PAIN IN PARKINSON’S DISEASE

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ABBREVIATIONS

- CCM: Corneal Confocal Microscopy
- CNBD: Corneal Nerve Branch Density
- CNFD: Corneal Nerve Fibre Density
- CNFL: Corneal Nerve Fibre length
- CT: C Tactile
- DB-HRV: Deep Breathing Heart Rate Variability
- DNIC: Diffuses Noxic Inhibitory Control
- HADS: Hospital Anxiety and Depression Scale
- IENFD: Intraepidermal Nerve Fibre Density
- KPPS: King’s PD Pain Scale
- LANSS: Leeds Assessment of Neuropathic Signs and Symptoms
- LARS: Lille Apathy Rating Scale
- NMS: Non-Motor Symptoms
- NMSS: Non-Motor Symptoms Scale
- PD: Parkinson’s Disease
- SCOPA-AUT: Scale for outcomes in Parkinson’s disease for autonomic symptoms
- SFMPQ: Short Form McGill Pain Questionnaire
Pain Characteristics in Parkinson’s Disease.
Submitted to the University of Manchester for the degree of Doctor of Philosophy by Lewis Kass-Iliyya. 2016

Abstract: Background: Pain is a very common symptom in Parkinson’s disease (PD). The underlying mechanism of pain in PD is poorly understood. Compared to PD, the characteristics of pain in other parkinsonian disorders such as Multiple System Atrophy (MSA) and Progressive Supranuclear Palsy (PSP) have not been studied. Musculoskeletal factors have been implicated in the generation of pain in PD. However, studies in PD have shown impaired central processing of nociceptive inputs. Recently, small fibre neuropathy has also been found to be common in PD with significantly reduced C-fibres density compared to controls. A subclass of C-fibres known as C tactile afferents (CT) mediate the pleasant sensation associated with gentle skin stroking (affective touch). CT afferents have recently been shown to have pain-inhibiting properties. These findings may implicate central sensitisation in pain generation in PD. Objectives: 1) To better understand the mechanisms of pain in PD and study the characteristics of pain in MSA and PSP compared to PD. 2) To quantify small fibre neuropathy in PD and explore its relation to pain utilising a novel diagnostic technique: corneal confocal microscopy (CCM). 3) To assess the perception of affective touch in PD and its relationship to pain. Methods: Four studies were conducted: Study 1: A cross sectional study of pain characteristics in PD, MSA and PSP. Study 2: A descriptive study of pain characteristics in a large cohort of early PD (disease duration < 3 years, n=1763). Study 3: A cross sectional study to quantify small fibre density in PD (n=26) compared to control subjects (n=26) using CCM and skin biopsies. Nerve density was correlated with non-motor symptoms in PD including pain. Study 4: A study to assess the CT-mediated perception of affective touch in PD and correlate it with clinical symptoms such as pain. Results: Study 1: Pain prevalence and intensity was significantly higher in MSA and PD compared to PSP, p < 0.05. Female sex and motor fluctuations but not motor severity were predictors for pain intensity in PD. Study 2: Pain was common in early PD (84.2%). Only a minority of PD patients (19.7%) reported that their pain improved with Levodopa therapy of their motor symptoms. Study 3: PD patients had significantly reduced small fibre nerve density on both CCM and skin biopsies compared to controls. Denervation correlated with autonomic symptoms but not with pain intensity. Study 4: Perception of pleasantness followed a linear relationship with nerve density and was abnormally enhanced in PD compared to control and correlated with pain at a very slow stroking velocity. Conclusions: Pain is common in early PD, does not respond to levodopa treatment and correlates with motor complications but not motor severity favouring central sensitisation. Pain is significantly less common in PSP compared to PD and MSA. Small fibre neuropathy does not appear to be an important cause of pain in PD but small fibre nerve density correlates with affective touch perception, which is enhanced in PD despite peripheral denervation. Corneal confocal microscopy identifies corneal denervation in PD offering a novel non-invasive way of assessing PD pathology.
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DEDICATION

I dedicate this work to my wife Heather for her unlimited support and patience through endless hours away from home and family.
PREFACE

This thesis is presented in the alternative format, which allows each chapter to be written in a paper style suitable for publication in peer-reviewed journals. At the time of writing, chapter 2, chapter 4 and chapter 5 of this thesis are published as the following papers:

Chapter 2:

Chapter 4:

Chapter 5:

The remaining chapters will form the basis of future publications.

The author has played the major role in all aspects of the production of the published work including the recruitment of participants, acquisition, analysis and interpretation of data as well as writing the papers.
CHAPTER 1

Introduction


1.1 Non-motor symptoms in Parkinson’s disease

Parkinson’s Disease (PD) is a neurodegenerative disease that has traditionally been recognised by its cardinal motor symptoms of rest tremor, bradykinesia, rigidity and postural instability (Jankovic, 2008). The motor syndrome is caused by degeneration of the dopamine producing cells in the substantia nigra of the midbrain (Greenfield and Bosanquet, 1953; Samii et al., 2004). These cells project to subcortical nuclei known as the basal ganglia and through a complex series of both inhibitory and excitatory neurotransmissions they control movements.

The motor symptoms of PD constitute a major part of the disease burden, which has been to a great extent successfully treated with dopaminergic medications. However, the motor syndrome is not the only manifestation of the disease. Numerous other symptoms such as bladder dysfunction, constipation, pain, fatigue, mood problems, orthostatic hypotension, sexual dysfunction, sleep disturbance and cognitive decline are well-recognised to be prevalent in PD (Chaudhuri et al., 2006). In fact it has been shown that these non-motor symptoms (NMS) can precede the motor syndrome and present a major challenge for both PD sufferers and clinicians especially in the advanced stages of the disease (O'Sullivan et al., 2008; Poewe, 2008). Table 1-1 summarises the different categories under which these symptoms can be classified (Park and Stacy, 2009).
NMS are increasingly identified as an important component of PD symptomatology as they have been shown to influence PD sufferers’ quality of life as much as, if not more than, the motor disability (Schrag et al., 2000; Witjas et al., 2002). Traditionally these symptoms have been under-recognised, under-treated and frequently ascribed to other conditions partly because they could present before the motor syndrome is apparent (O'Sullivan et al., 2008).

**Table 1-1 Non-motor symptoms in Parkinson’s disease**

<table>
<thead>
<tr>
<th>Symptom Group</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sensory symptoms</em></td>
<td>Pain, anosmia, restless legs</td>
</tr>
<tr>
<td><em>Autonomic symptoms</em></td>
<td>Constipation, urinary frequency and urgency, orthostatic hypotension, erectile dysfunction, excessive sweating, dry eyes</td>
</tr>
<tr>
<td><em>Sleep disturbances</em></td>
<td>REM sleep behaviour disorder, excessive daytime somnolence, insomnia</td>
</tr>
<tr>
<td><em>Neuropsychiatric symptoms</em></td>
<td>Depression, hallucinations, confusion, attention deficit, anhedonia, obsessional behaviour, delirium.</td>
</tr>
<tr>
<td><em>Cognitive decline</em></td>
<td>Subcortical dementia, slowed information processing, bradyphrenia, apathy</td>
</tr>
</tbody>
</table>

Table adapted from (Park and Stacy 2009)
While Levodopa has been successful in treating the motor syndrome its efficacy has not been established in non-motor symptoms especially those that are not related to motor fluctuations (Chaudhuri et al., 2006). This adds to the need of a better definition and understanding of the pathophysiology of these symptoms to develop targeted treatment.

From a neuropathological perspective nerve cell loss in the substantia nigra and the presence of abnormal aggregates of alpha-synuclein protein in the form of Lewy neurites and Lewy bodies define Parkinson’s disease (Forno, 1996; Spillantini et al., 1997). The manifestation of motor symptoms represents a late stage neuropathologically as the histological damage starts before the clinical symptoms become evident.

Braak et al proposed that the neuropathological process of sporadic PD follows a predetermined sequence affecting specific susceptible areas starting in the brain stem and eventually reaching the cerebral cortex and consequently they classified the neuropathology of PD into six stages Figure 1-1 (Braak et al., 2003):

- **Stage 1** affecting the medulla oblongata, dorsal IX/X motor nuclei and frequently the anterior olfactory nucleus as well as the intermediate reticular zone.
- **Stage 2** extending to the pontine tegmentum with lesions in the raphe nuclei, gigantocellular reticular nucleus and coeruleus-subcoeruleus complex.
• **Stage 3** reaching the midbrain and the pars compacta of the substantia nigra.

• **Stage 4** involving the basal prosencephalon and mesocortex

• **Stage 5 and stage 6** reaching the limbic structures and neocortex.

The first two stages correlate with the pre-motor phase of PD. The classical motor symptoms do not start to emerge until the substantia nigra is affected (stage 3). Stage 1 and stage 2 are widely implicated in the non-motor symptoms of PD because several of the brain stem structures affected are normally involved in autonomic function and sleep homoeostasis such as the serotonergic raphe nuclei, the noradrenergic locus coeruleus and the pedunculopontine nucleus (Chaudhuri et al., 2006).

While the Braak staging hypothesis explains a great proportion of the presentation pattern of both motor and non-motor symptoms in PD it does not explain varieties of Parkinsonism that present with cortical cognitive impairment as seen in dementia of Lewy bodies (Chaudhuri et al., 2006). Furthermore, on its own it does not adequately account for some of the common non-motor symptoms such as pain or autonomic dysfunction affecting bowel, bladder, cardiovascular system and sexual organs.
1.2 Pain perception

In order to better understand pain in PD it is important to review pain mechanisms in general. Pain was defined by Merskey et al in 1986 as an “unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” This is the definition of the International Association for the study of Pain (IASP). This definition acknowledges that pain is not universally caused by tissue injury as is seen in
conditions such as fibromyalgia. It also recognises the emotional dimension as well as the sensory dimension of pain experience (Melzack and Wall, 2008).

Pain is no longer thought of as a uniform experience. For several years it has been recognised that pain has multiple facets. Beyond the basic sensory discriminative aspect, pain experience has motivational-affective and cognitive-evaluative aspects. Ronald Melzack and Kenneth Casey proposed that pain intensity and unpleasantness are not simply determined by the magnitude of painful stimulus but “higher” cognitive activities can influence perceived intensity and unpleasantness (Melzack and Casey, 1968).

The sensory discriminative domain of pain encompasses the intensity, duration, quality and location of nociceptive stimulus. The motivational-affective domain involves the emotional and autonomic responses to pain and the cognitive dimension pertains to the conscious control of the two other dimensions (Chudler and Dong, 1995). It has become evident that there is no single pathway leading to a “pain centre” in the brain. Instead, the pain experience is a result of interaction of multiple systems and pathways influenced by multiple brain nuclei and higher cognitive functions.

Most of the times pain results from activation of specialised sensory nerve receptors known as nociceptors. These generate impulses in response to noxious stimuli, which are transmitted, to higher brain structures and pain is perceived.
Nociceptors can be activated by mechanical, thermal or chemical noxious stimuli. Pain sensation normally results from the activity of nociceptors and not from over-activation of other kinds of receptors (Willis and Westlund, 1997).

There are three types of peripheral nerve fibres based on their diameter:

- A group
  - These are sub-classified (in descending order) into alpha, beta, gamma and delta by their diameter and conduction velocity.
- B group
- C group

Group A have the largest diameters with the highest conduction velocity and group C have the smallest diameter with lowest conduction velocity. Group B falls in-between. The C group are unmyelinated nerve fibres.

Nociceptive fibres that conduct pain are on the whole small in diameter compared to fibres serving other non-noxious sensory or motor functions. Fibres that transmit pain information are either small myelinated (A-delta fibres) or unmyelinated (C fibres) (Table 1-2). About 60% of all sensory afferents are in the C group (Melzack and Wall, 2008).
### Table 1-2 Characteristics of different types of sensory nerve fibres.

<table>
<thead>
<tr>
<th></th>
<th>Myelinated</th>
<th>Thinly myelinated</th>
<th>Unmyelinated</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fibre type</strong></td>
<td>A-β</td>
<td>A-δ</td>
<td>C</td>
</tr>
<tr>
<td><strong>Diameter</strong></td>
<td>5-15 µm</td>
<td>1-5 µm</td>
<td>0.25-1.5 µm</td>
</tr>
<tr>
<td><strong>Velocity</strong></td>
<td>0.25-1.5 µm</td>
<td>6-30 m/sec</td>
<td>1.0-2.5 m/sec</td>
</tr>
<tr>
<td><strong>Responds to</strong></td>
<td>Light pressure</td>
<td>Light pressure</td>
<td>Light pressure</td>
</tr>
<tr>
<td></td>
<td>Heavy pressure</td>
<td>Heavy pressure</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heat (&gt;45°)</td>
<td>Heat (&gt;45°)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemicals</td>
<td>Chemicals</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cooling</td>
<td></td>
<td>Warmth</td>
</tr>
</tbody>
</table>

A different way of classifying sensory fibres is by their degree of sensitivity to noxious stimuli. Accordingly they can be placed into three categories (Chudler and Dong, 1995):

**Low Threshold Mechanoreceptors (LTM):** these only respond to non-noxious stimuli such as light touch and do not respond to stimulus intensities in the noxious range (A-β fibres).

**Wide Dynamic Range (WDR):** they respond to both non-noxious and more intense noxious stimuli.
**Nociceptive-Specific (NS)** also known as high threshold neurons; they only respond to noxious stimuli (A-δ and C fibres).

The smallest diameter thinly myelinated A-δ fibres are responsible for conducting fast, pricking pain whereas the small unmyelinated C fibres conduct dull burning pain that lingers afterward (Willis and Westlund, 1997). Both fibres transmit impulses to the dorsal horn of the grey matter in the spinal cord.

The grey matter of the spinal cord is divided on the basis of its cytoarchitecture into ten zones, known as Rexed’s laminae (Figure 1-2), which are numbered sequentially from dorsal to ventral sections. The tip of the dorsal horn corresponding to lamina I-III is also known as the substantia gelatinosa (Crossman and Neary, 2010).
Rexed laminae of the grey matter of the spinal cord. Laminae I, II and III where afferent sensory neurons terminate are also termed the substantia gelatinosa. Many opioid receptors, both pre- and post-synaptic are found on these nerve cells. These can be targeted for pain relief.

The fibres responsible for nociception, mentioned above, terminate at the substantia gelatinosa. These neurones are excitatory and use glutamic acid and the peptide substance P as neurotransmitters.

In the substantia gelatinosa, complex interactions occur with other types of afferent terminals, inter-neurons, and with descending pathways from the brain, which control the transmission of pain information to ascending pathways.

Afferent nociceptive C fibres synapse with second order neurons that form ascending spinothalamic pathways. These second order neurons are of the wide dynamic range variety (WDR) i.e. they receive direct synaptic input from
nociceptive terminals and also multisynaptic input from myelinated A-fibres (Non-noxious information) (Baron et al., 2010).

The non-nociceptive A fibres neurons project to deeper layers of the dorsal horn and through short inter-neurons indirectly project to the ascending WDR neurons. These inter-neurons use gamma-aminobutyric acid GABA as their neurotransmitter and exhibit inhibitory effects on the ascending pathway. Therefore activating afferent non-nociceptive neurons indirectly (through inhibitory inter-neurons) suppresses the transmission of pain information and has an analgesic effect. This explains why activating A fibres by rubbing a painful area can have mild analgesic effect (Melzack and Wall, 2008).

Furthermore, there are descending inhibitory pathways that synapse with the WDR neurons in the substantia gelatinosa. These descending pathways from higher centres in the brain stem exert pain inhibitory effect by controlling the transmission of pain information at the level of the substantia gelatinosa (Millan, 2002). Diffuse Noxious Inhibitory Control (DNIC) where pain perception in one body location is inhibited by painful stimuli applied to another remote location is thought to involve supraspinal inhibitory mechanisms (De Broucker et al., 1990).

All these interactions are supported by microglia that are present in the substantia gelatinosa and serve to facilitate neurotransmission.
The complex interactions between nerve terminals, spinal inter-neurons and descending pathways, that together regulate the transmission of nociceptive information to ascending spinothalamic and spinoreticular tracts, are explained by what is referred to as the gate control theory of pain (Melzack and Wall, 2008).

Beyond the initial afferent fibres and their synapses in the substantia gelatinosa, there are two neuroanatomical pathways that mediate pain transmission and perception (Figure 1-3). The antero-lateral pain pathway represented by the spinothalamic tract, which projects directly to the thalamus (Ventral Posterior Nucleus) and primary sensory cortex, sub-serving the discriminatory elements of pain (Ford, 2010). This is a fast conducting system that is thought to be the route by which sharp, pricking pain is conducted. It is highly organised somatotopically; consequently, the origin of sensory stimuli can be accurately localised (Crossman and Neary, 2010).

