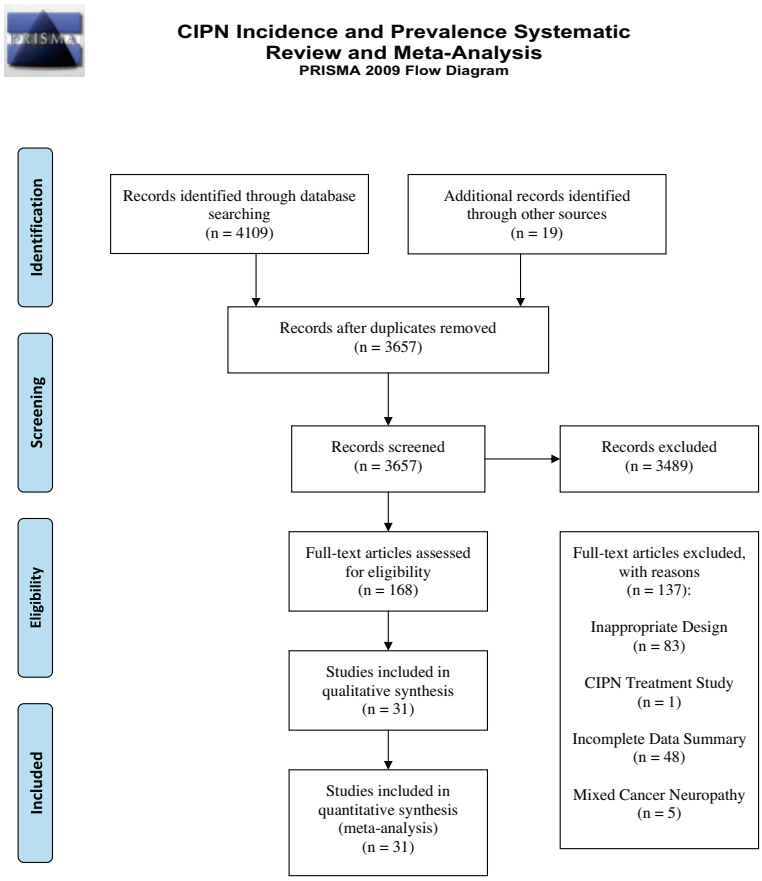

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 flow diagram.

cohort study also sought to validate genetic risk factor results in a control group. Nine of 10 RCTs (90%) described a sample size calculation. Of all included studies, 22 (71%) reported study participant dropout, giving reasons. In all, 14 of 31 study authors (45%) disclosed funders and/or whether they had a conflict of interest. Adherence of studies to reporting guidelines is summarized in Table 1. Of 31 studies, 26 (83.9%) used an assessment tool validated for CIPN. All studies reporting CIPN risk factors described methods used to identify these predictors.

3.3. CIPN incidence and prevalence

Of 4179 patients, 1960 developed CIPN (aggregate prevalence 48%). CIPN prevalence was 68.1% (95% CI = 57.7–78.4) within the first month of the end of chemotherapy, 60.0% (36.4–81.6) at 3 months, and 30.0% (6.4–53.5) at 6 months or later (Table 2). There was considerable heterogeneity in the estimates from different studies ($I^2 = 98.2$, $P < .001$). The time of assessment accounted for 36% of the observed heterogeneity (adjusted $R^2 = 0.365$, $P < .001$). An overview of the individual incidence reported in included studies is shown in Table 1. We did not include the cumulative dose (CD) of chemotherapy (actual dose received) in our meta-regression because standard and maximally tolerated doses would differ substantially from drug to drug (study-specific CD shown in Table 1). As expected, there was co-linearity between the cancer type and the chemotherapy used; because we reasoned that it is more likely that CIPN prevalence would be related to drug than to cancer type, we considered only chemotherapy type in our regression model (Table 3). The type of chemotherapy used accounted for 32% of the observed heterogeneity in our sample (adjusted $R^2 = 0.315$, $P < .04$).

Methods used to assess the presence or grade of CIPN were too diverse to include in the meta-regression. Of the 31 included studies, 8 defined CIPN according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), 1 study used the European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life Questionnaire 30 (QLQ – 30) combined with neurological examination, 1 used in-depth neurophysiological examination (NPS), 1 used a standard neurological examination, and 1 used the Total Neuropathy Score (TNSc). The remaining 18 studies used a combination of 2 or more of the above, and 1 study used skin biopsy (Table 3). To investigate any impact of neurophysiological

Table 1
Overview of included studies.

First author (year)	Study type and quality (CONSORT/STROBE score)	Incidence (95% CI)	Main cancer class (chemotherapy)	Dose (mg/m^2) (mean or cumulative)
Antonacopoulou (2009)*	Prospective cohort	58.8% (42.2–75.3)	Colorectal (oxaliplatin)	–
Argyriou (2006)	Prospective cohort (18/22)	61.5% (35.1–87.9)	Breast (paclitaxel)	1980
		42.8% (16.9–68.7)	Lung (cisplatin)	720
Argyriou (2007) [8]	Prospective cohort (19/22)	64% (45.2–82.8)	Colorectal (oxaliplatin)	1740
Argyriou (2007)	Prospective cohort (19/22)	69.2% (44.1–94.3)	Multiple solid (cisplatin and paclitaxel)	126·7
Argyriou (2012)	Prospective cohort (19/22)	83.3% (77.3–89.3)	Colorectal (oxaliplatin)	1646
Argyriou (2013)	Prospective cohort (20/22)	84.5% (79.4–89.5)	Colorectal (oxaliplatin)	1651
Attal (2009)	Prospective cohort (19/22)	66.6% (44.8–88.4)	Colorectal (oxaliplatin)	1278
Baldwin (2012)	Prospective cohort (20/22)	67.2% (64.1–70.3)	Breast (paclitaxel)	–
Cascinu (1995)	RCT (18/25)	64% (45.2–82.8)	Gastrointestinal (cisplatin)	–
Cascinu (2002)	RCT (16/25)	78.9% (60.6–97.3)	Colorectal (oxaliplatin)	783
Chaudhary (2008)†	Prospective cohort (13/22)	96.2% (89.2–103)	Multiple myeloma (bortezomib and thalidomide)	36
Dimopoulos (2011)	RCT (21/25)	46.7% (41.4–52.1)	Multiple myeloma (bortezomib)	38·4
Gandara (1995)†	RCT (18/25)	12.1% (5.6–18·5)	Ovarian and lung (cisplatin)	379
Ghoreishi (2012)	RCT (19/25)	59.2% (40.7–77.8)	Breast (paclitaxel)	–
Glendenning (2010)*	Cross sectional cohort (21/22)	20.1% (15.5–24.7)	Testicular (cisplatin and vincristine)	400
Gobran (2013)	RCT (13/25)	70% (53.6–86.4)	Colorectal (oxaliplatin)	763
Ishibashi (2010)	RCT (20/25)	93.7% (81.9–105)	Colorectal (oxaliplatin)	72·8
Johnson (2011)	RCT (23/25)	32.1% (29.1–34.9)	Multiple myeloma (thalidomide)	–
		19.6% (16.3–22.9)	(Vincristine)	–
Kawakami (2012)†	Prospective cohort (14/22)	76% (64.1–87.8)	Lung (cisplatin and paclitaxel)	–
Kemp (1996)	RCT (19/25)	67·5% (59.2–75.8)	Gynecological (cisplatin)	–
Krishnan (2005)	Prospective cohort (16/22)	50% (25.5–74.5)	Colorectal (oxaliplatin)	1200
Lin (2006)	Randomised trial (15/24)	90% (71.4–108)	Colorectal (oxaliplatin)	1200
Milla (2009)	Randomised trial (11/24)	92.8% (79.3–106)	Colorectal (oxaliplatin)	772
Pace (2003)	Randomised trial (11/24)	85.7% (67.4–104)	Multiple solid (cisplatin)	420
Pace (2007)	Prospective cohort (14/22)	92.8% (79.4–106)	Breast (paclitaxel)	1744
Pace (2010)	RCT (19/25)	41.6% (21.9–61.4)	Multiple solid (cisplatin)	450
Planting (1999)	Randomised trial (13/24)	13.5% (2.5–24.5)	Multiple solid (cisplatin)	401
Plasmati (2002)	Prospective cohort (15/22)	96% (88.3–103)	Multiple myeloma (thalidomide)	18
Van der Hoop (1999)	RCT (12/25)	41.6% (13.7–69.5)	Gynecological (cisplatin)	416
Von Schlippe (2001)	Prospective cohort (9/22)	17.2% (3.4–30.9)	Testicular (cisplatin)	–
Won (2012)	Prospective cohort (16/22)	40.6% (30.8–50.4)	Colorectal (oxaliplatin)	935

Abbreviation: RCT, randomized controlled trial (note that randomised trials, as opposed to RCTs, did not have blinding or placebo).
– Cumulative or average dose not reported. Reported cumulative dose refers to actual dose received.
* Abstract only available; STROBE assessment not possible. Where upper 95% confidence intervals exceeded 100, only 100% were recorded, as this is clinically interpretable.
† Study pooled incidence across chemotherapy types included.
‡ Study pooled incidence across cancer types.

assessment on the reported prevalence of CIPN, we conducted a post hoc sensitivity analysis. In all, 17 studies (449 patients) used NPS to assess for CIPN; 16 of these used NPS in combination with another assessment method. In these 17 studies, CIPN prevalence was higher; 73.3% (58.6–87.3) within 1 month of chemotherapy cessation, 70.1% (41.8–98.4) at 3 months, and 39.9% (3.9–76.0) at 6 months or more.

For publication bias, although Egger's test did not suggest asymmetry in the funnel plot at a confidence level of $P = .05$ (95% CI of intercept −0.64 to 7.8); trim and fill analysis did impute 14 theoretical missing studies. These 2 approaches to assess for publication bias are known to have different sensitivities [58].

3.4. CIPN risk factors

Eight of the included studies assessed risk factors for CIPN (Table 4) [8,9,21,26,33,34,48,65]. Four genome-wide association studies (GWAS), totaling 2671 patients, sought single nucleotide polymorphisms (SNPs) associated with CIPN [9,33,48,65]. All GWAS used validation datasets and conducted genotyping blinded to clinical status. These reported polymorphisms associated with a range of proteins, including voltage-gated sodium channels, Schwann cell function–related proteins, receptors for cell surface collagen, receptors involved in neuronal apoptosis, neuronal crest cell development, and an enzyme involved in pyruvate metabolism.

Four studies (701 patients) used statistical modeling to report clinical risk factors for CIPN [8,21,26,34]. Two of these studies included 50 patients or fewer. No study used a separate data set to validate candidate risk factors. Reported clinical risk factors for CIPN included baseline neuropathy, a history of smoking, decreased creatinine clearance, and specific sensory changes during chemotherapy treatment, including cold allodynia (pain in response to a nonpainful cold stimulus) and cold hyperalgesia (exaggerated pain in response to a painful cold stimulus, 20 °C).

4. Discussion

4.1. CIPN prevalence

This systematic review and meta-regression suggests a high overall prevalence of CIPN, maximum within the first month after treatment, and falling over time. Approximately one-third of patients can expect to have chronic CIPN 6 months or more after the end of chemotherapy; this has a significant negative impact on long-term quality of life for which effective treatment is needed.

The lack of uniformity in CIPN assessment methods make between-study comparisons difficult. Authors used 5 assessment methods (NCI-CTC, TNSc, EORTC QLQ-C30, neuro-physiological examination, which included nerve conduction studies and/or quantitative sensory testing, and neurological examination) alone or in combination. Of these, only the EORTC QLQ-C30 and quantitative sensory testing component of neurophysiological examination explicitly assess pain as a symptom of CIPN. It is known that although CIPN most frequently presents with pain, motor and other sensory symptoms may also be present [40]. Use of combinations of CIPN and pain assessment tools has been suggested as a

Table 2
Comparison of prevalence related to time of CIPN assessment.

Time of assessment (after cessation of chemotherapy)	Prevalence (95% CI)	Studies included	Total no. of patients in group
⩽1 mo	68.1% (57.7–78.4)	Antonacopolou 2009 Argyriou 2007 Argyriou 2012 Argyriou 2013 Baldwin 2012 Cascinu 1995 Cascinu 2002 Chaudhry 2008 Dimopoulos 2011* Gandara 1995 Ghoreishi 2012 Gobran 2013* Ishibashi 2010 Kawakami 2012 Krishnan 2005* Lin 2006 Milla 2009* Pace 2003 Pace 2007* Pace 2010 Van Der Hoop 1999 Won 2012	2085
3 mo	60.0% (36.4–81.6)†	Argyriou 2006 Argyriou 2007 Kemp 1996 Planting 1999 Plasmati 2007	234
⩾6 mo	30.0% (6.4–53.5)†	Johnson 2011‡ Attal 2009 Glendenning 2010 Von Schlippe 2001	1860

Abbreviations: CI, confidence interval; CIPN, chemotherapy-induced peripheral neuropathy.
* Studies included longer-term CIPN follow up but did not provide enough details at these later time points to allow use of data in the meta-regression.
† Wide confidence interval likely due to small number of studies assessing CIPN beyond this time point.
‡ Study considered CIPN only after induction therapy and not during maintenance.

strategy to improve detection and quantification of pain in CIPN [67]. There have been recent attempts to standardize CIPN assessment and reporting, and we encourage investigators to consider these when developing study protocols [15,16].

Three of the 5 largest studies in our sample did not include the mildest grades of CIPN [9,24,45]. The prevalence of CIPN is therefore likely to be higher than reported here. Early detection of mild CIPN might become important if effective prevention or management strategies become available. A lower incidence in these larger studies is an alternative explanation for the funnel plot asymmetry detected by trim and fill analysis [58].

Current clinical guidelines support use of NPS methods in the diagnosis of suspected CIPN [19,56]. Studies using this approach reported a higher prevalence of CIPN, but whether this is a clinically significant problem is not clear.

We found significant heterogeneity between studies. In meta-analyses aimed at providing a best estimate of, for instance, drug efficacy, significant heterogeneity usually limits the usefulness of pooled data. In contrast, because the etiology and epidemiology of CIPN are so poorly understood, we believe that investigating the sources of heterogeneity is important. Specifically, it might provide insight into the impact of length of assessment and chemotherapy type on the incidence and prevalence of CIPN. Furthermore, as expected, a substantial proportion of the heterogeneity that we observed was accounted for by chemotherapy type, which was related to the cancer type. Although the primary interest of many clinicians will be the prevalence of CIPN for specific chemotherapeutics, CIPN treatment decisions are routinely based on data from treatment trials that have recruited patients irrespective of the chemotherapy that they were prescribed [57].

4.2. Risk factors for CIPN

Four studies used multivariate statistical modeling to identify clinical risk factors for CIPN [8,21,26,34]. Despite using valid statistical approaches, these studies did not verify identified risk factors in new population datasets. Consequently, their results are probably affected by the statistical biases underpinning these types of predictive calculations [3,42]. To our knowledge, these are the only studies that describe baseline neuropathy, smoking, and decreased creatinine clearance as risk factors for CIPN. In contrast, description of sensory changes during chemotherapy treatment, including increased pain and nerve hyperexcitability, have previously been documented as predictors of CIPN [20,42]. The postulated mechanisms underpinning these sensory phenomena include axonal hyperexcitability and nociceptor sensitization. These processes may be important in CIPN development, and, to some degree, they fit with the mechanisms described in other neuropathic conditions related to systemic diseases, including human immunodeficiency virus (HIV) and multiple sclerosis [42,64]. There is ongoing debate about the relative importance of etiology in determining the underlying mechanisms of neuropathic pain [19,56,62].

Four studies reported genetic risk factors for CIPN. The functions of the identified genes fit with the postulated pathophysiological mechanisms underpinning CIPN [50]. The recent comprehensive review by Cavaletti et al. discusses these mechanisms in detail. All 4 included studies were, to some degree, affected by the universal limitations influencing pharmacogenetic studies: inadequate sample size, CIPN assessment tools, and use and size of a replication cohort. Despite these possible limitations, the potential clinical usefulness of pharmacogenetic studies in CIPN has recently been

Table 3
Studies stratified by drug type.