The medial spinoreticulothalamic pathway represents an additional, phylogenetically older route by which nociceptive sensory impulses ascend to higher centres (Crossman and Neary, 2010). It is a system of slow conducting fibres that terminate in the brain stem reticular formation, particularly in the medulla, with synapses in the parabrachial region, periaqueductal grey, hypothalamus, intralaminar and medial thalamic nuclei, as well as the insula, parietal operculum, anterior cingulate cortex, amygdala and hippocampus. This
pain pathway has intimate association with the autonomic nervous system and appears to sub-serve the autonomic, affective and cognitive dimensions of pain (Ford, 2010; Scherder et al., 2005).

Figure 1-3 The medial and lateral pain system. *Picture adapted from* (Bonaz, 2003)
The spinoreticulothalamic system is poorly organised somatotopically and is thought to be the route via which dull, aching pain is transmitted to a conscious level. Activation of both spinothalamic and spinoreticulothalamic fibres can be modulated by descending pathways from the brain.

PET imaging and functional MRI have demonstrated that multiple areas in the brain are involved in pain processing including the somatosensory cortex, insula, anterior cingulate cortex, prefrontal cortex and thalamus. Other regions such as the basal ganglia, cerebellum, amygdala, hippocampus have also been implicated highlighting a complex pain processing network in the brain (Tracey, 2008).

Pain is most commonly associated with tissue injury, or the threat of such injury, activating nociceptors and serving a protective function. However, this is not universally the case. Pain that is not caused by tissue injury but rather caused by a dysfunction or injury in the nervous system is known as neuropathic pain or primary pain. It is considered to be a separate pain category from the pain caused by the activation of nociceptors. Merskey and Bogduk defined it in 1994 as “pain due to a disturbance of function or pathological change in a nerve” (Merskey and Bogduk, 1994).

The international association for the study of pain (IASP) defined neuropathic pain as “pain initiated or caused by a primary lesion or dysfunction of the nervous system”. This was later re-defined by the Neuropathic Pain Special
Interest Group (NeuPSIG) of the IASP to “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system” (Treede et al., 2008).

Pain can persist after healing of the initial tissue injury and can be chronic and disabling. It can be triggered by innocuous stimulus such as touching the skin (Baron et al., 2010); this is thought to be caused by maladaptive plasticity which alters nociceptive signal processing so that pain is felt with minimal or no nociceptive stimulus (Costigan et al., 2009). When pain occurs due to a non-painful stimulus it is termed allodynia but when there is exaggerated pain perception to a stimulus that usually provokes pain the term hyperalgesia is used (Jensen and Finnerup, 2014). Conditions such as causalgia and phantom limb pain are example of such states (Melzack and Wall, 2008).

Furthermore, sometimes pain is encountered as a primary symptom with no evident injury or noxious stimulus to either connective tissue or neural tissue. Conditions such as fibromyalgia, low back pain, irritable bowel syndrome and migraine are examples. This is thought to results form autonomous amplification of nociceptive signals in the central nervous system (central sensitisation) (Staud and Rodriguez, 2006); such pain is termed dysfunctional pain syndrome (Costigan et al., 2009). This pain shares many of the features of neuropathic pain and it is also considered a maladaptive state (Costigan et al., 2009).
1.3 Pain in Parkinson’s disease

Pain is an important and common non-motor symptom in PD. Several descriptive and case-control studies have shown an increased frequency of pain in PD (Beiske et al., 2009; Brefel-Courbon et al., 2009; Defazio et al., 2008; Goetz et al., 1986; Lee et al., 2006; Negre-Pages et al., 2008).

Estimates of the prevalence of pain in PD vary between 40% (Negre-Pages et al., 2008) and 70% (Beiske et al., 2009). This variation is attributed to the differences in the methods used to measure pain as well as the lack of consensus on the definition, causes and classification of pain associated with PD.

While for many PD patients pain can be a minor inconvenience, in up to 10% of patients it is the most troubling symptom (Politis et al., 2010). Pain can also be the presenting feature of Parkinson’s disease in 15% of patients (O'Sullivan et al., 2008).

The exact mechanism of pain in Parkinson’s disease is not well understood. Traditionally, pain has been attributed to motor disability and peripheral factors specific to PD such as rigidity, dystonia, restless legs and postural abnormalities (Goetz et al., 1986; Tinazzi et al., 2006). However, there is evidence to suggest that pain maybe a result of abnormal central processing of nociceptive inputs in PD (Tinazzi et al., 2008). This is possible since the basal ganglia have an important role in pain perception (Chudler and Dong, 1995). Furthermore,
several studies have shown lower pain thresholds in PD patients compared to control subjects (Djaldetti et al., 2004; Schestatsky et al., 2007; Tinazzi et al., 2008). This could mean that PD patients have higher sensitivity to peripheral factors that would not cause the same degree of pain in non-parkinsonian individuals. In addition, around 10% of PD patients experience pain that cannot be explained by any peripheral factors (Beiske et al., 2009; Hanagasi et al., 2011). This is known as primary PD pain. It is predominantly felt on the affected side and has a burning, bizarre quality with unusual locations such as the face, pharynx, abdomen, pelvis, rectum and genitalia (Ford et al., 1996; Ford, 2010; Quinn et al., 1986; Snider et al., 1976). The cause of this type of pain is poorly understood; it is thought to result from dysfunction of the central processing of peripheral sensory input (Schestatsky et al., 2007).

1.3.1 The basal ganglia and pain

1.3.1.1 Basic anatomy of the basal ganglia

The basal ganglia are a group of grey matter nuclei situated deep in the cerebral hemispheres playing essential roles in orchestrating movements and posture. They consist of the caudate nucleus, the putamen and the globus pallidus (pallidum) (Figure 1-4).
Figure 1-4 A broad outline of the basal ganglia. The caudate nucleus and the putamen are best considered as a one functional unit. They are together termed the striatum and form the “input” region of the basal ganglia, receiving input from the whole cortical mantle. Picture adapted from (Calder et al., 2001).

The pallidum is subdivided into the external and internal pallidum. Although the substantia nigra is situated in the midbrain it has close functional and anatomical connections with the basal ganglia. The amygdala, which is located in the temporal lobe, is related to the basal ganglia from an embryological point of view but is functionally part of the limbic system.

Anatomically, the putamen and the pallidum are close to each other and together they are termed the lentiform nucleus. However, functionally the caudate nucleus and the putamen are best considered as a one unit. They are together called the
striatum forming the “input” region of the basal ganglia and receiving input from the whole cortical mantle.

At its rostral extremity the head of the caudate nucleus is continuous with the putamen through and beneath the anterior limb of the internal capsule. At this anterior level, the most ventral portion of the striatum is known as the nucleus accumbens NAcc (not illustrated), which has connections with the limbic system and is thought to have an important role in reward and pleasure experience (Costa et al., 2010; Crossman and Neary, 2010). The striatum and pallidum are the two functional units of the basal ganglia. They have important connections with the rest of the brain, in particular with the cerebral cortex, the thalamus, the subthalamic nucleus and the substantia nigra of the midbrain.

1.3.1.2 The role of the basal ganglia in pain

While the role of the basal ganglia in movement and posture has been well studied their role in pain and sensory processing is not clearly defined. Multiple animal studies have provided evidence of basal ganglia structures involvement in pain processing (Bernard et al., 1992; Chudler and Dong, 1995; Chudler and Lu, 2008; Greco et al., 2008; Saade et al., 1997). Dopamine is also involved in modulating pain perception and may contribute to descending pain inhibitory pathways (Wood, 2008).
The role of the basal ganglia in pain processing and modulation has been further studied using functional magnetic resonance imaging fMRI. These have provided evidence of pain-induced activation of almost all basal ganglia structures as well as cortical areas normally involved in pain (Borsook et al., 2010).

The nucleus accumbens, caudate nucleus, and putamen demonstrated pain-induced increase in activity on fMRI supporting their role in pain processing and modulation (Aharon et al., 2006; Becerra et al., 2001; Bingel et al., 2002; Davis et al., 2002; Freund et al., 2009). The contralateral pallidum and putamen have also been shown to display increased blood flow on painful thermal stimulation of the hand (Jones et al., 1991). Furthermore, hyper-perfusion of the basal ganglia was reported in migraine and cluster headache suggesting involvement with pain processing (Kawamura et al., 1991; Kobari et al., 1989).

Positron emission tomography (PET) imaging has demonstrated decreased putaminal 6-[18F] Fluorodopa (F-DOPA) uptake in patients with burning mouth syndrome suggesting a decreased dopaminergic activity which is thought to mediate the painful state by negating the pain inhibitory role of dopaminergic pathways (Jaaskelainen et al., 2001).

1.3.1.3 Hyperalgesia evidence from PD Patients

Several studies have described abnormal processing of nociceptive inputs in PD with decreased pain thresholds compared to controls (Defazio et al., 2013b).
Patients with unilateral PD with or without pain were experimentally found to have a lower threshold for heat pain when compared with matched controls. This reduced threshold was more pronounced when PD patients complained of pain beforehand and when the affected side was tested. The lower pain threshold remained present with “ON” and “OFF” conditions equally (Djaldetti et al., 2004).

Similarly, a study performed in pain-free PD patients found that cold pain threshold was lower in the “OFF” period compared to controls. This was, however, corrected by administration of Levodopa. No significant difference was observed between patients who were “ON” and normal controls, suggesting a role for dopamine in cold nociception (Brefel-Courbon et al., 2005). Furthermore, using PET imaging, the same study documented higher pain-induced cerebral blood flow in brain structures normally associated with pain such as the right insula, right prefrontal and left anterior cingulate cortices during “OFF” condition. This activation was reduced with the administration of Levodopa (Figure 1-5).
The difference in pain-induced regional cerebral blood between PD patients (top) and controls (bottom). The increased activation was attenuated with the administration of Levodopa. Picture adapted from (Brefel-Courbon et al., 2005)

The same group also reported that Levodopa increased pain threshold in pain-free PD patients by showing an increase in the nociceptive flexion reflex (RIII) threshold after the administration of Levodopa to PD patients in the “OFF” condition. This effect of Levodopa was not noted in healthy subject or PD patients who were in the “ON” condition (Gerdelat-Mas et al., 2007).

However, another study comparing the effect of apomorphine vs placebo on heat induced pain threshold in 25 PD patients found no effect on pain threshold or cerebral activity during noxious stimulations (Dellapina et al., 2011). The authors
suggested that other monoamines outside the dopaminergic system might be involved in pain processing in PD.

Abnormal laser-evoked neurophysiological potentials in PD patients were recorded by Tinazzi et al (Tinazzi et al., 2008). They demonstrated decreased amplitude of laser-evoked potentials recorded at the vertex in PD patients compared to controls. The meaning of this remains speculative but it demonstrates an altered processing of nociceptive impulses as several brain structures are involved in producing the N2/P2 potentials, which were recorded in the study (Tinazzi et al., 2008). The previously demonstrated pain threshold reduction in PD patients (Brefel-Courbon et al., 2005; Djaldetti et al., 2004) was also reproduced by this study.

In another similar study looking at central pain processing in PD patients Schestatsky et al found that PD patients who complained of primary central pain had lower threshold for heat and laser pinprick pain. However, in contrast to Tinazzi et al they found that Laser evoked potentials were increased in amplitude in PD patients with pain. They also demonstrated impaired autonomic response by showing reduced laser-induced sudomotor skin response to pain. These effects were more prominent on the affected side and they responded partially to Levodopa (Schestatsky et al., 2007).
1.3.2 Potential mechanisms of increased pain sensitivity in PD

Braak and colleagues have reported, in early pre-motor PD, evidence of neuropathological changes in layer I of the dorsal horn in the spinal cord as well as involvement of the parasympathetic and sympathetic pre- and postganglionic neurons. These two systems (i.e the pain system and the autonomic system) are closely interconnected and this may partly explain the pre-motor autonomic and pain symptoms in some PD patients (Braak et al., 2007).

Furthermore, the noradrenergic locus coeruleus of the pons projects nociceptive information to the thalamus (Voisin et al., 2005) as such the involvement of locus coeruleus in PD can play a role in altering pain processing.

Nociceptive inputs are modulated by higher centres, which can exert both inhibitory and facilitatory influence at a spinal level. Monoamines such as noradrenaline, serotonin and dopamine are the principal neurotransmitters involved in this action. Pain modulation is governed by the type of nociceptive input but also by other factors such as the attention, motivation and emotional state of the individual (Benarroch, 2008). It is possible that these endogenous pain control mechanisms are affected in PD due to degenerative processes involving brain stem structures (such as the raphe nucleus, periaqueductal grey and gigantocellular reticular nucleus) that exert such pain modulatory effects (Gebhart, 2004; Millan, 2002).
DNIC have been reported to be impaired in some chronic pain conditions such as osteoarthritis and fibromyalgia (Arendt-Nielsen et al., 2010; Lautenbacher and Rollman, 1997) however one study did not find this to be the case in PD (Mylius et al., 2009) therefore DNIC may not be an important cause of pain in PD.

Finally, small fibre neuropathy (both sensory and autonomic) has been reported in PD and is linked with autonomic dysfunction (Nolano et al., 2008; Wang et al., 2013). Sensory small fibre neuropathy is associated with pain in conditions such as diabetes (Lauria et al., 2012) although the exact mechanisms are not well understood. Small fibre neuropathy if commonly present in PD could represent another contributory factor to increased pain in this condition. Based on known clinical patterns, pain due to small fibre neuropathy would be expected to affect mainly the lower limbs and be associated with autonomic symptoms.

Table 1-3 summarises potential mechanisms of how pain arise in Parkinson’s disease.
<table>
<thead>
<tr>
<th>Pain mechanism</th>
<th>Potential associated features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral musculoskeletal factors</td>
<td>Pain improves with dopamine therapy and is worse in the “OFF” state.</td>
</tr>
<tr>
<td>(Postural instability, muscle rigidity and dystonia)</td>
<td></td>
</tr>
<tr>
<td>Degeneration of small fibre neurons</td>
<td>Prominent autonomic symptoms.</td>
</tr>
<tr>
<td>(both central and peripheral)</td>
<td>Lower limb pain with neuropathic features</td>
</tr>
<tr>
<td>Dopamine depletion</td>
<td>Pain improves with dopamine.</td>
</tr>
<tr>
<td></td>
<td>Decreased pain threshold (due to the analgesic effect of brain dopaminergic pathways)</td>
</tr>
<tr>
<td>Serotonine depletion</td>
<td>Anxiety and Depression.</td>
</tr>
<tr>
<td></td>
<td>Decreased pain threshold (due to loss of descending inhibitory inputs on the dorsal horn).</td>
</tr>
<tr>
<td>Noradrenaline depletion</td>
<td>Anxiety and Depression.</td>
</tr>
<tr>
<td></td>
<td>Decreased pain threshold (due to loss of descending inhibitory inputs on the dorsal horn).</td>
</tr>
</tbody>
</table>
1.3.3 Dopaminergic pathways and pain

There are four major dopaminergic pathways projecting from key dopaminergic areas in the brain, namely the ventral tegmental area (VTA), substantia nigra pars compacta and hypothalamus (Figure 1-6):

1- Mesocortical pathway: from the ventral tegmental area (VTA) to the cerebral cortex and in particular the frontal cortex. This pathway is associated with motivation and emotion and is thought to be dysfunctional in psychotic states.

2- Mesolimbic pathway: also known as the mesolimbic reward pathway connects the VTA to the limbic system via the nucleus accumbens (NAcc). It is believed to mediate reward, pleasure, addiction and motivation.

3- Nigrostriatal pathway: from the substantia nigra to the striatum. This is responsible for modulation of basal ganglia input and control of movements.

4- Tuberoinfundibular pathway: from the arcuate nucleus of the hypothalamus to the median eminence at the inferior boundary of the hypothalamus. It regulates the secretion of prolactin in the anterior pituitary.
From a PD perspective, it is thought that these pathways mediate several non-motor symptoms such as cognition, sleep and pain. Therefore dysfunctions of these pathways can explain some of the non-motor symptoms and their response to dopaminergic treatments (Chaudhuri and Schapira, 2009).

The mesolimbic dopamine pathway from the (VTA) to (NAcc) has pain-suppressing properties as activation of the neurons in this pathway by substance P or natural opioids (which can be triggered by stress or noxious stimuli) results
in suppression of tonic pain of similar magnitude to high dose morphine (Altier and Stewart, 1999).