	Study type (CONSORT/STROBE)	Main cancer class	CIPN severity report (count by grade if given)	CIPN assessment time points	CIPN assessment method(s)
Oxaliplatin: 72.3% (95% CI = 59.7–86.8)					
Antonacopoulou (2009)*	Prospective cohort	Colorectal	NR	Unclear	TNSc
Argyriou (2007) [8]	Prospective cohort	Colorectal	Grade I (6/16) Grade II (8/16) Grade III (2/16)	Baseline Cycles 4, 8, 12	TNSc NPS NCI-CTC
Argyriou (2012)	Prospective cohort	Colorectal	Grade I (38/125) Grade II (46/125) Grade III (41/125)	Baseline Cycles 3, 6 (FOLFOX) Cycles 4, 8 (XELOX)	TNSc NPS NCI-CTC
Argyriou (2013)†	Prospective cohort	Colorectal	Grade I (62/169) Grade II (46/169) Grade III (61/169)	Baseline Cycle 6, 12 (FOLFOX) Cycles 4, 8 (XELOX)	TNSc NCI-CTC
Attal (2009)	Prospective cohort	Colorectal	Sensory symptom counts described as means/ individual	Baseline Cycle 3, 6, 9 12 ± 2 mo after chemo end	NCI-CTC NPS (EORTC) QLQ-C30
Cascinu (2002)	RCT	Colorectal	Grade I (4/15) Grade II (6/15) Grade III (4/15) Grade IV (1/15)	Baseline Cycles 4, 8, 12 Within 2 wk of chemo end	NCI-CTC NPS
Gobran (2013)	RCT	Colorectal	Grade I (7/21) Grade II (0/21) Grade III (14/21) Grade IV (0/21)	Unclear if at baseline At each chemo cycle until end of chemo (variable no. of cycles) Longer follow-up for those with CIPN (but denominator unclear)	NCI-CTC
Ishibashi (2010)	RCT	Colorectal	Grade I (15/15) Grade II (1/15) Grade III (0/15) Grade IV (0/15)	Baseline At each chemo cycle until end of chemo	NCI-CTC
Krishnan (2005)	Prospective cohort	Colorectal	NR	No baseline Within 1 mo of chemo end only reported assessment	NCI-CTC NPS TNSc
Lin (2006)	Controlled trial	Colorectal	Grade I (1/9) Grade II (5/9) Grade III (3/9) Grade IV (0/9)	Baseline Cycles 4, 8, 12 Within 2 wk of end of chemo	NCI-CTC NPS
Milla (2009)	Controlled trial	Colorectal	Grade I (0/13) Grade II (9/13) Grade III (4/13)	Baseline Cycles 5, 9, 12 (Some followed up longer but denominator unclear)	NCI-CTC NES
Won (2012)	Prospective cohort	Colorectal	NR	Unclear if at baseline At each chemo cycle until end of chemo (variable no. of cycles)	NCI-CTC NES
Cisplatin: 42.2% (95% CI = 21.3–63.1)					
Argyriou (2006)*	Prospective cohort	Lung	Reported by age group only	Baseline Cycles 3, 6 3 mo after chemo end	PNS NPS
Cascinu (1995)	RCT	Gastrointestinal	Grade I (3/16) Grade II (10/16) Grade III (2/16) Grade IV (1/16)	Baseline After 9 and 15 wk of therapy Within 1 wk after end of chemo	NCI-CTC NPS
Gandara (1995)	RCT	Ovarian and lung	Only grade ⩾3 reported	Unclear if at baseline At each cycle until chemo end (variable no. of cycles) Study stopped early after interim analysis due to high toxicity in intervention group	NCI-CTC
Kemp (1996)	RCT	Gynecological	Grade I (31/81) Grade II (35/81) Grade III (15/81)	Baseline Cycles 4, 5, 6 Monthly after chemo for 3 mo	NCI-CTC NES
Pace (2003)	Controlled trial	Multiple solid	Grade I (6/12) Grade II (4/12) Grade III & IV (2/12)	Baseline After 6 cycles	TNSc NES
Pace (2010)	RCT	Multiple solid	Only grade ⩾3 reported	Baseline Every cycle for 3 cycles 1 mo after chemo end	TNSc NPS
Planting (1999)	Controlled trial	Multiple solid	Grade I (5/5)	Baseline Cycle 3, 6 3 mo after chemo end (Longer follow-up but no denominator info)	NCI-CTC NES
Van der Hoop (1999)	Controlled trial	Gynecological	Mean vibration threshold	Baseline Cycles 2, 4, 6 End of chemo	NES

Table 3 (*continued*)

	Study type (CONSORT/STROBE)	Main cancer class	CIPN severity report (count by grade if given)	CIPN assessment time points	CIPN assessment method(s)
Von Schlippe (2001)	Prospective cohort	Testicular	Grade I (4/5) Grade II (1/5)	Unclear if at baseline Every 6 wk for first 6 mo after chemotherapy Thereafter every 2 mo for median of 4 y (range 2–8 y)	NPS
Cisplatin or carboplatin and paclitaxel: 73% (95% CI = 36.2–109.7)					
Argyriou (2007)	Prospective cohort	Multiple solid	Mild (2/9) Moderate (6/9) Severe (1/9)	Baseline Cycle 3, 6 3 mo after chemo end	PNS NPS
Kawakami (2012)§	Prospective cohort	Lung	% Severity with cumulative dose	Baseline Daily during cycle 1 Cycle 2, 3, 4 Chemo end	NCI-CTC
Cisplatin and vincristine: 20.1% (95% CI = −26.2 to 66.5)					
Glendenning (2010)§	Cross-sectional cohort	Testicular	Only grade ⩾3 reported	Recruited patients at least 5 y post-treatment Assessed once for this prevalence study	(EORTC) QLQ-C30 NES
Paclitaxel: 70.8% (95% CI = 43.5–98.1)					
Argyriou (2006)†	Prospective cohort	Breast	Reported by age group only	Baseline Cycles 3, 6 3 mo after chemo end	PNS NPS
Baldwin (2012)	Prospective cohort	Breast	Only grade ⩾2 reported	Unclear if at baseline Cycles 4, 6 Within 1 mo of chemo end	NCI-CTC
Ghoreishi (2012)	RCT	Breast	Mild (10/16) Moderate (5/16) Severe (1/16)	Baseline 1 mo after chemo end	TNSc NPS
Pace (2007)	Prospective cohort	Breast	Mean neurotoxicity scores reported	Baseline After 12 wk of chemo After 24 wk of chemo	TNSc NPS
Vincristine: 19.6% (95% CI −26.6 to 65.9)					
Johnson (2011)†	RCT	Multiple myeloma	Grade ⩾ I 31.8% Grade ⩾ II 11% Grade ⩾ III 3.6%	Unclear if at baseline At each cycle For 6 months after chemo end for induction (ie, 36 wk from start of induction therapy)	NCI-CTC
Thalidomide: 63.5% (95% CI = 29.3–97.8)					
Johnson (2011)†	RCT	Multiple myeloma	Grade details not reported	Unclear if at baseline At each cycle For 6 mo after end of chemo for induction (ie, 36 weeks from start of induction therapy)	NCI-CTC
Plasmati (2002)	Prospective cohort	Multiple myeloma	Grade I (12/24) Grade II (6/24) Subclincial (6/24)	Baseline After 4 mo of chemo 3 mo after stem cell transplantation	NCI-CTC NPS
Bortezomib: 46.7% (95% CI = 0.3–93.1)					
Dimopoulos (2011)	RCT	Multiple myeloma	Grade I NR Grade II (64/159) Grade III (45/159) Grade IV (1/159)	Unclear if at baseline Every 3 wk until 1 mo after last chemo dose Longer follow-up but no denominator data	NCI-CTC
Bortezomib and thalidomide: 96.2% (95% CI = 49.7–143)					
Chaudhary (2008)	Prospective cohort	Multiple myeloma	Grade ⩾2 reported	Baseline Cycles 2, 4, 6, 8 End of chemo Note skin biopsy at baseline and end of chemo only	TNSc NPS Skin biopsy

Abbreviations: Chemo, chemotherapy; CIPN, chemotherapy-induced peripheral neuropathy; EORTC, European Organization for Research and Treatment of Cancer; CI, confidence interval; NCT-CTC, National Cancer Institute Common Toxicity Criteria; NES, neurological examination; NPS, neurophysiological examination (quantitative sensory testing and/or nerve conduction studies); NR, not reported; PNS, Modified peripheral neuropathy score; RCT, randomized controlled trial; TNSc, total neuropathy score.

* Abstract only available.

† Authors report both acute and chronic CIPN grade counts, only acute given here.

‡ Raw data obtained from author or reported in paper, allowing counts reported in single study to be split by chemotherapy type.

§ Studies pooled CIPN counts across chemotherapy types included.

described [10]. As suggested by Postma et al. adherence of future studies to standardized study design and methods will likely aid the advance of personalized oncology, possibly having an impact on CIPN prevalence in the future.

4.3. Limitations of this review

It is possible that we have omitted relevant studies despite our detailed search strategy, and we specifically excluded non–English language studies. Multivariate meta-regression would have allowed us to investigate interactions between various factors, but there are too few studies for this approach to be reliable. Because we expected there to be a broad range of CIPN assessment methods used, we did not plan to explore their impact. Our analysis of the impact of NPS as a component of the assessment of CIPN is post hoc and therefore should be interpreted with caution. We did not specifically seek out assessments for pain in CIPN in included studies and therefore

Table 4
CIPN risk factors.

Study	Category of risk factor reported	Data source of study	Sample size of study (N)	Risk factor details
Argyriou (2013)	Genetic	Prospective cohort	200	SNC4A-rs2302237 OR = 2.65 (1.15–6) SCN10A-rs1263292 OR = 0.39 (0.17–0.88)
Attal (2009)	Clinical	Prospective cohort	18	Cold allodynia OR = 39 (1.8–817) Cold hyperalgesia OR = 3.9 (1.0–1.20)
Baldwin (2012)	Genetic	Prospective cohort	855	FGD4-rs10771973 HR = 1.57 (1.30–1.91)
Dimopoulos (2011)	Clinical	RCT	340	Baseline neuropathy HR = 1.79 ($p < 0.01$)
Glendenning (2010)	Clinical and treatment-related	Cross-sectional cohort	293	Cisplatin dose increase OR = 1.91 (1.61–2.26) Carboplatin dose increase OR = 1.26 (1.04–1.52) Age at follow-up OR = 1.06 (1.04–1.08)
Johnson (2011)*	Genetic	RCT	970 + 550	ABCA1-rs363717 OR = 0.71 (0.52–0.98) ICAM1-rs1799969 OR = 0.67 (0.44–1.03) PPARD-rs2076169 OR = 0.60 (0.38–095) SERPINB2-rs6103 OR = 0.70 (0.52–0.95) SLC12A6-rs7164902 OR = 0.60 (0.44–0.80)
Kawakami (2012)	Clinical	Prospective cohort	50	Smoking history pack-years HR = 1.03 (1.0–1.05) Decreased creatinine clearance HR = 0.96 (0.92–0.99)
Won (2012)†	Genetic	Prospective cohort	96	TAC1-rs10486003 FOXC1-rs2338 ITGA1-rs830884 ACYP2-rs843748 DLEU7-rs797519

Abbreviations: CIPN, chemotherapy-induced peripheral neuropathy; HR, hazard ratio (95% confidence interval or significance level); OR, odds ratio (95% confidence interval); RCT, randomized controlled trial; SNP, single nucleotide polymorphism.
Note that Jonson et al. reported ORs for both populations included in their analysis. Only 1 set of ORs is reported here. All effect sizes reported here are directly from the cited studies.
* SNP association with CIPN grade $\geqslant 2$ only.
† Won et al. reported the overall predictive accuracy of the multiple logistic regression model yielding the 5 positive single nucleotide polymorphisms (SNPs), 72.8% (65.8–79.9), as opposed to ORs for individual SNPs.

are unable to quantify prevalence of painful CIPN explicitly in out analysis.

4.4. Strengths of this review

Our meta-analysis quantifies CIPN prevalence across most chemotherapy and cancer types. This allows our prevalence measures to be used by clinicians when deciding between chemotherapy types and regimens. It is also useful for planning future CIPN treatment studies. In addition, these findings may be useful for both resource allocation and research planning. Our pooled prevalence also allows direct estimation of economic costs of CIPN resulting from the chemotherapeutics and cancer types included in our review [51].

In this first meta-analysis investigating epidemiological measures of CIPN, we highlight the effect of the time of assessment, after chemotherapy cessation, on CIPN prevalence. This has implications for surveillance of CIPN at follow up, clinical care planning, and patient expectations. Specifically, our results may contribute to explaining the risks of developing CIPN, and its likely natural history, to patients at consent for chemotherapy. In broad terms, around two-thirds of patients will suffer from CIPN in the first month after chemotherapy, but in only one-half of these will CIPN have resolved by six months. Finally, we have confirmed the urgent need for a standardized approach to the diagnosis of CIPN, reaffirming ongoing efforts such as those of the chemotherapy-induced peripheral neuropathy outcome measures standardization study (CI-PERINOMS) group [67].

Conflict of interest statement

Marta Seretny, Gillian Currie, Emily Sena, Malcolm MacLeod, Robin Grant, and Marie Fallon declare no conflicts of interest. Lesley Colvin serves as an editor for the British Journal of Anaesthesia.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pain.2014.09.020.

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Appendix C

Strengths and Limitations of Observational Studies

Patient studies investigating CIPN utilize a cohort design. There are a number of strengths of this type of study design. These include the possibility of examining multiple outcomes. Secondly, strict control of the quality and nature of data recording is achievable. Finally, it is possible to utilize data acquired to identify subjects most at risk of a specific outcome, in this case CIPN development (Altman, 1991). All these factors are well suited to the realities of investigating CIPN in oncology patients.

However, cohort designs also have limitations. Bias, chance, random error, and confounding can influence these types of studies and should always be assessed. Bias occurs when the groups under study (i.e. exposed/unexposed) are affected by a distorting factor unequally, this is also referred to as systematic error. In contrast random error, also known as non-differential error, is a result of the distorting issue influencing both groups. In the CIPN literature the effects of bias are perhaps more important than those of random error, as the groups and measures used tend to be carefully chosen.

Chance and confounding are limitations of cohort studies, which can to be minimized by using high power for sample size calculations, as well as complex statistical modeling to control for confounding. However, neither of these approaches is infallible. Unknown confounders, in particular, are impossible to adjust for. In addition to confounding, effect modification should also be sought out and discussed in cohort research summaries. Within the context of prospective CIPN studies the impact of change and confounding is important as studies tend to be small and complex statistical models are not possible on small datasets of this size.

Perhaps the most significant problem affecting CIPN cohort studies is potential for loss to follow up. Subjects lost to follow up decrease the numbers in the study

and weaken the subsequent analysis (Altman, 1991).Loss to follow up among oncology patients included in CIPN cohort studies is common and rarely addressed in terms of statistical adjustment. The key concern with this is the possibility that patients lost to follow up are somehow different with regards to the neurotoxicity risk. This is a difficult problem to overcome and should be discussed in the presentation of CIPN study findings.

Another potential limitation of cohort studies results from misclassification of exposure or outcome. This can be minimized with the use of clear definitions. This is a major limitation in the CIPN literature as a concrete definition of CIPN diagnosis is still lacking. Progress has been made towards unifying how CIPN is measured and defined but this remains an ongoing issue in the CIPN research community (Cavaletti et al., 2013).

Finally, it is important to note that small single centre studies, which tend to represent the majority of CIPN epidemiological work, tend to have very limited generalisability. This is an important problem to consider and is one of the reasons that the systematic review published as part of this thesis aimed to give a statistical summary of all studies to calculate CIPN prevalence.

Due to the limitations listed here observational studies can only ever describe associations, as opposed to causal relationships. Despite these constraints in a condition such as CIPN where randomized controlled trials are never possible due to ethical limitations, evidence provided by robust observational studies, are both valid and useful. This is particularly true when observational studies utilize sensitive measuring instruments to diagnose CIPN development prospectively.

Appendix D

Scanner Noise Diagnostics

Identification of the source of the noise/radio frequency instability encountered in the scanner after introduction of new equipment was conducted using a systematic approach. A number of potential sources of noise were considered.

These included the following:

1) Medoc Pathway system
2) Any part of the physiological monitoring equipment
3) Neuronordic Laboratory (NNL) goggles used in the presentation of visual stimuli
4) Damage to the head coil
5) Damage to scanner hardware
6) Other sources including light bulbs in the scanner room
7) An interaction of one or more of the above.

Diagnostics were carried out by initially running scans on phantoms and later on healthy volunteers. During these scans equipment and cables were sequentially removed and added. Most diagnostic scans had to be completed out of hours in order to work around other research scans ongoing at CRIC.

Various companies involved in the provision of the equipment used at CRIC were consulted including: Medoc, Siemens and NNL. Colleagues from other centers, in particular Dr Jon Brooks in Bristol who helped with the physiological noise monitor (PNM) equipment set up were approached for advice by both Professor Roberts and or myself.