Dopamine release from terminals in the NAcc is important for this antinociceptive property as the administration of dopamine receptors antagonists blocked this analgesic effect (Altier and Stewart, 1999; Gear et al., 1999). Therefore, neuronal degeneration in the VTA in association with PD may compromise the efficacy of pain suppression properties of this pathway (Lim et al., 2008).

1.3.4 Neuropathy and PD
PD has traditionally been considered as a neurodegenerative disease of the brain and the basal ganglia with no involvement of the peripheral nervous system. In recent years, however, evidence of peripheral neuropathy associated with PD has been emerging (Mancini et al., 2013; Nolano et al., 2008). It seems that both small and large fibre neuropathies are observed more commonly in PD compared to healthy controls (Nolano et al., 2008; Rajabally and Martey, 2011).

The cause of this neuropathy is not completely understood but there are reports implicating a toxic effect of prolonged exposure to Levodopa causing increased levels of homocysteine and methylmalonic acid (Ceravolo et al., 2013; Toth et al., 2008; Toth et al., 2010).
An association between prolonged exposure to levodopa and large fibre neuropathy has been reported in several studies (Ceravolo et al., 2013; Rajabally and Martey, 2011; Toth et al., 2008). However, such connection could not be established in relation to small fibre neuropathy (Dabby et al., 2006; Doppler et al., 2014; Nolano et al., 2011). Therefore it is possible that neuropathy, particularly small fibre neuropathy, is a feature of PD itself. Nolano et al have demonstrated the presence of significant loss of epidermal nerve fibres and Meissner corpuscles in skin biopsies taken from PD patients (Nolano et al., 2008). Interestingly small fibre neuropathy was more pronounced on the affected side and it did not correlate with disease duration, age of the patient or pharmacological treatment. The skin biopsies also showed evidence of attempted nerve regeneration indicating a chronic process. Furthermore, 4 out of 18 patients in Nolano’s cohort were levodopa naïve and yet showed loss of cutaneous fibres as well as quantitative sensory testing abnormalities. Therefore it is possible that small fibre neuropathy is part of the spectrum of PD pathology. More recently, alpha-synuclein depositions were found in cutaneous autonomic nerves of PD patients introducing the prospect for using skin biopsies as a potential biomarker in PD (Donadio et al., 2014; Wang et al., 2013).

Small fibre neuropathy is associated with lower limb pain and autonomic dysfunction in conditions such as diabetes where this type of neuropathy is common (Latov, 2007; Lauria et al., 2012). There are multiple proposed mechanisms for this peripheral sensitisation including dysregulation of sodium
and potassium voltage gated channels as well as increased cytokines around injured and intact neurons leading to primary afferent hyper-excitability (Kim et al., 2002; Schafers et al., 2008; Wood et al., 2004). Therefore, if commonly present in PD, small fibre neuropathy represents an important potential cause for pain in PD. This type of pain would be expected to have neuropathic features, affect the lower limbs, and be associated with other autonomic symptoms.

1.3.4.1 C tactile afferents

Unmyelinated C fibres have traditionally been associated with conducting pain and temperature and for decades they were thought to respond exclusively to noxious stimuli. However, recent studies have discovered a class of low threshold mechanosensitive C-fibres in hairy skin known as C-tactile (CT) afferents (Olausson et al., 2010; Vallbo et al., 1999). CT afferents fire maximally when a gentle caressing or “stroking” is applied to the skin. Unlike myelinated afferents that respond linearly to stimulus velocity, CT afferents respond optimally to a stroking velocity of 1 – 10 cm/sec, which is also rated as the most pleasant. Stimulation velocities below and above this range (1-10 cm/sec) produce a sub-optimal CT response determined by lower firing rates (Loken et al., 2009; Morrison et al., 2010). Furthermore, fMRI studies have demonstrated that stimulation of CT fibres activates areas in the brain that are associated with pleasure, including the insular cortex, as opposed to stimulation of myelinated fibres which activates the somatosensory areas of the cortex (Olausson et al., 2002; Rolls et al., 2003). This emphasises the specialised role of CT afferents in
mediating the emotional component of the tactile experience that is known as the affective touch.

Recent studies reported a relationship between low threshold CT afferents and pain pathways with CT afferents playing a pain-inhibiting role (Liljencrantz et al., 2013; Liljencrantz and Olausson, 2014). Animal studies have indicated that CT afferents stimulation may inhibit C nociceptive inputs in the dorsal horn (Lu and Perl, 2003). Liljencrantz and colleagues used the heat capsaicin model of dynamic tactile allodynia applied to healthy subjects and those who lacked A beta afferents. The patients who lacked A beta afferents and only had CT fibres reported weaker sensation in the allodynic zone (Liljencrantz et al., 2013). Indeed emerging evidence suggests a complex relationship between pain and reward with common neural substrate underlying both states (Leknes and Tracey, 2008; Leknes and Bastian, 2014).

It is not known whether the perception of affective touch mediated by CT afferents is impaired in PD and if so whether a relationship exists between CT afferents denervation and pain.

1.3.4.2 Diagnosis of neuropathy

Large fibre neuropathy can be definitively diagnosed with conventional neurophysiological tests. However nerve conduction studies cannot detect small fibre damage (Hovagimian and Gibbons, 2011). In order to diagnose small fibre
neuropathy quantitative sensory testing, which measures thermal thresholds are normally used despite their limitations (Lauria et al., 2012). For definitive diagnosis a skin biopsy is needed (Devigili et al., 2008).

Skin biopsies are invasive and require considerable resources making them impractical for regular use. The cornea is richly supplied by small nerve fibres making it an ideal target to study peripheral nervous system pathology (Muller et al., 2003). Corneal confocal microscopy (CCM) has been developed over the last decade as a rapid, non-invasive technique that offers \textit{in-vivo} visualisation of corneal nerves and has been shown to be a valid surrogate measure of peripheral nerve damage and regeneration in diabetes (Quattrini et al., 2007; Tavakoli et al., 2013) as well as other conditions characterised by neuropathy such as Fabry’s disease, idiopathic small fibre neuropathy and Charcot-Marie-Tooth (Tavakoli et al., 2009; Tavakoli et al., 2010; Tavakoli et al., 2012).

1.3.5 Classification of pain in PD

Due to the multiple aetiology of pain in PD there have been attempts to classify pain in PD according to putative causes. Snider et al in 1976 recognised the presence of primary sensory symptoms, pain being the most prominent, and they distinguished between primary pain that is a manifestation of PD itself and secondary pain that is a result of the motor complications of the disease (Snider et al., 1976).
Nègre-Pagès and colleagues in their cross-sectional (DoPaMiP) survey of pain in Parkinson’s disease classified pain into pain unrelated to PD and pain related to PD and sub-classified the latter into pain directly related to PD (cannot be explained by any other condition) and pain indirectly related to PD (caused by another condition but made worse by PD) (Negre-Pages et al., 2008).

The most commonly used classification of pain in PD is the one proposed by Blair Ford. He classified pain according to its aetiology into the following categories (Ford, 2010):

1. Musculoskeletal Pain
2. Dystonic Pain
3. Radicular-Neuropathic Pain
4. Central-Neuropathic Pain
5. Akathisia-Related Pain

More recently, Chaudhuri et al proposed a modified classification of pain in PD, which overlaps with the previous classifications but includes more subcategories of specific potential causes (Figure 1-7) (Chaudhuri and Schapira, 2009). It is worthy of note that all these classifications depend on the clinician’s judgement as well as the patient’s description of their pain and their perception of its possible causes and thus may be open to misinterpretation.
A proposed classification of pain in Parkinson’s disease by Chaudhuri and Schapira:

- Musculoskeletal pain
- Parkinson’s disease-related chronic pain (might respond to dopaminergic therapy)
  - Central pain
  - Visceral pain
- Fluctuation-related pain (dopaminergic therapy responsive)
  - Dyskinetic pain
  - “Off” period dystonia-related pain
  - “Off” period generalised pain
- Nocturnal pain (usually dopaminergic therapy responsive)
  - Pain related to restless legs syndrome or periodic limb movement
  - Nocturnal akinesia-linked pain
- Coat-hanger pain (pain around the shoulder area; rare in Parkinson’s disease and linked to postural hypotension)
- Oro-facial pain
  - Temporo-mandibular joint pain
  - Bruxism-related pain
  - Burning mouth syndrome (might be levodopa responsive)
- Peripheral limb or abdominal pain
  - Drug induced
    - Peripheral oedema-linked pain
    - Lower bowel pain associated with retroperitoneal fibrosis

Figure 1.7 Proposed classification of pain in Parkinson’s disease by Chaudhuri and Schapira.
1.3.6 Causes of pain in PD

Pain in PD is most probably multifactorial with several potential mechanisms. There are some predictors that have been reported to be associated with increased risk of pain in PD. Pain is more frequently encountered in patients who have younger disease onset, longer dopamine therapy and more motor fluctuations (Negre-Pages et al., 2008; Tinazzi et al., 2006). The most common type of pain reported has been musculoskeletal, followed by dystonic and neuropathic pain (Beiske et al., 2009; Hanagasi et al., 2011). Lim et al suggested that dyskinesia and pain in PD share a common pathophysiology given that dyskinetic patients are more likely to experience pain. Furthermore, patients experiencing dyskinesia and pain benefited more from Levodopa compared to other stable responders (Lim et al., 2008). However, not all studies reported a correlation between motor complications, disease duration and pain. Hanagasi et al found that pain did not correlate with duration of disease, disease stage or duration of dopaminergic treatment (Hanagasi et al., 2011). Likewise, Beiske et al did not find a correlation between pain and disease duration, age of onset or severity of the disease. Female sex has been reported as a predictor for pain in PD (Beiske et al., 2009; Zambito Marsala et al., 2011). The conflicting results of these studies highlight the need for further descriptive studies with large cohorts to identify pain predictors.

Pain unrelated to motor complications which is less common (primary central pain) can be bizarre in its quality and location involving the mouth, rectum,
vagina, chest, abdomen and testes. The description of this type of pain supports neuropathic mechanisms and central sensitisation (Clifford et al., 1998; Ford et al., 1996).

An alternative method of studying pain in PD is to classify it into nociceptive and neuropathic. Nociceptive causes of pain in PD include osteoarthritis, frozen shoulder and dystonic cramps. These are all common factors in PD therefore it is reasonable to expect that nociceptive mechanisms will be common. However, previous studies have shown that patients with PD have a lower threshold for pain as well as abnormal central processing of sensory inputs (Brefel-Courbon et al., 2005; Djaldetti et al., 2004; Hara et al., 2013; Mylius et al., 2011; Tinazzi et al., 2008). This indicates that neuropathic mechanisms and central sensitisation are expected to play an important role in PD pain. We do not currently know how much central sensitisation and neuropathic mechanisms are contributing to pain in PD. Common causes of neuropathic pain include small fibre neuropathy (such as in diabetes), spinal cord lesions, thalamic lesions and primary pain syndrome such as fibromyalgia.

There are several potential mechanisms by which central sensitisation can occur in PD:

*Central dopaminergic depletion:* Dopaminergic pathways, in particular, the mesolimbic pathway (from the ventral tegmental area to the limbic system via
the nucleus accumbens) has a role in pain processing in addition to its role in mediating pleasure experience (Altier and Stewart, 1999; Hagelberg et al., 2004; Villemure et al., 2012) so it is possible that dysfunction of this pathway is contributing to pain in PD.

Degeneration of other monoamine nuclei in the brain stem: PD affects not only the dopaminergic neurons but also other monoaminergic neurons. The serotonergic nucleus raphe and the noradrenergic locus coeruleus are involved early on in the course of the disease. These have been implicated in several non-motor symptoms associated with PD (Chaudhuri et al., 2006). In addition, these two nuclear complexes have a pain modulating effect on the dorsal horn of the spinal cord (Benarroch, 2008). Therefore, degeneration of these pathways might explain the decreased pain threshold in PD. Furthermore, given the key role of these monoaminergic pathways in the control of mood, one would expect a link between anxiety and depression and pain caused by degeneration of these neurons (Radat et al., 2013).

Small fibre neuropathy: Peripheral neuropathy is emerging as a new, previously unrecognised, feature of PD. Both small and large fibre neuropathy is observed more commonly in PD compared to age matched controls (Nolano et al., 2008; Rajabally and Martey, 2011). Small fibre neuropathy is known to cause lower limb pain and autonomic dysfunction in conditions such as diabetes (Lauria et al., 2012). Therefore, if commonly present, small fibre neuropathy represents an
important potential cause for neuropathic pain in PD. This type of pain would be expected to have strong neuropathic features, affect the lower limbs, and be associated with other autonomic symptoms.

1.3.7 Pain assessment in PD

Understanding the causes of pain in PD is essential for providing effective treatment. Defining the type of pain is governed by the clinician’s assessment as well as the patient’s description. This has created a need for reliable methods to categorise and diagnose pain in PD.

The visual analogue scale is used to assess the intensity of pain by asking the patient to place a mark on a 10cm straight line that represent the spectrum between no pain at one end and the worst pain ever at the other end. After that a measurement with a ruler is made to obtain a numerical intensity of the pain. This method is effective in assessing the intensity of pain but it leaves out other variables in the pain experience therefore on its own it is not adequate.

The short form of McGill pain questionnaire (SFMPQ) is widely used. This has 15 pain adjectives eleven of which cover the sensory aspect of pain and the other four address the affective aspect. The patient is asked to rank their pain according to one of four categories for each word (0-None, 1-Mild, 2-Moderate, 3-Severe). A score is derived for sensory, affective and overall pain intensity (Melzack, 1987) (Appendix A).
Several tools and scales have been developed to diagnose neuropathic pain with high sensitivity and specificity. This is based on the characteristic qualities of pain and examination of the painful area. Neuropathic pain is commonly associated with abnormal sensations of crawling, burning and shooting qualities (dysaesthesia) that can be spontaneous or stimulus-induced. Allodynia (pain in response to non-nociceptive stimuli) and hyperalgesia (increased pain sensitivity to nociceptive stimuli) are other characteristics of neuropathic pain (Baron et al., 2010). When looked for specifically, these characteristics and signs gives high accuracy in distinguishing pain with probable neuropathic mechanisms from pain secondary to nociceptive mechanisms. Although these scales should not substitute clinical judgment they are useful tools for the non-specialist and for research purposes.

The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) (Appendix B) is widely used and has a high sensitivity and specificity. It contains five questions about pain and two brief examinations to determine the presence of allodynia and altered pinprick threshold. It is designed to enable the non-specialist to distinguish neuropathic from nociceptive pain with high sensitivity and specificity (Bennett, 2001). Other comparable screening tools used to identify neuropathic pain include the “Douleur Neuropathique en 4 questions (DN4)” (Bouhassira et al., 2005) and pain DETECT (Freynhagen et
Chaudhuri et al have produced a PD specific pain scale. This is the King’s PD pain Scale (KPPS) which has been recently validated (Chaudhuri et al., 2015). The KPPS consists of 14 items covering all aspects of potential causes of pain in PD. The KPPS has the advantage of grade rating the various types of pain in PD as well as providing an overall pain score.

1.3.8 Pain in atypical parkinsonism

Atypical parkinsonian disorders are a group of conditions, which present with an akinetic rigid syndrome that can be difficult to distinguish from Parkinson’s disease especially in the early stages (Litvan et al., 2003). Atypical parkinsonian disorders include: Multiple System Atrophy, Progressive Supranuclear Palsy, Dementia with Lewy Bodies and Corticobasal Degeneration. These conditions are more aggressive than PD causing significant disability in a relatively short duration. Furthermore, the motor syndrome is poorly responsive to dopaminergic treatment (Arena et al., 2016; Wenning et al., 2004), which makes symptomatic management of non-motor symptoms an important goal to improve the quality of life in these devastating conditions.
Non-motor symptoms such as gastrointestinal symptoms, pain, urinary problems and postural instability are common in secondary and atypical parkinsonism (Colosimo et al., 2010).

While there are several studies addressing pain in Parkinson’s disease, there are very few reports on the prevalence and characteristics of pain in atypical parkinsonian disorders such as Multiple System Atrophy (MSA) and Progressive Supra-nuclear Palsy (PSP).

In one study the researchers retrospectively looked at histories of pain in the case notes of 100 patients with probable MSA. They reported pain in 47% of patients, the commonest type being musculoskeletal, followed by sensory pain and dystonic pain (Tison et al., 1996). As pain information was gathered retrospectively from case notes rather than prospectively the prevalence is probably an under-estimate of the problem. Furthermore, the classification of pain was solely based on the descriptions of patients and the investigators assessment of case notes.