A combination of the diagnostic scans and liaison with the above companies and researchers identified an interaction between equipment as being responsible for the noise issue affecting the data. Specifically the following three components were implicated:

1) One of the cables used for the physiological monitoring (connecting the pulse oximetry to the Biopac), running through the waveguide, was introducing noise. This was earthed and is no longer a problem.

2) The Medoc pathway contains ferrous material and introduces RF interference. Although this did not seem to be the case on installation of the equipment when phantom scans were performed to ensure acceptably low noise levels. Noise from the pathway, possibly acceptable when used in isolation, was found to be amplified by the NNL goggles as described below.

3) The NNL goggles were found to amplify any noise present in the scanner room. This is because they contain ferrous material in the cable connecting them to their control tower. Additionally, the cable gets damaged over time when exposed to a magnetic field. NNL, as a company have no solution for this problem at present. A projector screen could overcome this issue but was not feasible in CRIC when the CIPN fMRI experiment was being set up.

Additionally, in order to get a more comprehensive understanding of the impact of this noise on the functional data, independent component analysis (ICA) was carried out using MELODIC in FSL. ICA results confirmed that the impact on the thermal data acquisition when the Medoc pathway was in used was likely unacceptable (see figure D.1). Punctate data seemed unaffected by noise to the same degree.

Following diagnostics and after research team consensus it was decided that thermal stimuli would not be given during the CIPN fMRI study.

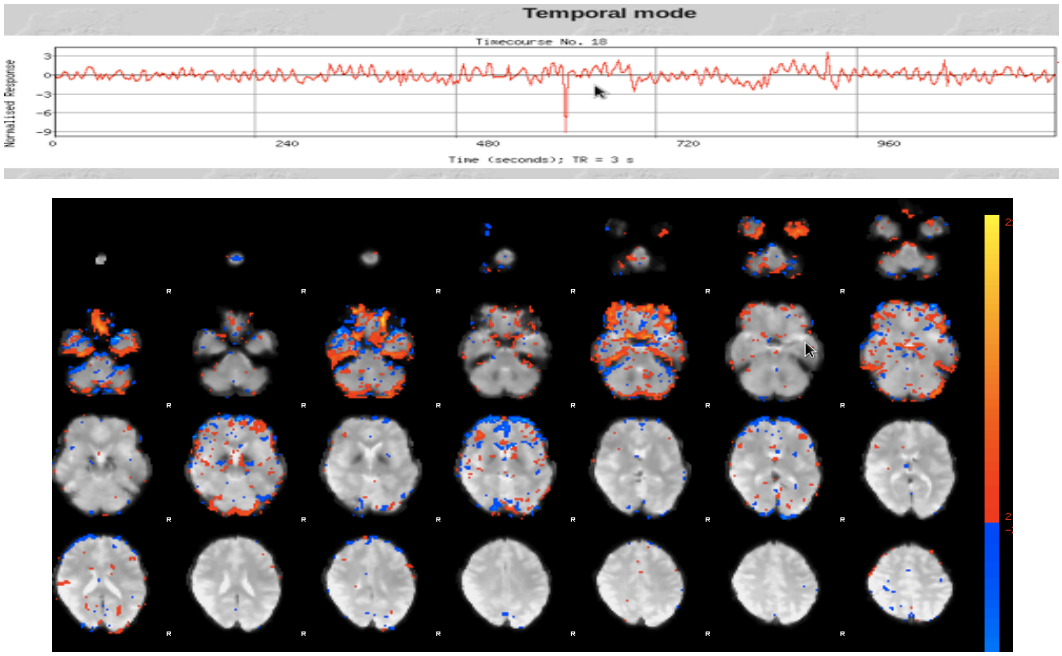

Figure D.1. Time course and spatial map showing high frequency noise in the thermal fMRI data, most likely related to interaction between Medoc Pathway and NNL goggles**.**

Appendix E

CIPN ethical approval letter (final substantial amendment)

Lothian NHS Board

South East Scotland Research Ethics Committee 02

Waverley Gate
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Telephone 0131 536 9000
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Date 22 March 2013
Your Ref
Our Ref

Enquiries to: Joyce Clearie
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22 March 2013

Prof Marie Fallon
Edinburgh Cancer Research Centre
Western General Hospital
Edinburgh Cancer Research Centre
Western General Hospital
Edinburgh
EH4 2XR

Dear Prof Fallon

Study title:	**A prospective study of chemotherapy induced neuropathy**
REC reference:	**09/S1103/43**
Amendment number:	**AMO4 SA**
Amendment date:	**11 March 2013**
IRAS project ID:	**31300**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Participant Consent Form: PCF	4	11 March 2013
Participant Information Sheet: PIS	5	11 March 2013
Protocol	6	11 March 2013

Notice of Substantial Amendment (non-CTIMPs)		11 March 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

09/S1103/43:	**Please quote this number on all correspondence**

Yours sincerely

Mr Thomas Russell
Chair

E-mail: joyce.clearie@nhslothian.scot.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Karen Maitland, Research and Development*
Mrs Elspeth Currie

Appendix F

MINT3 Ethical Approval Letter

Scotland A Research Ethics Committee

Research Ethics Service
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NHS SCOTLAND

Dr Marta Seretny
Edinburgh Cancer Research UK Centre
University of Edinburgh
Western General Hospital
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Date: 2 December 2013
Your Ref.:
Our Ref.: 13/SS/0201
Enquiries to: Walter Hunter
Extension: 35680
Direct Line: 0131 465 5680
Email: walter.hunter@nhslothian.scot.nhs.uk

Dear Dr Sertny

Study title: **Using Functional Magnetic Resonance Imaging to investigate the efficacy of menthol in chemotherapy induced peripheral neuropathy (CIPN): The MINT3 FMRI Study**
REC reference: **13/SS/0201**
Protocol number: **2013/MINT3_FMRI**
EudraCT number: **2013-003968-31**
IRAS project ID: **139424**

Thank you for your letter dated 20 November 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

I have considered the response on behalf of the Committee.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mr Walter Hunter, walter.hunter@nhslothian.scot.nhs.uk.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation [as revised], subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites listed in the application, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
REC application: IRAS Form 3.5		10 October 2013
Protocol	2.0	26 November 2013
Investigator CV: Dr Serenty		
Investigator CV: Professor Fallon		10 October 2013
Participant Information Sheet	2	26 November 2013
Participant Information Sheet: MRI	1	09 October 2013
Participant Consent Form	1	09 October 2013
GP/Consultant Information Sheets	1	09 October 2013
Response to request for further information		20 November 2013

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.
The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

REC reference number: 13/SS/0201-Please quote this number on all correspondence

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Ian Zealley
Committee Chairman
cc: Professor Fallon
Ms Marianne Laird
Ms Karen Maitland, NHS Lothian

Appendix G

Testosterone Hormone Levels and CIPN Development

Background:

There is evidence suggesting that low testosterone hormone levels are associated with greater pain experience (Vincent et al., 2013). Sex hormone impact on pain perception is a complex and evolving field of research (Bartley and Fillingim, 2013). The impact of sex hormones on CIPN development is unknown.

The following research questions were explored:

1) Is there a difference between testosterone level in patients who developed CIPN and those who did not?
2) Does testosterone level correlate with % BOLD signal change in the RVM, PAG, MPRF or Thalamus?
3) Does adjusting for testosterone help explain observed sex differences in the ROI analysis (chp 5)?

Methods:

Salivary testosterone levels was collected from patients at the time of the scan and analysed by colleagues at the department of Clinical Biochemistry, University Hospital South Manchester NHS Foundation Trust. Testosterone results are reported in pmol/L with a reference range of 5.3-46pmol/L.

Statistical analyses presented below were split by sex and testosterone level was log transformed as the variable was non-normally distributed. Mean difference was compared using a two-sided independent sample t test. Correlation was explored using Kendall's tau non-parametric correlation recommended for use in a small data set. Repeat measures ANOVA was used to explore the association between CIPN development and % BOLD signal change to punctate stimuli, in the MPRF, RVM, PAG and Thalamus. Testosterone level was introduced as a

factor in this analysis to assess if this variable explained any of the interactions seen.

Results:

Of the 30 patients in the CIPN study, 27 patients were included in this analysis: 15 female and 12 male. Exclusions were due to three patients having corrupted salivary sputum samples inappropriate for analysis. Mean age and testosterone level data are shown in Table G.1.

	Female n=15		**Male** n=12	
	No CIPN (n=4)	**CIPN** (n=11)	**No CIPN** (n=8)	**CIPN** (n=5)
Mean Age (95% CI)	55.2 (48.8-61.6)	61.6 (56.9-66.3)	59.6 (55.5-63.7)	64.2 (47.3-81)
MeanTestosterone (95% CI)	11.01 (-3.5-25.4)	15 (7.3-22.7)	150.1 (108-191.4)	180.7 (146-215.3)

Table G.1 Summary of age and testosterone levels between the No CIPN and CIPN group split by sex.

Group comparison of mean testosterone level:

There was no difference in mean testosterone level between female patients who developed CIPN and those who did not (p=0.55). There was also no difference in mean testosterone between males who developed CIPN and those who did not (p=0.15). Figure G.1 shows the spread of these data points for the two groups.

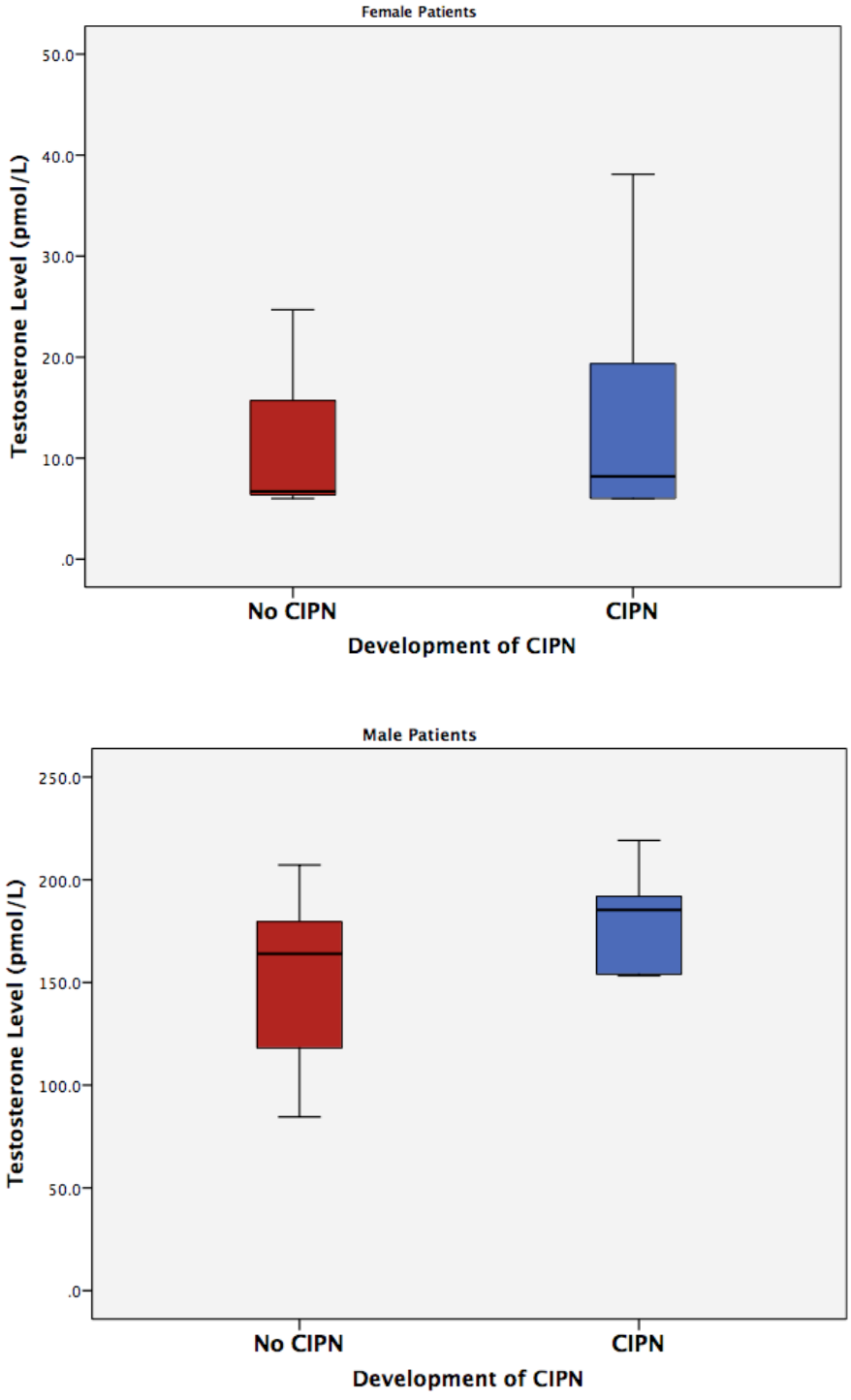

Figure G.1 Testosterone Level Split by Sex. Blue denotes CIPN patients, red denotes non CIPN patients. Error bars represent 95% confidence intervals. Median represented by sold line in box plot. Please note the differences in the scale and spread of the y-axis. This relates to known higher testosterone levels in men, which occur physiologically.

Correlation of testosterone level and ROI % BOLD signal change

Assessment of correlations between salivary testosterone level and % BOLD signal

change in the ROIs, showed no significant correlations in men. In women there was a negative correlation between % BOLD signal change in the left RVM and testosterone level (τ = -0.42, p=0.04) and a positive correlation between % BOLD signal change in the right thalamus and testosterone level (τ = 0.45, p=0.03).

Correlation of testosterone level and ROI % BOLD signal change, according to CIPN/NO CIPN classification

When the group was split into those who developed CIPN and those who did not, females who developed CIPN had a negative correlation between serum testosterone level and % BOLD signal change in the left RVM (τ = -0.58, p=0.03) and right RVM (τ = -0.63, p=0.02). Males had no correlation between ROI % BOLD signal change and CIPN development.

Testosterone as a covariate in the repeat measures ANOVA investigating ROIs

Testosterone did not explain any of the observed variance in the increased MPRF activation seen in females with CIPN or the decreased thalamic activation seen in males.

Discussion

There is tentative evidence that testosterone is correlated with % BOLD signal change in response to punctate stimuli in the RVM in women with cancer. This is a region of the descending pain modulatory system previously reported to be influenced by low testosterone levels in healthy females (Vincent et al., 2013). There is no clear relationship with this and CIPN development *per se*. Without measures of sharpness in a group with clinically insignificant pain ratings, and unknown menopausal status further interpretations of these findings are not possible. These results however suggest further work is warranted in terms of exploring the impact of testosterone on pain perception in general.

Appendix H

CIPN20 Questionnaire

EORTC QLQ CIPN-20

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

	During the past week	Not at all	A little	Quite a bit	Very much
1	Did you have tingling fingers or hands?	1	2	3	4
2	Did you have tingling feet or toes?	1	2	3	4
3	Did you have numbness in your fingers or hands?	1	2	3	4
4	Did you have numbness in your toes or feet?	1	2	3	4
5	Did you have shooting or burning pain in your fingers or hands?	1	2	3	4
6	Did you have shooting or burning pain in your toes or feet?	1	2	3	4
7	Did you have cramp in your hands?	1	2	3	4
8	Did you have cramp in your feet?	1	2	3	4
9	Did you have problems standing or walking because of difficulty feeling the ground under your feet?	1	2	3	4
10	Did you have difficulty distinguishing between hot and cold water?	1	2	3	4
11	Did you have a problem holding a pen, which made writing difficult?	1	2	3	4
12	Did you have difficulty manipulating small objects with your fingers (for example fastening small buttons)?	1	2	3	4
13	Did you have difficulty opening a jar or bottle because of weakness in your hands?	1	2	3	4
14	Did you have difficulty walking because your feet dropped forward?	1	2	3	4
15	Did you have difficulty climbing the stairs or getting up out of a chair because of weakness in your legs?	1	2	3	4
16	Were you dizzy when standing up form a lying or standing position?	1	2	3	4
17	Did you have blurred vision?	1	2	3	4
18	Did you have difficulty hearing?	1	2	3	4
	Please answer the following question only if you drive a car	**Not at all**	**A little**	**Quite a bit**	**Very much**
19	Did you have difficulty using the pedals?	1	2	3	4
	Please answer the following question only if you are a man	**Not at all**	**A little**	**Quite a bit**	**Very much**
20	Did you have difficulty getting or maintaining an erection?	1	2	3	4

Appendix I

MINT3 Baseline CRF

MINT3 fMRI STUDY

Eudract No. 2013-003968-31

A randomised, double-blind, controlled exploratory fMRI study of menthol gel versus placebo in the treatment of chemotherapy induced peripheral neuropathy.