Pain was also reported in MSA patients assessed for factors influencing health-related quality of life and it was found to be a common symptom ranging from 50% to 76% depending on the type of MSA (MSA-Parkinsonian type having more pain than MSA-Cerebellar type)(Schrag et al., 2006).
Studies looking at pain in PSP are scarce. Stamelou and colleagues have found that PSP patients have lower electrical pain thresholds compared to healthy controls. This was ascribed to possible degeneration of the descending inhibitory inputs from higher pain inhibiting locations such as the periaqueductal gray (Stamelou et al., 2012).

In a multicentre Italian study looking at the prevalence of non-motor symptoms in all parkinsonian disorders pain was found to be very common in MSA. Indeed, pain prevalence was higher in MSA (over 70%) than PD (61%) and PSP (40%) (Colosimo et al., 2010).

In view of the paucity of data on pain in these conditions a comparative study on pain characteristics in atypical parkinsonian disorders using validated pain measures is warranted.
1.4 Research Objectives and Hypotheses

The individual objectives and hypotheses of each study will be given in the relevant chapters but general overarching hypotheses and objectives are given here.

1.4.1 General objectives

1. To determine the prevalence, severity and characteristics of pain in atypical parkinsonian disorders compared to Parkinson’s disease.

2. To determine the prevalence, severity and characteristics of pain in a large cohort of patients with early Parkinson’s disease using validated pain scales.

3. To correlate the information about pain with other data relating to demographics, disease characteristics and identify predictors of pain in PD.

4. To evaluate the role of confocal corneal microscopy in diagnosing small fibre neuropathy in PD compared to skin biopsies.

5. To evaluate the relation between small fibre neuropathy and pain as well as other autonomic symptoms and dysfunctions in PD.

6. To evaluate the perception of affective touch in PD and its potential relationship to small fibre neuropathy and pain.
1.4.2 General hypotheses

1. Pain is common in MSA and PSP as well as PD however due to the different underlying neurodegenerative substrates pain characteristics and origin may be different in atypical Parkinsonism compared to PD.

2. Pain is common in early as well as advanced PD (arguing for a lesser role for motor impairment in pain generation in PD).

3. Neuropathic pain mechanisms play an important role in pain associated with parkinsonian disorders.

4. Confocal corneal microscopy is a valid surrogate technique in diagnosing small fibre neuropathy in PD and it offers a non-invasive alternative to skin biopsy.

5. Small fibre neuropathy contributes to pain in PD especially neuropathic pain.

6. The perception of affective touch is impaired in PD compared to controls and correlates with small fibre neuropathy.

7. There is a relation between affective touch perception and pain in PD with impairment in affective touch potentially influencing pain intensity.
1.5 The Thesis

The body of the thesis will consist of the following studies:

- **Pain in Multiple System Atrophy and Progressive Supranuclear Palsy compared to Parkinson’s Disease:** An abridged version of this study has been published as the following paper: *Brain Behav. 2015 May;5(5). Pain in multiple system atrophy and progressive supranuclear palsy compared to Parkinson's disease. Kass-Iliyya L, Kobylecki C, McDonald KR, Gerhard A, Silverdale MA*. The **first author role:** Identify and consent suitable participants; clinical examination of patients to quantify disease severity; quantify and qualify pain and affective symptoms using validated scales; analyse and interpret data; draft the manuscript.

- **Pain characteristics in a large cohort of patients with early Parkinson’s disease:** This study is conducted nationally and is a work in progress. It is a result of collaboration with Parkinson's Repository of Biosamples and Networked Datasets (ProBaND) study. This study will form basis for future publication. Full details of the methods are provided in the relevant chapter. The **author’s role:** Recruit patients locally at Salford Royal Hospital Site; analyse the initial results.

- **Small Fibre Neuropathy in Parkinson’s Disease: A Clinical, Pathological and Corneal Confocal Microscopy Study:** This study has
been published as the following paper: *Parkinsonism Relat Disord.* 2015 Dec;21(12):1454-60. *Small fiber neuropathy in Parkinson's disease: A clinical, pathological and corneal confocal microscopy study.* Kass-Iliyya L, Javed S, Gosal D, Kobylecki C, Marshall A, Petropoulos IN, Ponirakis G, Tavakoli M, Ferdousi M, Chaudhuri KR, Jeziorska M, Malik RA, Silverdale MA. The first author role: Identify and consent suitable participants; clinical examination of patients to quantify PD severity; quantify pain, autonomic symptoms, non-motor symptoms; clinical examination of patients to identify signs of neuropathy; perform quantitative sensory testing; perform autonomic function testing; perform skin biopsies; refer patients to optometry for corneal confocal microscopy; analyse and interpret data; draft the manuscript.

perform affective touch assessment; analyse and interpret data; draft the manuscript.

A concluding chapter summarising future work is presented afterward.
CHAPTER 2

Pain In Multiple System Atrophy And Progressive Supranuclear Palsy Compared to Parkinson’s Disease
2.1 Abstract

Background: Pain is an important and common non-motor symptom in Parkinson’s disease (PD). The pathophysiology of pain in PD is not well understood. Pain characteristics have rarely been studied in atypical parkinsonian disorders such as Multiple System Atrophy (MSA) and Progressive Supranuclear Palsy (PSP). We therefore aimed to evaluate pain intensity, location, and associated symptoms in atypical parkinsonian disorders compared to PD.

Methods: Twenty-one patients with MSA (14 with MSA-P, 7 with MSA-C), 16 patients with PSP and 65 patients with PD were screened for pain using question 1.9 of the MDS-UPDRS. Pain intensity was quantified using the short form McGill Pain Questionnaire (SFMPQ). Pain locations were documented. Motor disability was measured using part III of the UPDRS. Motor fluctuations were measured with part IV of the UPDRS. Affective symptoms were assessed using the Hospital Anxiety and Depression Scale (HADS). Results: Pain was significantly more common and more severe in MSA and PD compared to PSP \((p < 0.01)\). Pain was significantly more prevalent in MSA-P (100%) compared to MSA-C (43%) \((p < 0.01)\). Pain was significantly more severe in female patients in both PD and MSA. There was a significant correlation between pain intensity and HADS scores but not motor severity. The main predictors of pain intensity in PD were female sex and the presence of motor fluctuations. Conclusions: Pain is significantly more common and more intense in PD and MSA than in PSP. Differences in distribution of neurodegenerative pathologies may underlie these differential pain profiles.
2.2 Introduction

Pain is an important and common non-motor symptom in Parkinson’s disease (PD) (Beiske et al., 2009). Pain in PD has been associated with various factors, including affective disorders, motor fluctuations and female sex (Beiske et al., 2009; Negre-Pages et al., 2008). The underlying mechanism of pain in PD is not fully understood but impaired central processing of nociceptive inputs is thought to be important (Brefel-Courbon et al., 2005; Djaldetti et al., 2004; Schestatsky et al., 2007; Tinazzi et al., 2008). Pain characteristics have been frequently studied in PD, however, there is very little literature addressing pain in atypical parkinsonian disorders. A retrospective study identified that half of patients with MSA reported pain (Tison et al., 1996). Pain in MSA and PSP has also been reported in epidemiological and questionnaire based studies of factors influencing quality of life in parkinsonian disorders (Schrag et al., 2006; Schrag et al., 2010; Winter et al., 2011). In a study of the epidemiology of non-motor symptoms in atypical parkinsonism pain has been documented to be present (Colosimo et al., 2010). When tested experimentally, pain thresholds were reported to be lower in patients with Multiple System Atrophy (MSA) and Progressive Supranuclear Palsy (PSP) compared to healthy controls (Perrotta et al., 2013; Stamelou et al., 2012). A comparative study of pain characteristics in atypical parkinsonian disorders using validated pain scales has never been reported.
Chronic pain can be classified as nociceptive (due to tissue injury), or neuropathic (secondary to a lesion or dysfunction of the somatosensory system) (Baron et al., 2010; Costigan et al., 2009; Treede et al., 2008; Woolf et al., 1998). Pain in PD has been classified into five different categories based on putative causes: musculoskeletal, radicular-neuropathic, central, akathisia-related and dystonic (Ford, 2010). Clinical studies have shown that correct classification of pain based on history alone may be inaccurate (Rasmussen et al., 2004). Thus pain scales such as the Short Form McGill Pain Questionnaire (SFMPQ) and the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) scale are needed to quantify pain intensity and distinguish pain mechanisms (neuropathic or nociceptive).

In this study we aimed to evaluate the prevalence, intensity and location of pain in atypical parkinsonian disorders compared with Parkinson’s disease using validated pain scales. We also sought to examine the relationship between pain and disease severity, demographic features and affective symptoms.

2.3 Methods:

Consecutive patients were recruited from movement disorder clinics at the Greater Manchester Neurosciences Centre. Patients with clinically probable diagnosis of PD, MSA or PSP according to published criteria and with a mini-mental state examination score of 24 and above were included (Gilman et al.,
Patients with MSA were divided according to their clinical features into those with MSA-Parkinsonian type (MSA-P) and those with MSA-Cerebellar type (MSA-C). Medical notes were reviewed and patients with known painful conditions such as neuropathy, radiculopathy, or severe osteoarthritis were not included. Ethical approval was obtained from the Cumbria and Lancashire Research Ethics Committee (Ref. Number 09/H1016/61). All participants gave their informed written consent.

Assessments were carried out in the medication “ON” state. Patients were screened for the presence of pain using question 1.9 of the Unified Parkinson’s Disease Rating Scale (UPDRS), which determines the degree of pain or uncomfortable feelings over the past week (0 = no pain, 1 = slight pain, 2 = mild pain, 3 = moderate pain, 4 = severe pain) (Goetz et al., 2008). Scores of 1 and above were considered positive for the presence of pain.

Pain intensity was quantified using the SFMPQ (Melzack, 1987). The site of pain was determined by asking patients to locate their pain on a body map (Appendix C). The contribution of neuropathic mechanisms was evaluated using the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) scale (Appendix B). The LANSS scale determines the likelihood of neuropathic pain by a series of five questions and a brief sensory examination to detect allodynia and altered pin-prick threshold. A score of ≥ 12 is suggestive of neuropathic pain. The LANSS has a sensitivity and specificity of over 80% in detecting neuropathic
pain (Bennett, 2001) comparable to other neuropathic pain scales such as painDETECT (Freynhagen et al., 2006) and DN4 (Bouhassira et al., 2005).

Age of patients, sex, disease duration, use of analgesia and pain response to dopamnergic therapy were recorded. Motor disability in all patients was assessed using part III of the Unified Parkinson’s Disease Rating Scale (UPDRS-III). Motor complications (dyskinesia, motor fluctuations, off state dystonia) were assessed using part IV of the Unified Parkinson’s Disease Rating Scale (UPDRS-IV). Affective symptoms were quantified using the hospital anxiety and depression scale (HADS) (Zigmond and Snaith, 1983) (Appendix D).

2.3.1 Statistical analysis

SPSS version 20 (IBM) was used to analyse data. Means were compared using student t test or one-way ANOVA with post hoc Bonferroni corrections as appropriate. Categorical data were compared using Chi square test. Pearson’s correlation was used to test correlation between potentially dependent variables. Standard multiple regression and logistic regression analyses were used to evaluate predictors of pain intensity and predictors of neuropathic pain in PD. P value of < 0.05 was considered statistically significant.
2.4 Results

Sixty-five patients with PD, 21 patients with MSA (14 MSA-P, 7 MSA-C) and 16 patients with PSP were enrolled in the study. The demographics of patients in each group are summarised in Table 2-1.

Table 2-1 Demographic and clinical data of patients with Parkinson’s disease, multiple system atrophy and progressive supranuclear palsy.

<table>
<thead>
<tr>
<th></th>
<th>PD, n=65</th>
<th>MSA, n=21</th>
<th>PSP, n=16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex M/F</td>
<td>39/26</td>
<td>9/12</td>
<td>7/9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.9 ± 1.2 *</td>
<td>63.6 ± 1.6 *</td>
<td>73 ± 1.7</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>7.2 ± 0.6 *$</td>
<td>3.2 ± 0.3</td>
<td>3.9 ± 0.6</td>
</tr>
<tr>
<td>UPDRS-III</td>
<td>22.5 ±1.3 *$</td>
<td>37.5 ± 2.9</td>
<td>38.1 ± 2.9</td>
</tr>
<tr>
<td>UPDRS-IV</td>
<td>4.3 ± 0.5</td>
<td>4.5 ± 0.5</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Pain present (%)</td>
<td>58/65 (89%) *</td>
<td>17/21 (81%) *</td>
<td>4/16 (25%)</td>
</tr>
<tr>
<td>SFMPQ pain score</td>
<td>16.4 ± 1.5 *</td>
<td>17.6 ± 3.1 *</td>
<td>3.7 ± 1.7</td>
</tr>
<tr>
<td>Neuropathic pain (LANSS ≥ 12) (%)</td>
<td>19/58 (33%)</td>
<td>3/17 (18%)</td>
<td>0</td>
</tr>
<tr>
<td>Pain improves with dopaminergic therapy (%)</td>
<td>29/58 (50%)</td>
<td>8/17 (47%)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>Regular Analgesia (%)</td>
<td>28/65 (43%)</td>
<td>11/21 (52%)</td>
<td>5/16 (31%)</td>
</tr>
<tr>
<td>Neuropathic pain analgesia (%)</td>
<td>9/65 (15%)</td>
<td>4/21 (19%)</td>
<td>1/16 (6%)</td>
</tr>
<tr>
<td>HADS</td>
<td>13.2 ± 0.8</td>
<td>14.7 ± 1.8</td>
<td>17.3 ± 1.7</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM unless otherwise specified. * $ p < 0.05 vs PSP; $ p < 0.05 vs MSA. UPDRS, Unified Parkinson’s Disease Rating Scale; SFMPQ, Short form McGill Pain Questionnaire; LANSS, Leeds Assessment of Neuropathic Symptoms and Signs; HADS, Hospital Anxiety and Depression Scale.
Pain was significantly more prevalent in PD and MSA (89%, 81% respectively) compared to PSP patients (25%) \((p < 0.01)\) (Table 2-1). Overall pain intensity was significantly greater in MSA and PD compared to PSP \((p < 0.01)\) (Figure 2-1).

![Figure 2-1](image_url)

**Figure 2-1** Comparison of pain intensity between parkinsonian disorders as defined by the short form McGill pain questionnaire. Mean scores and SEM are shown. *\(p < 0.05\) vs PSP. # \(p < 0.05\) vs males in the same diagnostic group.

Pain was significantly more common in MSA-P \((n=14, 100\%)\) compared to MSA-C \((n=3, 43\%)\) \((p < 0.01)\). There was a trend towards overall mean pain intensity scores being higher in MSA-P \((21.7 \pm 3.5)\) than MSA-C \((9.3 \pm 5.1)\) \((p = 0.056)\). Of the patients who reported pain, neuropathic pain as assessed by the LANSS scale was found in 19 PD \((33\%)\) and 3 MSA \((18\%)\) patients (all MSA-P). No patient fulfilled the criteria for neuropathic pain in the PSP group. Pain intensity was significantly higher in female patients compared to male patients in all groups \((p < 0.01)\) (Figure 2-1). Twenty-eight patients with PD \((43\%)\) were on
regular analgesia with nine patients (15%) taking neuropathic pain treatments (Gabapentin, Pregabalin, or Amitriptyline). Eleven patients with MSA (52%) were on regular analgesia and four patients were taking neuropathic pain treatments (19%). Five patients with PSP were using regular analgesia (31%) and one patient was on amitriptyline. There was no significant statistical difference between these proportions (2-1). Fifty per cent of PD patients with pain reported improvement with dopaminergic medications compared to 47% of MSA patients and 25% of PSP patients (Table 2-1). The distribution of pain was similar between groups with lower limb pain being the most common followed by upper limb pain, neck pain and back pain. Neck and shoulder pain (coat-hanger pain) prevalence was comparable between PD and MSA (Figure 2-2).