Case Report Form

Patient's Initials __ __ __ Subject No. _ _ _ _ _

Date of Birth ___ / ___ / ___ Gender ____

CRF 1 Completed by: *(Signature)* ----------	*Print Name Here:* *(Block Capitals)* ----------
Date Completed: (dd/mm/yy) __ __ /__ __ /__ __	*Version 1, 10/04/2016, Page 1*

MINT 3 fMRI PT'S DOB: __/__/__ Trial No: _____

ELIGIBILITY CHECKLIST

		YES	NO
	INCLUSION CRITERIA		
a.	Has the patient received any neurotoxic chemotherapy?	☐	☐
b.	Has the patient experienced post treatment Chemotherapy Induced Peripheral Neuropathy (CIPN) pain for a minimum of 3 months?	☐	☐
c.	Does the patient report a distressing or uncomfortable neuropathic symptom (such as pain or tingling) with a score of ≥ 4 on a scale of 0-10 with 0 being none?	☐	☐
d.	Is the patient ≥ 18 years of age?	☐	☐
e.	Does the patient's Oncology team agree to the patient's study participation?	☐	☐
f.	Is the patient able to provide written informed consent to participation in the study after explanation of the study protocol?	☐	☐
g.	Does the patient have the ability to complete questionnaire assessments in the English language?	☐	☐
h.	In the opinion of the Investigator, is the patient able to comply with study procedures?	☐	☐
i.	Is the patient's neuropathy confined to the distal extremities (distal to elbows and/or knees)?	☐	☐
	EXCLUSION CRITERIA		
a.	Does the patient have a pre-existing history of peripheral neuropathy due to any cause other than chemotherapy (diabetes, alcohol, toxin, hereditary etc)?	☐	☐
b.	Does the patient have any contraindication to using topical therapy or menthol?	☐	☐
c.	Does the patient have any other neurological condition which may influence findings (such as Multiple Sclerosis or residual signs/symptoms from a previous stroke)?	☐	☐
d.	Does the patient have any skin condition which would prevent assessment of the relevant areas affected by peripheral neuropathy?	☐	☐
e.	Does the patient suffer from significant psychiatric illness which would hinder their completion of the study?	☐	☐
f.	Does the patient have a general medical condition which is unstable or rapidly deteriorating, such that they are unlikely to be able to contribute to the study?	☐	☐
g.	In the opinion of the Research Team or their usual medical team, would the patient be unable to complete the study protocol for any other reason?	☐	☐
h.	Is the patient currently undergoing treatment of ≤ 30 days duration with anticonvulsants, tricyclic antidepressants, MAO inhibitor, or other neuropathic pain medication agents such as carbamazepine, phenytoin, valproic acid, gabapentin, lamotrigine or amifostine?	☐	☐
i.	Has the patient had topical lidocaine patches/ gel or capsaicin patches/ cream (to the limb extremities) applied within the last 30 days?	☐	☐
j.	Does the patient have any other medical condition which, in the opinion of the treating physician/ allied health professional, would make this protocol unreasonably hazardous for the patient?	☐	☐
k.	Is there any contraindication to the patient having an MRI eg aneurysm clips, other metal work in body, claustrophobia?	☐	☐

SIGNATURES					
Medic Confirming Eligibility					
Name		Signature		Date	
Person Taking Consent					
Name		Signature		Date	

__CRF 1__ Completed by: *(Signature)* ---------- *Print Name Here:* *(Block Capitals)* ----------

Date Completed: (dd/mm/yy) __ __ /__ __ /__ __

Non-Completion of Trial

Please complete if the patient did not complete the trial.

Status:

- Patient withdrew consent to continue with trial ☐
- Patient unable to attend fMRI scan ☐
- Patient unable to tolerate scan (scan stopped early) ☐
- Unable to contact patient ☐
- Withdrawn by clinician ☐
- Patient died ☐

(*If patient died)*
Date of death | | | | (dd mm yy)

Status change date: | | | | (dd mm yy)

Patient Demographics: *Cancer Details*

Cancer Diagnosis Date | | | |
dd mm yy

Primary tumour site *(tick one box only)*

Bladder ☐	Gynaecological ☐	Oesophageal ☐
Bone ☐	Head and neck ☐	Pancreatic ☐
Brain ☐	Leukaemia ☐	Prostate ☐
Breast ☐	Liver ☐	Renal ☐
Colorectal ☐	Lymphoma ☐	Testicular ☐
Dermatological ☐	Lung ☐	Thyroid ☐
Endocrine ☐	Mesothelioma ☐	Unknown ☐
Gastric/Stomach ☐	Myeloma ☐	Other ☐

If 'other' please specify here:

Current Status *(tick one box only)*

No evidence of disease ☐ Local disease ☐ Loco-regionally advanced ☐ Metastatic disease ☐

If 'Metastatic disease' is ticked,
please specify Site(s) of metastases *(tick all that apply)*

Brain ☐	Lung ☐	Bone ☐
Liver ☐	Lymph nodes ☐	Other ☐

***CRF 1** Completed by:* *Print Name Here:*
(Signature) ---------- *(Block Capitals)* ----------

Date Completed: (dd/mm/yy) __ __ /__ __ /__ __

If 'other' please specify here:

Patient Demographics: *Previous Cancer Treatment*

Radiotherapy History

Has the patient received radiotherapy? Yes ☐ No ☐

If yes please give date of last radiotherapy: ☐☐ ☐☐ ☐☐

dd mm yy

Chemotherapy History

Has the patient received chemotherapy? Yes ☐ No ☐

If yes please list below:

Chemotherapy (including hormonal therapy)				
Regimen	**Total No. Cycles**	**Start Date** (dd/mm/yy)	**Stop Date** (dd/mm/yy)	**Cumulative Dose** (mg)
Paclitaxel (weekly)				
Carboplatin/Paclitaxel: 3 weekly				
Carboplatin/Paclitaxel: weekly				
Carboplatin/Oxaliplatin				
Docetaxel (3 weekly)				
Bortezumib				
Other (specify):				

Patient Demographics: *Medical & Surgical History*

For how many months has the patient had peripheral neuropathy? ☐

Relevant Medical History

Condition *(please specify within category)*	**Start Date** (dd/mm/yy)	**Stop Date** (dd/mm/yy)
Cardiovascular:		
Respiratory:		
Renal:		
Hepatic:		
Neurological:		
Gastrointestinal:		
Endocrine:		
Musculoskeletal:		
Dermatalogical:		
Mental Health:		
Other:		

Relevant Surgical History

Procedures *(please specify within category)*	**Date** (dd/mm/yy)
Abdominal:	
Breast:	
Gynaecological:	
Lung:	
Colorectal:	
Other:	

Current Medications & Trial Compliance

Current Medications

Drug Name *(specify code according to drug charts attached)*	**Dose**	**Unit** (g/mg etc)	**Freq** (OD/BD/ PRN etc)	**Freq Count** (for PRN)	**Route** (PO/SC etc)	**Start Date** (dd/mm/yy)	**Stop Date** (dd/mm/yy)

Compliance

Tube ID	**Start Date** *(dd/mm/yy)*	**Weight on Issue** *(g)*	**<= 25 C storage instructed?**	**End Date** *(dd/mm/yy)*	**Weight on Return** *(g)*	**<= 25 C storage confirmed?**

CRF 1 *Completed by:* *Print Name Here:*
(Signature) ---------- *(Block Capitals)* ----------

Date Completed: (dd/mm/yy) __ __ /__ __ /__ __

Patient Demographics: *Additional Data*

Weight (in kg) ☐ **Height** (in cm) ☐

NART Score (National Adult Reading Test) ☐ **DSST Score** (Digit Symbol Substitution Test) ☐

GCOS Score (General Causality Orientations Scale) ☐ **Skin Temperature** (degrees C) (after first application of gel) ☐

fMRI Scan Dates

First Scan Date ☐☐☐ (dd mm yy) Second Scan Date ☐☐☐ (dd mm yy)

Dominant Hand Left ☐ Right ☐

Randomisation

Drug Pack Number ☐

***CRF 1** Completed by:* *(Signature)* ---------- *Print Name Here:* *(Block Capitals)* ----------

Date Completed: (dd/mm/yy) __ __ /__ __ /__ __

Appendix J

Quantitative Sensory Testing Protocol planned for the MINT3 Study

Modality	Description
Mechanical: *Threshold detection*	- Standardised Von Frey monofilament - 0.008-300g - Up down application for touch detection threshold
Pain threshold detection	- Blunted 30-gauge needle attached to weights - Weights in grams: 8,10,16,20,32,64 or 128 - Patient reports when application sharp or painful
Thermal: *Threshold detection*	- Thermal Sensory Analyzer (TSA II, 2001 MEDOC) - For warm detection temp start= 32C (increasing to max 50C) - For cool detection temp start= 32C (decreasing to min 0C) - Patient presses button when either sensation first felt - Four tests run for each and mean taken
Pain threshold detection	- Above thermode used - Start temperature for hot pain and cold pain as above - Maximum temperatures as above - Report button press as above - Four test average taken as above

The above protocol is the full QST protocol carried out outside the scanner. This will serve as the basis to establish a modified version for application of thermal and mechanical pain within the scanner. This will be individualised for each patient. Pain rating of the noxious stimuli will be taken at baseline and during scanning. Background pain will be noted after QST and also in the scanner.