Figure 2-2 Comparison of pain location between parkinsonian disorders.
Pain intensity scores correlated significantly with total HADS scores in both PD ($r = 0.579, p < 0.01$) and MSA ($r = 0.734, p < 0.01$). The anxiety and depression sub-scores also correlated significantly with pain intensity in PD (Anxiety: $r = 0.528, p < 0.01$, depression: $r = 0.477, p < 0.01$) and MSA (Anxiety: $r = 0.743, p < 0.01$, depression: $r = 0.567, p = 0.007$)

2.4.1 Predictors of pain in PD

Based on previous literature of pain predictors in PD motor characteristics and patients demographics were included in the model. Depression and anxiety scores were excluded, as they are not expected to cause pain despite their strong association with it. Standard multiple regression was used to assess the ability of age, sex, disease duration, motor severity (UPDRS-III) and motor complications (UPDRS-IV) to predict pain intensity in PD. The total variance explained by the model as a whole was 27.7%, $F(5, 59) = 4.52, p = 0.001$. Motor fluctuations and female sex were the main predictors of pain intensity followed by shorter disease duration. There was no relationship to motor severity or age. (Table 2-2)
Table 2-2 Standard regression results of predictors of pain intensity measured by the short form McGill pain questionnaire in PD.

<table>
<thead>
<tr>
<th></th>
<th>b</th>
<th>SE-b</th>
<th>Beta</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>12.6</td>
<td>9.46</td>
<td></td>
<td>0.190</td>
</tr>
<tr>
<td>Age</td>
<td>-0.095</td>
<td>0.14</td>
<td>-0.077</td>
<td>0.512</td>
</tr>
<tr>
<td>Female sex</td>
<td>8.04</td>
<td>2.75</td>
<td>0.34</td>
<td>0.005</td>
</tr>
<tr>
<td>PD duration</td>
<td>-0.81</td>
<td>0.035</td>
<td>-0.38</td>
<td>0.024</td>
</tr>
<tr>
<td>UPDRS III</td>
<td>0.001</td>
<td>0.128</td>
<td>0.001</td>
<td>0.992</td>
</tr>
<tr>
<td>UPDRS IV</td>
<td>1.267</td>
<td>0.468</td>
<td>0.43</td>
<td>0.009</td>
</tr>
</tbody>
</table>

2.4.2 Predictors of neuropathic pain in PD

Direct logistic regression was performed to assess the impact of a number of factors on the likelihood that PD patients would experience neuropathic pain defined as a LANSS score of ≥12. The model contained five independent variables (age, sex, PD duration, UPDRS-III and UPDRS-IV). The full model containing all predictors was statistically significant, \( \chi^2(5, N = 65) = 14.77, p = 0.011 \), indicating that the model was able to distinguish between PD patients who experience and did not experience neuropathic pain. The model as a whole explained between 20.3% (Cox & Snell R square) to 29% (Nagelkerke R square) of the variance in pain status (neuropathic, non-neuropathic), and correctly classified 75.4% of cases. As shown in Table 2-3, only two of the independent variables made a unique statistically significant contribution to the model (female sex and motor complications). The strongest predictor of experiencing neuropathic pain was female sex, recording an odds ratio of 4.21. This indicated that PD patients who had neuropathic pain were over four times more likely to be
females than those who were not controlling for all other factors in the model.

The other significant predictor was motor complications with higher UPDRS IV scores predicting higher likelihood of neuropathic pain.

Table 2-3 Logistic regression predicting likelihood of reporting neuropathic pain.

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>S.E</th>
<th>Wald</th>
<th>p value</th>
<th>Odds Ratio</th>
<th>95% C.I. for Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.48</td>
<td>2.14</td>
<td>0.24</td>
<td>0.172</td>
<td>0.95</td>
<td>0.89 – 1.02</td>
</tr>
<tr>
<td>Female Sex</td>
<td>1.44</td>
<td>0.66</td>
<td>4.78</td>
<td>0.029</td>
<td>4.21</td>
<td>1.16 – 15.3</td>
</tr>
<tr>
<td>PD duration</td>
<td>-0.02</td>
<td>0.009</td>
<td>3.22</td>
<td>0.073</td>
<td>0.98</td>
<td>0.97 – 1.00</td>
</tr>
<tr>
<td>UPDRS-III</td>
<td>0.02</td>
<td>0.031</td>
<td>0.61</td>
<td>0.435</td>
<td>1.02</td>
<td>0.96 – 1.09</td>
</tr>
<tr>
<td>UPDRS-IV</td>
<td>0.25</td>
<td>0.12</td>
<td>4.59</td>
<td>0.032</td>
<td>1.29</td>
<td>1.02 – 1.62</td>
</tr>
</tbody>
</table>

HADS scores were significantly higher in PD patients with neuropathic pain (17.37 ± 1.5) compared to those with nociceptive pain (11.71 ± 0.98), p = 0.002.

The sub-scores of anxiety were significantly higher in PD patients with neuropathic pain (10.8 ± 0.9) compared to those with nociceptive pain (6.7 ± 0.7), p < 0.001. Depression scores were also higher in PD patients with neuropathic pain (6.5 ± 3) compared to those with nociceptive pain (4.9 ± 3.4) but this did not reach statistical significance (p = 0.078).
2.5 Discussion

Our main finding is that pain is significantly more intense and prevalent in PD and MSA compared to PSP. Pain is also more burdensome in MSA-P than MSA-C. Despite the high prevalence of pain in PD and MSA only half of these patients were taking regular analgesia indicating that pain related to parkinsonian syndromes might be under-recognised and under-treated. Different anatomical patterns of neurodegeneration affecting pain pathways as well as different pathological substrates of synucleinopathies versus tauopathies may explain this discrepancy in pain profiles. Neurodegeneration affecting the basal ganglia would be expected to alter pain perception given the involvement of these structures in pain processing (Borsook et al., 2010). The greater involvement of the basal ganglia in MSA-P compared to MSA-C could account for the observed difference in pain prevalence between the two subtypes of MSA. Cognitive factors related to fronto-temporal impairment such as blunting of affect in PSP could potentially reduce pain perception (Carlino et al., 2010). Although patients with frank dementia were excluded from our study more sensitive tests of frontal lobe function were not performed.

Neck and shoulder pain (coat-hanger pain) have been considered to be a feature of MSA (Bleasdale-Barr and Mathias, 1998; Mathias et al., 1999). We found neck and shoulder pain to be similarly prevalent in PD. The results suggest that coat-hanger pain may not necessarily constitute a “red flag” for MSA.
When assessed using the LANSS scale a small proportion of patients’ pain had probable neuropathic mechanisms. This is also supported by the number of patients who were taking regular neuropathic pain treatments (Table 2-1). Although no comprehensive assessments were made to distinguish neuropathic pain from nociceptive pain it is likely that central mechanisms play at least a partial role in the generation of pain in parkinsonian disorders. Furthermore, reduced threshold to experimental pain is reported in all three conditions suggesting involvement of central mechanisms (Brefel-Courbon et al., 2005; Perrotta et al., 2013; Stamelou et al., 2012). The role of central sensitisation in pain generation is also supported by the lack of correlation between motor disability and pain intensity, which argues against pain being a mere function of muscle rigidity and dystonia. In our PD cohort, one of the main predictors of neuropathic pain was the presence of motor fluctuations and dyskinesia. Motor fluctuations results from central sensitisation to external dopamine, which occurs due to plastic changes in the striatum (Nutt, 2007). In our study we confirmed previous reports showing that pain is more common in PD patients who experience motor fluctuations (Tinazzi et al., 2006) however we are the first to demonstrate a relationship between motor complications and neuropathic pain, which further supports a centrally driven pathophysiology for pain in PD.

Over half of our patients with PD and MSA reported improvement of their pain with dopaminergic medications. Thus optimising dopaminergic treatment may be
important in MSA for the management of non-motor symptoms such as pain even when the motor symptoms are poorly responsive to treatment.

Female sex was a predictor for pain intensity and the presence of neuropathic pain in our PD cohort. Also, female patients experienced more intense pain in our MSA group. Increased severity and prevalence of pain in female patients has been reported in many other chronic pain conditions although the pathophysiology is not well established (Isacson and Bingefors, 2002; Unruh, 1996). Previous reports in PD have found that female sex was a predictor for pain (Beiske et al., 2009; Scott et al., 2000), equally Tison et al noted significantly increased pain reporting in female MSA patients (Tison et al., 1996). It is possible that central pain mechanisms play a role in explaining this sex difference especially given that centrally mediated pain conditions such as chronic pain syndrome, fibromyalgia and migraine are more common in females (Popescu et al., 2010; Rasmussen et al., 1991; Wolfe et al., 1995).

The strong correlations between pain intensity and affective symptoms may reflect common pathophysiological substrates or a secondary effect of pain. This has been documented in previous reports investigating pain in PD (Negre-Pages et al., 2008). We have documented significantly higher scores for affective symptoms in patients who experienced neuropathic pain compared to those who experienced nociceptive pain. This may reflect a causal relationship but can also result from neurodegeneration of serotonergic and adrenergic neurons, which can
influence both pain inhibiting pathways and affect. Depression is equally common in both PSP and MSA and has been found to have a significant influence on patients’ perception of their quality of life (Schrag et al., 2010). This highlights the importance of adequate management of affective symptoms for pain control and improved subjective health status.

There are a number of limitations to our study, the main one being the relatively small sample size. This is largely due to the relative rarity of atypical parkinsonian disorders and the exclusion of patients with dementia, limiting the number of suitable PSP patients. Motor disability was not evenly matched between the three groups, as atypical parkinsonian syndromes are more aggressive conferring higher motor scores and PD patients were assessed in the “ON” state in an outpatient setting. Assessing PD patients in the “OFF” state would have probably been more reflective of the degree of motor impairment. This was difficult given the cross-sectional design of the study, which was conducted in an outpatient setting. However, contrary to what one would expect, pain intensity in PSP and MSA was either less or matching pain intensity in PD despite higher motor disability. Therefore the discrepancy in motor scores is unlikely to have contributed to the difference in reported pain. Disease duration and age were also different between the groups reflecting the natural disease course and age groups at which these conditions present. Due to sample size we could not correct for these variables and we accept this as another limitation to our study.
To our knowledge, this is the first study to prospectively evaluate pain in atypical parkinsonian disorders using validated pain scales. Further work with larger cohorts is needed to understand the causes of pain in parkinsonian disorders and alleviate the burden of this common and disabling symptom.
CHAPTER 3

Pain Characteristics In A Large Cohort of Patients With Early Parkinson’s Disease.
3.1 Abstract

**Background:** Pain is a common non-motor in Parkinson’s disease (PD) with a prevalence of over 70%. The underlying mechanism of pain in PD is poorly understood but motor factors and central sensitisation are thought to be important. Pain prevalence and characteristics are not known in early PD when motor symptoms are mild. **Objective:** To determine the prevalence, location, quality, and response to levodopa of pain in a large cohort of patients with early PD of less than 3-year duration. **Methods:** One thousand, seven hundred and sixty three PD patients from across the UK had their pain intensity assessed using the King’s PD Pain Scale (KPPS). The contribution of neuropathic mechanisms was determined using the Leeds Assessment of Neuropathic Signs and Symptoms (LANSS) scale. Pain location was determined using a body map. Pain response to Levodopa and use of analgesia were recorded. **Results:** One thousand, four hundred and eighty five PD patients (84.2%) reported pain. The most common location of pain was in the lower limbs followed by back pain, neck pain, arm pain and head pain. Mean pain intensity as measured by the KPPS was 11.27 ± 0.312 (range 0 – 81). The most common pain category was musculoskeletal, followed by radicular, central neuropathic, akathisia related and dystonic. Neuropathic pain as defined by the LANSS scale was documented in 140 patients (7.9%). Two hundred and seventy two PD patients (19.7%) reported that their pain improved with Levodopa with over 80% stating that their pain did not differ between the “ON” and “OFF” state. **Conclusions:** Pain prevalence in early PD is comparable to advanced PD suggesting a lesser role for motor
disability in pain generation. Pain characteristics may be different in early PD with lower prevalence of clinically evident neuropathic pain and dystonic pain compared to advanced PD.
3.2 Introduction

Pain is an important and common non-motor symptom in Parkinson’s disease (PD) with a prevalence of up to 70% (Beiske et al., 2009; Brefel-Courbon et al., 2009; Defazio et al., 2008; Negre-Pages et al., 2008). In approximately 10% of PD patients pain is cited as the most troubling symptom (Politis et al., 2010). The pathophysiology of pain in PD is poorly understood but several studies suggest an underlying impaired central processing of nociceptive inputs. Pain in PD has been categorised based on putative causes into musculoskeletal, dystonic, neuropathic (central and radicular) and akathisia related. There is a significant overlap between these categories and to date there is no consensus on the origin of pain in PD. The prevailing perception in clinical practice is that pain in PD is caused by peripheral factors relating to muscle rigidity and postural instability but little attention is given to potential central sensitisation. This has led to under-recognition and under-treatment of pain in PD with more priority given to treating the motor syndrome. The aim of this study is to increase understanding of pain mechanisms in PD and examine the contribution of central sensitisation through studying pain characteristics and correlations in a large sample of well phenotyped patients with early PD. To date there are no well-powered studies that have addressed these important questions using validated scales to quantify and qualify pain. Other objectives include identifying genetic biomarkers of painful PD phenotypes. This study is running alongside the Parkinson’s Repository of Biosamples and Network Datasets (ProBaND) study and is a work in progress.
The hypothesis is that central sensitisation plays a prominent role in pain generation in PD. We predict that this is important in all types of pain in PD and not only the clinically defined neuropathic pain.

In April 2012, the PRoBaND study was launched. This is a large multi-centre UK-wide longitudinal study that will prospectively record the clinical features and progression rate of patients with early Parkinson’s disease over a period of five years. Variations in motor severity, associated cognitive impairment, response to treatment and non-motor features will be documented in details. Blood samples are obtained to assess known gene mutations linked to the condition. The phenotypic variations will be mapped to the genetic information in an effort to understand the aetiology of the varied presentation and progression of the condition and identify biomarkers aiding the development of novel therapies.

In conjunction with the PRoBaND study the “Parkinson’s Pain Study” was launched. This study will gather detailed information about pain in those who are already participating in PRoBaND. The data on pain presented in this chapter are preliminary descriptive data that will in the future be complemented by data from the PRoBaND study. At the time of writing this thesis the PRoBaND data are not available yet.
3.3 Methods

3.3.1 Ethics and funding

The study received a favourable opinion from the National Research Ethics Service Committee North West - Preston. Ref No. 13/NW/0554. The study is funded by Parkinson’s UK.

3.3.2 Patients

All patients in the PRoBaND study (whether they have pain or not) were eligible to participate in the Parkinson’s pain study. Therefore, inclusion and exclusion criteria are the same as those for the PRoBaND study:

3.3.2.1 Inclusion Criteria:

1- Diagnosis of Parkinson’s disease, based on UK Brain Bank criteria (Hughes et al., 1992) and made within the preceding 3 years.

2- Age ≥18 to < 90 years.

3- Subject is able and willing to provide informed consent.

3.3.2.2 Exclusion Criteria:

1- Patient has severe co-morbid illness that would prevent full study participation.

2- Patient has features indicating another type of degenerative parkinsonism, e.g. progressive supranuclear palsy.

3- Drug induced parkinsonism (Drug unmasked PD is allowed)
4- Symmetrical lower body parkinsonism attributable to significant cortical and/or subcortical cerebrovascular disease (patients with ‘incidental’ small vessel disease on brain imaging are allowed).

5- Negative or normal functional imaging of the presynaptic dopamine system.

6- The presence of UK Brain Bank exclusion criteria will be recorded at baseline, allowing for the presence of 1 or 2 exclusion criteria (e.g. dopamine antagonist Drug used; more than one affected relative) (if justified e.g. by abnormal SPECT).

All participants have given informed written consent.

3.3.3 Pain assessments

Assessment of pain was completed at any point throughout the time course of patients’ participation in PRoBaND. Patients were asked whether they have experienced pain over the last three months and if so to locate their pain on a body map (Appendix C). Information on whether the pain is worse in the “OFF” or “ON” state and whether it improves with Levodopa or any other medications was obtained. Patients were asked to score their pain intensity over the last month from 0 – 10. A visual analogue scale (VAS) with the descriptor “no pain” for 0 and the descriptor “worst pain ever” for 10 was used. Pain was considered of low intensity if VAS score was below 5. VAS scores above 5 over the last months were considered to indicate pain of moderate to severe intensity. Pain intensity was further assessed with the Short Form of McGill Pain Questionnaire (SFMPQ) (Appendix A). Additionally the newly devised King’s PD pain scale
(KPPS) (Chaudhuri et al., 2015) was used to assess pain intensity across the sub-categories of pain in PD (musculoskeletal, central neuropathic, radicular neuropathic, dystonia related and akathisia related). KPPS has the advantage of quantifying pain specific to PD across several categories. KPPS has seven domains and 14 items, each item is scored by severity (0-3) multiplied by frequency (0-4), resulting in a sub-score of 0 to 12, with a total possible score range from 0 to 168. The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) scale was used to distinguish neuropathic pain from nociceptive pain (Appendix B).

3.3.4 **PD demographics and data from the PRoBaND study** *(The data from the PRoBaND study are not available yet but the methods are mentioned here as they will form part of future publications)*:

General information about patients such as demographics, social history, vital signs, body mass index, past medical history, family history and drug history including Levodopa equivalent dose will also be obtained. Detailed information about PD phenotype will be available from the PRoBaND study. These are fairly extensive and cover all the motor and non-motor spectrum of PD symptoms using validated questionnaires.

They include:

1- Disease severity as assessed by the Movement Disorders Society Sponsored United Parkinson’s Disease Rating Scale (UPDRS) (Goetz et al., 2008).
2- Cognitive assessment using the Montreal Cognitive Assessment test (MoCA) (Nasreddine et al., 2005).
3- Blood samples are obtained for genetic analysis.
4- Parkinson’s disease quality of life 8 item version PDQ8 (Peto et al., 1998).
6- Epworth sleep scale (Johns, 1991).
7- REM sleep behaviour disorder screening questionnaire (Stiasny-Kolster et al., 2007)
8- Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983)
9- Questionnaire of Impulsive-Compulsive Disorders in Parkinson’s disease Rating Scale QUIP-RS (Weintraub et al., 2012)
10- Constipation questionnaire
11- The scale for outcomes in Parkinson’s disease for autonomic symptoms (SCOPA-AUT) (Visser et al., 2004).
12- Non-motor symptoms scale NMSS (Chaudhuri et al., 2007)
13- PD sleep scale PDSS (Chaudhuri et al., 2002)
14- The presence of anosmia is assessed using a special smell testing kit.
15- Big Five Inventory for personality testing BFI
16- Mini Environmental Risk Questionnaire for Parkinson’s Disease Patients Baseline (MERQ-PD-B)
3.3.5 Statistical analysis

Information on prevalence of pain, pain intensity, localisations of pain and treatment response are presented as descriptive statistics. All data are presented as Mean ± SEM. For future analysis, linear regression analysis will be used to document whether associated symptoms, including motor symptoms, autonomic features and levodopa-equivalent dose predict the severity of pain.

3.4 Results

One thousand, seven hundred and sixty three PD patients with disease duration of less than 3 years were recruited from neurology clinics across the UK over a period of three years.

3.4.1 Pain prevalence, intensity and location:

One thousand, four hundred and eighty five PD patients (84.2%) reported pain of any intensity (VAS > 0) and 715 patients (40.6%) reported pain of moderate to severe intensity (VAS > 5). The most common location of pain was in the lower limbs followed by back pain, neck pain, arm pain and head pain (Figure 3-1).
Figure 3-1 Pain locations in Parkinson’s disease.

Average pain intensity in PD patients who reported pain as measured by the SFMPQ was $6.56 \pm 0.15$ (range: $0 - 39$, $n = 1763$). The frequency and distribution of pain intensity is shown in Figure 3-2.
Figure 3-2 The distribution of pain intensity in Parkinson’s disease. SFMPQ: Short Form McGill Pain Questionnaire.

Using the King’s PD scale (KPPS), subcategories of pain in PD were documented. Mean pain intensity in PD patients as measured by the KPPS was 11.27 ± 0.312 (range 0 – 81, n = 1763). Average pain intensity in each of the 14 items of the KPPS is shown in (Figure 3-3). The frequency of the five categories of pain in PD as defined by Blair Ford (musculoskeletal, neuropathic central, neuropathic radicular, akathisia related and dystonic) (Ford, 2010) can be surmised using the KPPS. The most commonly described category was musculoskeletal, followed by radicular, central neuropathic, akathisia related and dystonic (Figure 3-4).
Figure 3-3 Mean and SEM of pain intensity in each of the 14 items of the King’s PD pain scale (data from 1763 PD patients).
Neuropathic pain was assessed to be present using the KPPS in 382 patients (21.7%) however when assessed by the LANSS scale neuropathic pain was documented only in 140 patients (7.9%).

3.4.2 Response to medications:
Six hundred and twenty eight PD patients (35.6%) were taking regular analgesia of which 586 patients (33.2%) were taking normal analgesia (Paracetamol, NSAIDs, Opioids) compared to 42 patients (2.4%) who were taking neuropathic pain treatment (Amitriptyline, Gabapentin, Pregabalin).
3.4.2.1 Levodopa

Data on 1382 PD patients with pain who were treated with levodopa were available. Two hundred and seventy two PD patients (19.7%) reported that their pain improved with Levodopa compared to 1110 patients (80.3%) who said that their pain did not improve with Levodopa treatment. Of those patients who had chronic pain with VAS intensity above 5, data on 704 patients were available. Five hundred and forty patients (76.7%) reported no response to levodopa compared to 150 patients (21.3%) who said their pain improved with levodopa.

3.4.3 Relation to PD state

Data on 1365 PD patients with pain were available. One thousand, one hundred and twenty nine patients (82.7%) reported no difference in pain intensity between the “ON” and the “OFF” state compared to 216 patients (15.8%) who reported worse pain when “OFF” and 20 patients (1.5%) who reported worse pain when “ON” (Figure 3-5).
3.5 Discussion

This is the largest study ever conducted on the characteristics of pain in Parkinson’s disease. Pain of moderate to severe intensity (VAS > 5) was reported in over 40% of PD patients and pain of any intensity (VAS > 0) was reported in over 80% of patients. These percentages are well above the rates of chronic pain in the general population of similar age group who have pain prevalence ranging from 15% to 30% (Blyth et al., 2001; Breivik et al., 2006; Eriksen et al., 2003; Johannes et al., 2010). Therefore this study provides strong evidence of increased prevalence of pain in PD.
Several studies have reported increased prevalence of pain in PD compared to controls (Defazio et al., 2008; Negre-Pages et al., 2008) however this is the first large-scale study to show increased prevalence of pain in patients with early PD (disease duration of less than 3 years). Motor disability and complications are expected to be mild in early PD; nevertheless our data showed that pain prevalence is comparable to PD patients who have longer disease duration (Beiske et al., 2009; Lee et al., 2006). Furthermore, only a small percentage of patients reported improvement of their pain when they are “ON” (15.8%) and over 70% of PD patient reported no pain improvement with levodopa therapy. These findings make pain in PD less likely to results solely from motor impairment and more likely to involve up-regulated central processing of nociceptive stimuli. This is supported by previous studies of experimental pain in PD (Brefel-Courbon et al., 2005; Djaldetti et al., 2004; Gerdelat-Mas et al., 2007).

Pain in PD has been categorised into various types including musculoskeletal, dystonic, central neuropathic, radicular neuropathic and akathisia-related. This study is the first to assess the intensity of pain in each category using the newly devised and validated King’s PD scale. In our PD population musculoskeletal pain was the most common, which is congruent with previous reports (Defazio et al., 2008; Lee et al., 2006). Musculoskeletal pain was defined as “pain around the joints” and was quantified on a scale from 1-12. Other categories of pain in PD were quantified using the same scale (Figure 3-3). Neuropathic pain was
documented in 21.7% of our patients using the King’s PD pain scale. This was defined as a pain deep within the body, burning pain and constant dull aching pain. However, using the LANSS scale only 7.9% of patients fulfilled the definition of neuropathic pain (score ≥12) suggesting that fewer patients with early PD had clinically testable neuropathic pain. Additionally, pain intensity measured by the SFMPQ was lower in this cohort of early PD (mean SFMPQ = 6.56 ± 0.15) in comparison to our previous group of 65 PD patients (mean SFMPQ = 16.4 ± 1.5) who had much longer disease (mean disease duration = 7.2 ± 0.6 years). Another notable observation is that dystonic pain was less prevalent in this study of early PD compared to previous literature (Lee et al., 2006; Tinazzi et al., 2006) likely reflecting lower prevalence of dystonia.

Taken together these findings indicate that while pain is equally common in early PD compared to late PD, pain intensity becomes higher with longer disease duration, which could reflect either worsening motor disability or worsening central sensitisation or both. Given the available evidence from our previous cohort and other reports of the strong relation of pain to motor fluctuations (UPDRS-IV), a central sensitisation phenomena, as opposed to motor disability (UPDRS-III) it is likely that central factors are more important in pain generation than peripheral factors although the latter may still play a causative role with a centrally upregulated pain processing state in PD. This would be consistent with the several studies that documented a reduced pain threshold to experimental
pain in PD (Brefel-Courbon et al., 2005; Djaldetti et al., 2004; Gerdelat-Mas et al., 2007).

The question of how important central sensitisation is in pain generation in PD and why the so-called “musculoskeletal” pain is so common in PD is yet to be answered conclusively. Data from the PRoBaND study including motor severity and complications are not available at the time of writing this chapter but if motor disability and muscle rigidity were important causes of pain in PD one would expect a very strong correlation between UPDRS-III and the intensity of pain that is defined as musculoskeletal. If such correlation were modest or not present it would strongly suggest a lesser role for motor factors in pain generation and a more important role for central sensitisation. If this was the case it is also expected that pain would correlate with genetic polymorphisms that affects central sensitisation such as Catechol-O-methyltransferase (COMT) polymorphism. COMT plays an important role in regulating the levels of dopamine within the brain. Non-PD subjects who carry lower activity COMT polymorphisms have lower pain thresholds and higher chance of developing chronic pain (Diatchenko et al., 2005). One would predict, that the influence of COMT polymorphisms on the development of neuropathic pain would be much higher in PD than in the general population.

Additioally more data will be available including patients’ demographics, affective symptoms, autonomic symptoms, quality of life measures and
cognition, all of which will further clarify the predictors of various pain syndrome in PD and increase our understanding of this common and disabling symptom.
CHAPTER 4

Small Fibre Neuropathy In Parkinson’s Disease: A Clinical, Pathological And Corneal Confocal Microscopy Study
4.1 Abstract

Autonomic and somatic denervation is well established in Parkinson’s disease (PD). **Objectives:** (1) To determine whether corneal confocal microscopy (CCM) can non-invasively demonstrate small nerve fibre damage in PD. (2) To identify relationships between corneal nerve parameters, intraepidermal nerve fibre density (IENFD) and clinical features of PD. **Methods:** Twenty-six PD patients and 26 controls underwent CCM of both eyes. 24/26 PD patients and 10/26 controls underwent skin biopsies from the dorsa of both feet. PD patients underwent assessment of parasympathetic function [deep breathing heart rate variability (DB-HRV)], autonomic symptoms [scale for outcomes in Parkinson’s disease – autonomic symptoms (SCOPA-AUT)], motor symptoms [UPDRS-III “ON”] and cumulative Levodopa dose. **Results:** PD patients had significantly reduced corneal nerve fibre density (CNFD) with increased corneal nerve branch density (CNBD) and corneal nerve fibre length (CNFL) compared to controls. CNBD and CNFL but not CNFD correlated inversely with UPDRS-III and SCOPA-AUT. All CCM parameters correlated strongly with DB-HRV. There was no correlation between CCM parameters and disease duration, cumulative Levodopa dose or pain. IENFD was significantly reduced in PD compared to controls and correlated with CNFD and UPDRS-III. However, unlike CCM measures, IENFD correlated with disease duration and cumulative Levodopa dose but not with autonomic dysfunction. **Conclusion:** CCM identifies corneal nerve fibre pathology, which correlates with autonomic symptoms, parasympathetic deficits and motor scores in patients with PD. IENFD is also
reduced and correlates with CNFD and motor symptoms but not parasympathetic deficits, indicating it detects different aspects of peripheral nerve pathology in PD.
4.2 Introduction

Parkinson’s disease (PD) is a neurodegenerative disease recognised clinically by its motor symptoms of rest tremor, bradykinesia, rigidity and postural instability. The motor syndrome results from degeneration of dopaminergic neurons in the substantia nigra. Abnormal aggregates of alpha-synuclein in neuronal cells form the histopathological hallmark of PD.

A substantial body of evidence demonstrating peripheral nerve pathology has challenged the traditional view of PD as a disorder of the central nervous system. Phosphorylated alpha-synuclein deposits have been demonstrated in the autonomic nerves of the colon, cardiac plexus and more recently cutaneous C fibres (Dabby et al., 2006; Donadio et al., 2014; Fujishiro et al., 2008; Lebouvier et al., 2010; Wang et al., 2013).

Several studies have shown clinical and histopathological evidence of small and large fibre peripheral neuropathy in PD (Ceravolo et al., 2013; Doppler et al., 2014; Nolano et al., 2008; Rajabally and Martey, 2011; Toth et al., 2008). To date there is no consensus on the underlying pathophysiology of peripheral neuropathy in PD. Cumulative Levodopa exposure has been proposed as a potential cause for large fibre neuropathy via homocysteine accumulation and reduced levels of folate as well as vitamin B12 (Ceravolo et al., 2013; Rajabally and Martey, 2011; Toth et al., 2008). However several studies have found no relation between peripheral denervation and Levodopa exposure suggesting that
peripheral nerve involvement may be an intrinsic feature of the disease, especially in relation to small fibre neuropathy (Dabby et al., 2006; Doppler et al., 2014; Nolano et al., 2011). Furthermore, the identification of alpha-synuclein in cutaneous nerves has led to the consideration of skin tissue as a potential biomarker in PD (Donadio et al., 2014; Wang et al., 2013).

The cornea receives its innervation from the ophthalmic branch of the trigeminal nerve. Corneal nerves have an important neurotrophic role in maintaining corneal integrity. Small unmyelinated nerve fibres enter the cornea from the periphery and course through the corneal stroma before they penetrate the sub-epithelial Bowman’s membrane, giving rise to the sub-basal nerve plexus (Muller et al., 2003). This nerve plexus is of particular relevance for defining neuropathic changes since it supplies the overlying corneal epithelium. We have developed corneal confocal microscopy (CCM) as a rapid, non-invasive technique for in vivo visualisation of corneal nerves and shown it to be a valid surrogate measure of diabetic somatic and autonomic neuropathy (Quattrini et al., 2007; Tavakoli et al., 2015). CCM can also detect early neuropathy in patients with recently diagnosed type 2 diabetes and subjects with impaired glucose tolerance (Asghar et al., 2014; Ziegler et al., 2014). Furthermore, CCM has a high sensitivity in detecting early nerve regeneration following simultaneous pancreas and kidney transplantation in diabetic neuropathy (Tavakoli et al., 2013). We have further extended the role of CCM to detect small fibre neuropathy in Fabry’s disease,
idiopathic small fibre neuropathy and Charcot-Marie-Tooth 1A (Tavakoli et al., 2009; Tavakoli et al., 2010; Tavakoli et al., 2012).

One study that included 4 patients with PD reported no significant reduction in corneal nerve density compared to controls (Reddy et al., 2013). However, assessment of corneal nerve morphology was not the primary aim of the study and the small number of PD participants precludes meaningful conclusions regarding the utility of CCM in PD.

Given the strong evidence for small fibre neuropathy in PD, we hypothesised that CCM would allow rapid, non-invasive detection of corneal nerve pathology and that this would be related to intraepidermal nerve fibre density and clinical symptoms in PD, in particular those related to pain and autonomic dysfunction.

4.3 Methods

4.3.1 Ethical approval

NRES Committee / North West (Ref. No 12/NW/0086) approved the study.

4.3.2 Subjects

Thirty-three patients (22 males, 11 females) fulfilling the UK Brain Bank criteria for the diagnosis of Parkinson’s disease were recruited from neurology clinics. Patients with a known history of cancer, chemotherapy, diabetes, alcoholism, vitamin deficiencies, coeliac disease and autoimmune conditions were excluded.
All patients underwent an oral glucose tolerance test and fasting blood screening to include: vitamin B12, folate, methylmalonate, and homocysteine to exclude other causes of neuropathy. Six patients (5 males, 1 female) were excluded due to impaired glucose tolerance, leaving 27 patients who fulfilled the inclusion criteria. CCM was not undertaken in 1 patient due to previous refractive eye surgery. Twenty-six age-matched healthy volunteers served as controls. All participants gave their written informed consent.

4.3.3 Disease duration, severity and Levodopa exposure

PD duration was calculated from reported symptoms onset until the date of assessment. UPDRS-III was used to assess motor severity. Patients were examined in the “ON” state in an outpatient setting. All patients except one were on Levodopa therapy. Cumulative Levodopa dose was calculated from the date of first prescription until the date of assessment.

4.3.4 Non-motor symptoms

Non-motor symptoms were quantified using the non-motor symptoms scale NMSS (Chaudhuri et al., 2007). Pain was quantified separately using the recently devised King’s PD pain scale (Chaudhuri et al., 2015) as well as the short form McGill Pain Questionnaire (SFMPQ).