Appendix K

Common Toxicity Criteria Details Used for Chemotherapy Dose Cessation or Reduction

- Also to document any neurotoxicity persisting at time next treatment due. Both are important in managing dose delays and dose reductions.
- Laryngospasm should be recorded as a separate note
- **Colorectal = CR and Upper GI = UGI**

OXALIPLATIN neurotoxicity		**1-7 days** (ie. asymptomatic on treatment day)	**>7 days** (ie. asymptomatic on treatment day)	**Persistent** (on treatment day)
	Modified CTCAE v4 grade	**a**	**b**	**c**
Loss of deep tendon reflexes or paraesthesia (does not interfere with function)	**1**	No change	No change	**CR: Omit Oxaliplatin this cycle** and restart when symptoms totally resolved. Continue Capecitabine or 5FU alone* **UGI: Switch Oxaliplatin to Carboplatin**, if Cycle 5/6 then consider discontinuation
Moderate symptoms; limiting instrumental ADL**	**2**	**CR & UGI: Continue Oxaliplatin but with dose reduction** (CapOx/EOX/EOF 130 to 100 mg/m^2 or 100 to 80mg/m^2, OxMdG 85 to 75mg/m^2)	**CR & UGI: Continue Oxaliplatin but with dose reduction** (CapOx/EOX/EOF 130 to 100 mg/m^2 or 100 to 80mg/m^2, OxMdG 85 to 75mg/m^2)	**CR: Omit Oxaliplatin this cycle** and restart with dose reduction when symptoms totally resolved. (CapOx 130 to 100 mg/m^2 or 100 to 80mg/m^2, OxMdG 85 to 75mg/m^2) Continue Capecitabine or 5FU alone* **UGI: Switch Oxaliplatin to Carboplatin**, if Cycle 5/6 then consider discontinuation
Severe symptoms; limiting self care ADL***	**3**	**CR: Continue Oxaliplatin but with dose reduction** (CapOx 130 to 100 mg/m^2 or 100 to 80mg/m^2, OxMdG 85 to 75mg/m^2) If recurs again at this grade, discontinue Oxaliplatin permanently **UGI: Switch Oxaliplatin to Carboplatin**, if Cycle 5/6 then consider discontinuation	**CR: Discontinue Oxaliplatin permanently** Continue Capecitabine or 5FU alone* **UGI: Switch Oxaliplatin to Carboplatin**, if Cycle 5/6 then consider discontinuation	**CR: Discontinue Oxaliplatin permanently** Continue Capecitabine or 5FU alone* **UGI: Switch Oxaliplatin to Carboplatin**, if Cycle 5/6 then consider discontinuation
Life-threatening consequences; urgent intervention indicated	**4**	**CR: Discontinue Oxaliplatin permanently** Continue Capecitabine or 5FU alone* **UGI: Discontinue Chemotherapy**	**CR: Discontinue Oxaliplatin permanently** Continue Capecitabine or 5FU alone* **UGI: Discontinue Chemotherapy**	**CR: Discontinue Oxaliplatin permanently** Continue Capecitabine or 5FU alone* **UGI: Discontinue Chemotherapy**
Acute laryngo-pharyngeal dysaesthesia or jelly legs		Increase infusion to 4 hours. If recurrent, increase to 6 hours		

* **CR ONLY:** If stopping Oxaliplatin for neurotoxicity alone, consider escalating Capecitabine/5FU (other toxicities allowing)
**Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
***Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden

Oxaliplatin neurotoxicity guidance. Written by the GI Team, ECC. July 2011. Next review 2013. (Replaces previous PNQ guidelines).

Reference: Cassidy J et al. Journal of Clinical Oncology 2004;22(11):2084-9

Revised by Dr L Dawson, in consultation with Edinburgh Cancer Centre colorectal team. Updated by: D Clifford June 2011, Checked by Dr S Clive June 2011. Review date: June 2014. Reg No Update Sept 2013

Patients Name: **CHI No:** **Diagnosis:**
Address: **Date of Birth:** **Stage:**
Consultant: **Allergies:**

Chemotherapy Regimen: Carboplatin & Paclitaxel for Gynaecological Cancer (3 weekly)

	Drug	Dose/m^2	Route and Schedule of Administration	Days of Cycle Given	Cycle Frequency	Comment here on any non-standard doses or schedules
1.	Paclitaxel	175mg/m^2	iv over 3 hours via 0.22 micron filter & non- PVC givingset	day 1	21 days	Give paclitaxel first
2.	Carboplatin	AUC6 (AUC 5 if GFR by EDTA)	mg iv over 30 mins	day 1	21 days	

Investigations Baseline: full blood count (FBC), liver function tests (LFTs), creatinine, magnesium, CA125. Baseline CT ECG if over 60 or previous cardiac history or drugs with QTc prolongation. EDTA if eGFR <50. If using EDTA value use AUC 5.
Weekly FBC cycle 1. FBC, LFTs, Magnesium and Ca125 before each course; CT scan after cycle 6.

Toxicities **Common:** fatigue, emesis, myelosuppression, total alopecia, arthralgia, peripheral neuropathy.
Uncommon: flushing or rash, mucositis, diarrhoea, LFT changes, arrhythmias, anaphylaxis, ototoxicity, GFR changes, blood clots.
Unknown: effects on fertility.

Dose modifications **Delay treatment if neutrophils < 1.0**, platelets < 100 on day of treatment. **If treated with neutrophils< 1.5, give prophylactic ciprofloxacin days 5-12**.
Reduce dose of paclitaxel and carboplatin by 25% for grade 4 platelets, complicated or prolonged grade 4 neutropenia, or other grade 3 toxicity.
Reduce paclitaxel by 25% for grade 2 neurotoxicity, **withdraw paclitaxel** for grade 3 neuropathy, serious hypersensitivity or Bilirubin/ALT>3x ULN

Special Precautions **Hypersensitivity common - premedication essential.** Impaired hepatic function . Conduction abnormalities. **Consider secondary prophylaxis in neoadj & adju patients as per ECC GCSF policy**

Contraindications Pregnancy, lactation, severe hepatic dysfunction, prior severe hypersensitivity. eGFR <20

Carbo Dose use Calvert formula mg = AUC x (GFR + 25) Cap the GFR used in Calvert formula at maximum 125ml/min

GFR in Calvert formula **1.EDTA if eGFR <50, AUC 5** if EDTA used in Calvert Formula
2.Wright formula used when EDTA has not been measured, AUC 6 if not using EDTA
Wright formula for calculating GFR in females = $\frac{[6580 - (38.8 \times \text{Age})] \times \text{BSA} \times 0.832}{\text{Serum Creatinine}}$

Calculation Use **same dose** each cycle unless dose reduction required for toxicity or creatinine has increased above normal limits.
Calculate GFR using creatinine in µmol/l, age in years and BSA using Dubois and Dubois. Use same dose each cycle unless creatinine has changed by >10µmol or weight changed >5kg (or 10% from baseline if original weight<50kg) or if dose reduction required for toxicity. If actual creatinine is <60µmol/l, use 60µmol/l in the calculation and check the nadir bloods.

Patient consent signed? YES/NO

EVALUATION OF RESPONSE

Which disease sites should be followed? _____
How are these assessed? Ca125 each cycle, and CT before treatment and after 6 cycles. If marker –ve, repeat CT after 3.
When should patient attend clinic? After cycles 3 & 6.