4.3.5 Autonomic symptoms and function

Autonomic symptoms were assessed using the scale for outcomes in Parkinson’s disease - autonomic symptoms (SCOPA-AUT) (Visser et al., 2004) (Appendix E). Deep breathing heart rate variability (DB-HRV) provided an estimation of
cardiac parasympathetic dysfunction and was assessed with an ANX 3.0 autonomic nervous system device (ANSAR Medical Technologies Inc., Philadelphia, PA). Cardiovascular sympathetic function was assessed by measuring blood pressure and heart rates in the supine position and after standing up for 5 minutes.

4.3.6 Evaluation of neuropathy

Nerve conduction studies were performed on all PD patients. Patients with evidence of a large fibre neuropathy underwent assessment of immunoglobulins and protein electrophoresis to rule out the presence of a paraproteinaemic neuropathy. The neuropathy disability score (NDS) was obtained in all participants. NDS is a bedside measure to stratify diabetic neuropathy (Young et al., 1993). A clinician assesses vibration perception using a tuning fork, temperature perception using a cold and warm metal rods, pinprick perception using Neurtips™ and ankle reflexes using a tendon hammer producing a score from 0-10. Quantitative Sensory Testing was assessed using the MEDOC TSA II (Medoc Ltd., Ramat-Yishai 20095, Israel) device on the dorsum of the left foot. A probe (thermode) would heat or cool the skin until the patient clicks a button to stop it. Quantitative sensory testing included heat sensation threshold, heat-induced pain threshold, cold threshold and cold-induced pain threshold. Vibration perception threshold was measured using a Neurothesiometer (Horwell, Scientific Laboratory Supplies, Nottingham, UK) on the hallux of both feet. The quantitative stimulus (heating, cooling or vibration) was repeated three times in each category and an average of three readings was used for analysis.
4.3.7 Skin biopsies

Two 3-mm punch skin biopsies were taken from the dorsa of both feet. The biopsies were immediately fixed in 4% paraformaldehyde, cryoprotected in graded solutions of sucrose, frozen and cut on a cryomicrotome (HM450, Microm International, Germany). Six 50 µm sections per biopsy were immunostained using anti-human PGP 9.5 antibody and nerve fibres were demonstrated using SG chromogen (Vector Laboratories, Peterborough, U.K.). A pathologist blinded to participants’ details performed tissue analysis. Intraepidermal nerve fibre density (IENFD), i.e., the number of nerve fibres crossing basement membrane, was quantified according to established criteria and expressed as number per millimetre of epidermal length (Lauria et al., 2010). The mean between right and left IENFD was calculated for each patient and used for analysis.

4.3.8 Corneal confocal microscopy

Corneal microscopy was performed on both eyes using a Heidelberg Retina Tomograph III with a Rostock Cornea Module (HRT III RCM; Heidelberg Engineering GmbH, Heidelberg, Germany), as previously described (Tavakoli et al., 2013). Four to six high-resolution (1-2 µm) images of the sub-basal plexus of each eye were obtained for all participants. A trained investigator who was blinded to participants’ details analysed corneal images separately. Corneal Nerve Fibre Density (CNFD): The number of main nerves per square millimetre, Corneal Nerve Branch Density (CNBD): The number of branches emanating
from each main nerve per square millimetre and Corneal Nerve Fibre Length (CNFL): The length of all nerve fibres and branches (mm per square millimetre) were quantified and the mean derived from the right and left eye for each parameter. Quantification was undertaken using semi-automated, purpose-written, proprietary software (CCMetrics; M.A. Dabbah, Imaging Science and Biomedical Engineering, Manchester, UK). To estimate the error in measuring CNFD, CNBD and CNFL, we acquired images and determined each of these parameters in 15 subjects on two occasions separated by at least 48 hours. The coefficient of variation of these parameters was 12% for CNFD, 24% for CNBD and 9% for CNFL.

4.3.9 Statistical analysis

IBM SPSS version 22 was used to compute the results. All parameters were normally distributed except for cumulative Levodopa dose, vibration and cold perception thresholds as well as heat and cold induced pain thresholds. Independent samples t-test or Mann–Whitney U test were used to compare means as appropriate. Cohen d was calculated to measure effect size. Two-tailed Pearson’s correlation or Spearman’s correlations were used as appropriate to determine relationships between continuous variables. Categorical data were compared with Chi square test. P value of < 0.05 was considered statistically significant. P value was not corrected for multiple comparisons.
4.4 Results

4.4.1 Study population

A total of 27 PD patients and 26 controls were enrolled in the study. There was no significant difference in demographics between PD patients and control subjects (Table 4-1).

4.4.2 Pain characteristics in the PD population

When asked about pain experience over the last month only two PD patients said they were pain free with 25 patients reporting pain (92.6%). Pain was mostly reported in the limbs with 17 patient reporting pain in the arms (63%) and 16 patients reporting pain in the legs (59.2%). This was followed by neck pain in 12 patients (44.4%) and back pain in 11 patients (40.7%). Pain was reported in the groin in 2 patients (7.4%) and in the head in 3 patients (11.1%).

Using Ford’s pain categories in PD, pain was described as musculoskeletal in 18 patients (67%), central in 16 patients (59%), dystonic in 15 patient (56%), akathisia related in 11 patients (41%) and radicular in 9 patients (33%).

8 patients (29.6%) fulfilled the definition of neuropathic pain as measured by the LANSS.
Out of the patients who reported pain 12 patients (48%) said that their pain improved with dopamine therapy and 13 (52%) reported no improvement.

4.4.3 Corneal and intraepidermal nerve fibres

Twenty-six PD patients and 26 controls underwent CCM and 24 PD patients and 10 controls underwent skin biopsies. Both CNFD and IENFD were significantly lower in PD patients compared to controls (CNFD mean difference: -5.6 no./mm$^2$, 95% CI [-9.2, -2], $P = 0.003$; IENFD mean difference: -5.9 no./mm, 95% CI [-7.9, -3.9], $P < 0.001$). However, CNBD was significantly higher in PD patients compared to controls (CNBD mean difference: 86.6 no./mm$^2$, 95% CI [55.9, 117.2]), $P < 0.001$). CNFL was also significantly higher in PD patients (CNFL mean difference: 3.2 mm./mm$^2$, 95% CI [0.3, 6.1], $P = 0.031$) (Figure 4-1 & 4-2).

Both IENFD and CCM parameters displayed asymmetry between sides in PD patients. However, when CCM measures and IENFD were separated according to the clinically more affected side (defined by UPDRS-III scores) and the clinically less affected side there was no significant difference in CCM measures or IENFD.
<table>
<thead>
<tr>
<th></th>
<th>PD patients (n = 27)</th>
<th>Controls (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>10 females, 17 males</td>
<td>11 females, 15 males</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.0 ± 1.5 (49–77)</td>
<td>60.1 ± 1.3 (51–73)</td>
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<tr>
<td>Disease duration (years)</td>
<td>6.6 ± 0.9 (0.7–21)</td>
<td>1.3 ± 0.5 (0.7–33)</td>
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<td>UPDRS-III</td>
<td>26.6 ± 2.3 (10–53)</td>
<td>21.0 ± 3.0 (0–53)</td>
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<tr>
<td>SCOPA-AUT</td>
<td>15.2 ± 1.3 (4–30)</td>
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<tr>
<td>NMSS</td>
<td>59.9 ± 5.2 (2–117)</td>
<td></td>
</tr>
<tr>
<td>King’s PD pain scale</td>
<td>21.0 ± 3.0 (0–53)</td>
<td></td>
</tr>
<tr>
<td>SFMPQ</td>
<td>21.0 ± 2.5 (0–51)</td>
<td></td>
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<tr>
<td>NDS</td>
<td>3 ± 0.5 (0–9)</td>
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<tr>
<td>DB-HRV</td>
<td>15.6 ± 1.3 (6–28)</td>
<td></td>
</tr>
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<td>Cumulative Vodopar dose (5)</td>
<td>158.7 ± 14.6 (201–252)</td>
<td>70 ± 14.6 (50–80)</td>
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<tr>
<td>SFprQ</td>
<td>20.7 ± 0.9 (11.4–30)</td>
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<tr>
<td>King’s PP pain scale</td>
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<td>NMDA</td>
<td>3 ± 0.6 (0–5)</td>
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<tr>
<td>UPDBs-III</td>
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<tr>
<td>Disease Duration (years)</td>
<td>11.3 ± 1.5 (7–16)</td>
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<tr>
<td>Age (years)</td>
<td>60.1 ± 1.5 (31–73)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>10 females, 17 males</td>
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</tr>
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</table>

Table 4: Demographics, clinical characteristics and quantitative measures of neuropathy in patients with Parkinson’s disease (PD) and controls.
Figure 4.1 Representative examples of 50 µm sections from skin biopsies immunostained for PGP9.5. Healthy control (A) shows numerous long branching intraepidermal nerve fibres (red arrows) reaching upper layers of epidermis and well developed sub-epidermal nerve plexus (green arrows). Biopsy from a patient with Parkinson’s disease (B) showing scant, faintly staining intraepidermal nerve fibres (red arrows), some branches with axonal swellings (red star) and a biopsy from another patient with Parkinson’s disease (C) showing an area of epidermis with only one short intraepidermal nerve fibre and signs of degeneration in the sub-epidermal nerve plexus (red stars). Note short nerve fibres, barely crossing epidermal basement membrane (B, C, blue arrows). A-C at the same magnification, scale bar = 100 µm. Corneal confocal image of a healthy control (D) compared to a patient with Parkinson’s disease (E) showing a reduction in overall corneal nerve fibre density and increased corneal nerve branch density. Quantification of corneal nerve fibre density, branch density and fibre length is calculated using a semi-automated process. Main fibres are highlighted in red, branches are highlighted in blue and branch origins are represented by the green dots (F&G).
**Figure 4-2** Mean ± SEM of corneal nerve fibre density (CNFD) (A), intraepidermal nerve fibre density (IENFD) (B), corneal nerve fibre branch density (CNBD) (C) and fibre length (CNFL) (D) in Parkinson’s disease (PD) compared to controls with significance level and effect size.

### 4.4.4 Clinical, neurophysiological, quantitative sensory and autonomic measures of neuropathy

NDS, DB-HRV and sensory thresholds all showed impairment in PD compared to controls (Table 4-1). Overall, there was no significant postural drop in blood pressure in PD (defined as mean systolic drop of > 20 mm Hg and/or diastolic drop of > 10 mm Hg) (Table 4-1). Three out of 27 PD patients (11.1%) had large fibre axonal neuropathy on nerve conduction studies and 2 of these patients had raised methylmalonate. However, methylmalonate was raised in a further 4 PD
patients with normal nerve conduction studies. Homocysteine was raised in 5 (18.5%) patients all of whom had normal neurophysiology. All patients had normal vitamin B12 and folate levels. Protein electrophoresis was normal in patients with large fibre neuropathy.

4.4.5 Relation between corneal nerve parameters, intraepidermal nerve fibres and clinical features.

CNFD correlated positively with IENFD in PD patients (Pearson’s $r = 0.464$, $P = 0.026$). Correlations between CCM parameters, IENFD and clinical data are summarised in Table 4-2. CNBD and CNFL but not CNFD correlated inversely with UPDRS-III. There was no correlation between any of the corneal nerves parameters and disease duration, cumulative Levodopa dose, non-motor symptoms, pain scales or sensory thresholds. When asked about pain experience over the last month only two PD patients said they were pain free. However, there was an inverse correlation between heat-induced pain threshold and SFMPQ (Spearman’s rho -0.450, $P = 0.036$). A significant positive correlation was found between DB-HRV and all corneal nerve parameters, but not IENFD (Table 4-2). However, IENFD correlated with disease duration, disease severity and cumulative Levodopa dose. Both CNFD and IENFD correlated with NDS. CNFD, IENFD and NDS were independent of vitamin B12, folate, methylmalonate and homocysteine levels.
Table 4-2 Correlations between corneal nerve measures, intraepidermal nerve fibre density and various clinical and demographic data of PD patients.

<table>
<thead>
<tr>
<th></th>
<th>CNFD</th>
<th>CNBD</th>
<th>CNFL</th>
<th>IENFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.362</td>
<td>0.002</td>
<td>-0.104</td>
<td>-0.097</td>
</tr>
<tr>
<td>Disease duration</td>
<td>-0.284</td>
<td>-0.181</td>
<td>-0.210</td>
<td><strong>-0.416</strong> *</td>
</tr>
<tr>
<td>Cumulative L-dopa dose</td>
<td>-0.301</td>
<td>-0.243</td>
<td>-0.170</td>
<td><strong>-0.476</strong> *</td>
</tr>
<tr>
<td>UPDRS-III</td>
<td>-0.337</td>
<td><strong>-0.445</strong> *</td>
<td><strong>-0.416</strong> *</td>
<td><strong>-0.441</strong> *</td>
</tr>
<tr>
<td>SCOPA-AUT</td>
<td>-0.373</td>
<td><strong>-0.439</strong> *</td>
<td><strong>-0.405</strong> *</td>
<td>-0.267</td>
</tr>
<tr>
<td>DB-HRV</td>
<td>0.537**</td>
<td>0.681***</td>
<td>0.708***</td>
<td>0.174</td>
</tr>
<tr>
<td>NMSS</td>
<td>-0.042</td>
<td>-0.204</td>
<td>-0.047</td>
<td>-0.353</td>
</tr>
<tr>
<td>King’s PD pain scale</td>
<td>-0.360</td>
<td>-0.232</td>
<td>-0.243</td>
<td>-0.281</td>
</tr>
<tr>
<td>SFMPQ</td>
<td>-0.053</td>
<td>-0.249</td>
<td>-0.117</td>
<td>-0.071</td>
</tr>
<tr>
<td>NDS</td>
<td><strong>-0.522</strong> **</td>
<td>-0.328</td>
<td>-0.360</td>
<td><strong>-0.451</strong> *</td>
</tr>
<tr>
<td>Vibration Threshold</td>
<td>-0.133</td>
<td>-0.278</td>
<td>-0.293</td>
<td>-0.354</td>
</tr>
<tr>
<td>Cold Threshold</td>
<td>-0.090</td>
<td>-0.242</td>
<td>-0.144</td>
<td>0.227</td>
</tr>
<tr>
<td>Heat Threshold</td>
<td>-0.385</td>
<td>-0.240</td>
<td>-0.282</td>
<td>-0.399</td>
</tr>
<tr>
<td>Cold Pain Threshold</td>
<td>0.025</td>
<td>-0.303</td>
<td>-0.265</td>
<td>-0.369</td>
</tr>
<tr>
<td>Heat Pain Threshold</td>
<td>-0.336</td>
<td>-0.169</td>
<td>-0.247</td>
<td>-0.094</td>
</tr>
</tbody>
</table>

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. $p$ value is not corrected for multiple comparisons. Pearson's correlation was applied in all measures except for Levodopa dose, vibration threshold, cold and heat pain thresholds where the non-parametric Spearman's correlation was applied. CNFD: Corneal Nerve Fibre Density, CNBD: Corneal Nerve Branch Density, CNFL: Corneal Nerve Fibre Length, IENFD: Intraepidermal Nerve Fibre Density, UPDRS-III: Unified Parkinson’s Disease Rating Scale, SCOPA-AUT: Scale for Outcomes in Parkinson’s Disease - Autonomic Symptoms, DB-HRV: Deep Breathing Heart Rate Variability, NMSS: Non-Motor Symptoms Scale, SFMPQ: Short Form McGill Pain Questionnaire, NDS: Neuropathy Disability Scale.
4.5 Discussion

This is the first study to systematically characterise corneal nerve changes in PD using the non-invasive technique of corneal confocal microscopy. Compared to controls, PD patients demonstrated a reduction in CNFD, a marked increase in CNBD and an increase in CNFL. This pattern of corneal nerve pathology is notably different from diabetic neuropathy, Fabry’s disease, chronic inflammatory demyelinating polyneuropathy and Charcot-Marie Tooth 1A, where a reduction of all three corneal nerve parameters has been documented (Quattrini et al., 2007; Schneider et al., 2014; Tavakoli et al., 2009; Tavakoli et al., 2012). Thus the reduced CNFD with a markedly increased CNBD appears to be unique in PD. Pathologically this represents small fibre neuropathy, characterised by the reduced CNFD. However, the increased CNBD is likely to reflect attempted nerve regeneration. CNBD correlated inversely with motor severity suggesting that nerve regeneration occurs in the early stages of the disease and declines as the disease becomes more advanced. Worthy of note is that we are not the first to report increased nerve branching in PD as this was reported before on skin biopsies (Nolano et al., 2008). Indeed, increased corneal nerve branch density was the first measure to increase after simultaneous pancreas and kidney transplantation or continuous subcutaneous insulin infusion in type 1 diabetes (Azmi et al., 2015; Tavakoli et al., 2013).

We confirm the results of a number of studies, which have shown a reduction of distal IENFD in PD patients (Doppler et al., 2014; Nolano et al., 2008; Wang et
al., 2013). However, we are the first to report a reduction of CNFD, which correlates with IENFD indicating widespread involvement of small nerve fibres in PD. This is further supported by the significant impairment of modalities relating to both parasympathetic and sensory functions (DB-HRV, thermal perception and pain thresholds). We did not document significant postural drop in blood pressure or heart rate (sympathetic cardiovascular function) in our PD population. Given that postural hypotension is a manifestation of severe sympathetic dysfunction this may reflect the disease stage of most of our PD cohort with relatively few patients in very advanced stages (Table 4-1).

The change in corneal nerve parameters was independent of age, cumulative Levodopa dose, methylmalonate, homocysteine, B12 and folate levels, suggesting that damage to corneal nerves is a result of an intrinsic disease process rather than external factors. Furthermore, unlike diabetes where corneal nerve damage was symmetrical (Petropoulos et al., 2013) we found asymmetry in corneal nerve parameters between sides in PD patients suggesting that corneal neuropathy in diabetes is due to a global metabolic process, whereas in PD may be due to a more localised degenerative process consistent with PD pathology.

Corneal nerve changes strongly correlated with autonomic symptoms (SCOPA-AUT) and parasympathetic dysfunction (DB-HRV), but did not correlate with disease duration or cumulative Levodopa dose. However, IENFD correlated with disease duration, and cumulative Levodopa dose but not with autonomic
symptoms or parasympathetic deficits (Table 4-2). These findings suggest that CCM and skin biopsies may be reflecting different aspects of neuronal pathology in PD and indeed corneal nerves changes rather than IENFD are more closely associated with autonomic pathology. In support of this we have recently shown that CCM has a very high sensitivity and specificity for the diagnosis of diabetic autonomic neuropathy and correlates with autonomic symptoms and deficits (Tavakoli et al., 2015). The moderate correlation between IENFD and CNFD emphasises that skin biopsy and CCM are visualising different aspect of small fibre pathology. Indeed in a recent study of 134 patients with early diabetic neuropathy the association between IENFD and CCM pathology was even lower (Ziegler et al., 2014). It would be interesting to investigate whether corneal denervation is present during the pre-motor phase when autonomic symptoms are commonly reported which can potentially add to the sensitivity and specificity of pre-motor features such as anosmia and REM sleep behaviour disorder in diagnosing pre-motor PD.

Previous studies of skin biopsies in PD have revealed preferential deposition of phosphorylated alpha-synuclein in autonomic cutaneous nerves, with a higher yield for alpha-synuclein from proximal as opposed to distal sites (Donadio et al., 2014; Wang et al., 2013). IENFD reduction, on the other hand, follows a length-dependent pattern (Doppler et al., 2014). This has led to the suggestion that alpha-synuclein deposition and a length-dependent reduction in IENFD are different pathological processes (Doppler et al., 2014). Distal skin denervation in
our PD patients was more pronounced than corneal denervation (Figure 4-2). Taken together one may conclude that the difference of alpha-synuclein deposition between proximal and distal sites may reflect neuronal availability with distal axons being more vulnerable to degeneration compared to proximal sites.

Similar to previous reports we found otherwise unexplained clinical and neurophysiological evidence of large fibre neuropathy in a proportion of our PD patients but we could not establish a link between levodopa exposure or vitamin deficiencies and large fibre neuropathy. The question of Levodopa induced neuropathy in PD remains a subject of debate. While there have been reports linking large fibre neuropathy with prolonged exposure to Levodopa, (Ceravolo et al., 2013; Rajabally and Martey, 2011) evidence for such a link with small fibre neuropathy is lacking (Dabby et al., 2006; Donadio et al., 2014; Doppler et al., 2014; Nolano et al., 2011). Although we have found a correlation between IENFD and cumulative Levodopa dose, establishing causality is difficult as reduced IENFD also correlated with disease duration and severity. Correcting for these factors using multiple regression analysis may address this question, but our study was not powered for this. However, given the lack of relationship between changes in IENFD and methylmalonate, homocysteine or B12 as well as the asymmetry between skin biopsies, our results suggest that cutaneous denervation may be due to an intrinsic disease process rather than an extrinsic toxicity due to Levodopa.
Pain is very common in PD, affecting up to 70% of PD patients (Beiske et al., 2009). Pain characteristics in this cohort were comparable to our previous studies with musculoskeletal pain being the most common type. Similar to the previous chapter neuropathic pain was reported in a significant proportion of patients. A discrepancy from the cohort of early PD patients, discussed in the previous chapter, is the higher proportion of patients reporting improvement with levodopa (48% compared to 19.7%). This highlights that levodopa may become more important in pain management as the disease becomes more advanced. We found no correlation between self-reported pain scores and small fibre nerve density suggesting that small fibre pathology in itself may make little contribution to pain in PD and that central sensitisation maybe more important. This is further supported by the inverse correlation between heat-induced pain threshold and SFMPQ indicating a reduced pain threshold in painful PD phenotypes. Notably, average heat pain thresholds were higher in PD patients compared to controls (Table 4-1), which is at odds with previous reports of reduced pain threshold in PD (Brefel-Courbon et al., 2005; Djaldetti et al., 2004). This could be explained by sensory neuropathy in our PD population, which may have influenced heat pain threshold and as such will need to be taken in context.

Both CNBD and CNFL as well as IENFD correlated negatively with UPDRS-III. These findings strengthen the argument that skin biopsy and CCM may be useful biomarkers in PD. Of course our study was not designed to assess the utility of

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these measures as biomarkers and is limited by the cross sectional design and the assessment of UPDRS-III in the “ON” state, potentially affecting the ability to interpret the motor severity data. Furthermore, skin tissue co-immunostaining with vasoactive intestinal peptide (VIP) or tyrosine hydroxylase would have been required to detect sympathetic adrenergic fibres and differentiate autonomic from sensory nerve involvement. These limitations, however, did not affect our primary objective, which was to assess corneal nerve pathology in PD compared to healthy controls and this was demonstrated with large effect size.

Corneal confocal microscopy, a rapid non-invasive imaging technique, allows in vivo visualisation and quantification of corneal nerves. This enables longitudinal assessment of small nerve fibre pathology, which is not readily achieved using skin biopsies in PD. Further longitudinal studies using UPDRS-III “OFF” scores are needed to establish its relationship to motor deficits. Whether CCM can indeed fulfil the criteria of a non-invasive biomarker to detect sub-clinical disease, predict progression and the effect of treatment in PD, remains to be answered.
CHAPTER 5

The Perception Of Affective Touch In Parkinson’s Disease And Its Relation To Small Fibre Neuropathy.
5.1 Abstract

**Background:** Affective touch sensation is conducted by a sub-class of C-fibres in hairy skin known as C-Tactile (CT) afferents. CT afferents respond maximally to gentle skin stroking at velocities between 1-10 cm/sec. Parkinson’s disease (PD) is characterised by markedly reduced cutaneous C-fibres. It is not known if affective touch perception is influenced by C fibre density and if affective touch is impaired in PD compared to healthy controls. **Hypothesis:** We predicted that perceived pleasantness to gentle stroking in PD would correlate with C afferent density and that affective touch perception would be impaired in PD compared to healthy controls. **Methods:** Twenty-four PD patients and 27 control subjects rated the pleasantness of brush stroking at an optimum CT stimulation velocity (3cm/sec) and two sub-optimal velocities (0.3cm/sec & 30cm/sec). PD patients underwent quantification of C-fibre density using skin biopsies and corneal confocal microscopy. **Results:** All participants rated stroking velocity of 3cm/sec as the most pleasant with significantly lower ratings for 0.3cm/sec and 30cm/sec ($p < 0.001$). There was a significant positive correlation between C-fibre density and pleasantness ratings at 3cm/sec and 30cm/sec ($p < 0.05$) but not 0.3cm/sec. Mean pleasantness ratings were consistently higher in PD patients compared to control subjects across all three velocities. **Conclusions:** This study shows that perceived pleasantness to gentle touch correlate significantly with C-fibre density in PD further validating the role of CT afferents in affective touch perception. The higher perceived pleasantness in PD patients compared to controls suggests
central sensitisation to peripheral inputs, which may have been enhanced by dopamine therapy.
5.2 Introduction

Cutaneous sensory modalities such as pain and touch are fundamental for normal interaction between organisms, their environment and one another. While the emotional aspect of pain has been acknowledged for a long time (Melzack and Casey, 1968) the affective dimension of touch has only been recognised in recent years (Morrison et al., 2010). Vallbo et al, using the technique of microneurography, identified and characterised a population of low threshold mechanosensory C- fibres, named C-Tactile afferents (CT). Unlike the more classically described C-fibres, CT afferents did not code for pain or itch, but responded optimally to low force/velocity gentle touch (Vallbo et al., 1999). CT afferents fire maximally when a gentle caressing or “stroking” is applied to the skin. In contrast to myelinated afferents that respond linearly to stimulus velocity, CT afferents respond optimally to a stroking velocity of 1 – 10 cm/sec, which is also rated as the most pleasant. Stimulation velocities below and above this range (1-10cm/sec) produce a sub-optimal CT response determined by lower firing frequency (Loken et al., 2009; Morrison et al., 2010). Furthermore, fMRI studies have demonstrated that stimulation of CT afferents activates areas in the brain that are associated with pleasure, including the insular cortex, as opposed to stimulation of myelinated fibres which activates the somatosensory areas of the cortex (Olausson et al., 2002; Rolls et al., 2003). This emphasises the specialised role of CT afferents in mediating the affective component of the tactile experience.
The current view of CT afferents is that they provide the neurobiological basis for the formation and maintenance of social bonds and attachment relationships. They are proposed to be integral to an affiliative reward system that also involves several neuropeptides and neurotransmitters including serotonin, dopamine, opioids and oxytocin (Berridge and Robinson, 1998; Deakin, 1996; Le Merrer et al., 2009; Lee et al., 2009; Walker and McGlone, 2013). Moreover, impaired processing of affective touch has been associated with autistic spectrum disorders (Cascio et al., 2012; Kaiser et al., 2016; McGlone et al., 2007). Recent evidence also suggests that there may be a relationship between low threshold CT afferents and pain pathways with CT afferents playing a pain-inhibiting role (Liljencrantz et al., 2013). Consequently, it is possible that pathological variations in peripheral nerve structures or central neurotransmitters may cause impaired processing of affective stimuli.

Parkinson’s disease (PD) is a neurodegenerative condition characterised by both central monoamine depletion and reduced number of C-afferent fibres (Kassi-Iliyya et al., 2015; Nolano et al., 2008). Thus PD presents a good model for investigating the potential effects of central and peripheral neural impairment on affective touch processing. It is not known if affective touch perception is influenced by C-fibre density and if affective touch is impaired in PD compared to healthy controls. PD is also characterised by a range of non-motor symptoms including pain, depression and apathy that may contribute to anhedonia (Aarsland et al., 2009; Defazio et al., 2013a).
In this study, we assessed perceived pleasantness in response to skin stroking in a cohort of PD patients who had their C-fibre afferents quantified by two different methods: skin biopsy and corneal confocal microscopy. We predicted that affective touch perception would correlate positively with C-fibre afferent density and that pleasantness would be reduced in Parkinson’s disease compared to age-matched healthy controls. We also sought to investigate the potential relationship between perceived pleasantness from CT afferent stimulation and other self-reported symptoms such as depression, apathy and pain.

5.3 Methods

5.3.1 Participants

Twenty-four PD patients (age 51-78 years, mean age 63.7; 14 males) and 27 healthy volunteers (age 50-71 years, mean age 63.6; 11 males) took part in the study. Nerve conduction studies were performed on all PD participants and none had significant large fibre or demyelinating neuropathy. Assessments were undertaken without withdrawing dopaminergic therapy (the “ON” state). Healthy controls were recruited via the Salford citizen scientist project (http://www.citizenscientist.org.uk). Control participants were selected to be free of all significant medical problems, including pain. The study was approved by NRES Committee London - Bromley (Ref No. 15/LO/0252). All participants
provided their written informed consent. The conduct of the study adhered to the tenets of the declaration of Helsinki.

5.3.2 Affective touch evaluation

A goat-hair 70 mm wide artist brush was used to deliver strokes by a trained investigator. Experiments were undertaken in a quiet room on an examination couch. Strokes were delivered on each limb across a 10cm section of the skin. The lateral aspects of both forearms and shins were chosen to deliver brush strokes. One optimum velocity for CT afferents stimulation (3cm/sec) and two sub-optimal velocities (0.3cm/sec and 30cm/sec) were used to deliver brush strokes. For speed accuracy the investigator followed a moving bar across 10cm on a computer screen, which was not visible to the participant. The purpose-written programme was developed in LabVIEW (National Instruments, Texas, USA); project no. 2013-40, department of medical physics, Salford Royal NHS Foundation Trust. The programme randomised velocities of 0.3 cm/sec, 3 cm/sec and 30 cm/sec. Each velocity was randomised three times producing a total of nine strokes per limb. The programme was designed so the same velocity is not randomised three times in a row to minimise CT afferent adaptation. There was a pause of 10 seconds between consecutive brush strokes to prevent CT afferents fatigue. Study participants rated pleasantness by placing a mark on a 100mm visual analogue scale (VAS) with the descriptor: “neutral/normal” at the lowest end and the descriptor: “very pleasant” at the highest end. The ratings were measured with a ruler and the mean of the three ratings for each velocity was
calculated. The overall average rating for each velocity across all four limbs was used for the final analysis.

5.3.3 Small fibre neuropathy

Small nerve fibre quantification was only undertaken in the PD population. Twenty PD patients underwent skin biopsy and corneal confocal microscopy.

5.3.3.1 Skin biopsies

Two 3-mm punch skin biopsies were taken from the dorsa of both feet. The biopsies were immediately fixed in 4% paraformaldehyde, cryoprotected in graded solutions of sucrose, frozen and cut on a cryomicrotome (HM450, Microm International, Germany). Six 50 µm sections per biopsy were immuno-stained using anti-human PGP 9.5 antibody (Abcam, Cambridge, U.K.) and nerve fibres were demonstrated using SG chromogen (Vector Laboratories, Peterborough, U.K.). A pathologist blinded to the participants’ clinical details performed tissue analysis. Intraepidermal nerve fibre density, i.e., the number of nerve fibres crossing basement membrane, was quantified according to established criteria and expressed as number per millimetre of epidermal length (Lauria et al., 2010). The mean between right and left intraepidermal nerve fibre density was calculated for each patient and used for analysis.

5.3.3.2 Corneal Confocal Microscopy

Corneal confocal microscopy is a non-invasive technique that allows in-vivo visualisation and quantification of corneal nerves. It is well established as a non-invasive surrogate method for studying small fibre neuropathy correlating
significantly with skin biopsies (Chen et al., 2015; Quattrini et al., 2007). Corneal confocal microscopy was performed on both eyes using a Heidelberg Retina Tomograph III with a Rostock Cornea Module (HRT III RCM; Heidelberg Engineering GmbH, Heidelberg, Germany), as previously described (Tavakoli et al., 2013). Four to six high-resolution (1-2 μm) images of the sub-basal plexus of each eye were obtained for all participants. A trained investigator who was blinded to participants’ details analysed corneal images separately.

Corneal Nerve Fibre Density: The number of main nerves per square millimetre was quantified and the mean derived from the right and left eye. Corneal nerve fibre quantification was undertaken using semi-automated, purpose-written, proprietary software (CCMetrics; M.A. Dabbah, Imaging Science and Biomedical Engineering, Manchester, UK).

5.3.4 Affective symptoms, apathy and pain

5.3.4.1 Depression and anxiety:

Affective symptoms were quantified using the Hospital Anxiety and Depression (HADS) Scale (Zigmond and Snaith, 1983). The HADS consists of 14 items, 7 of which relate to anxiety and the other 7 relate to depression. Each item is scored between 0-3. The overall score ranges from 0 to 42 with higher scores representing a greater degree of affective symptoms (Appendix D).
5.3.4.2  *Apathy:*

All participants underwent assessment of apathy using the Lille Apathy Rating Scale (LARS) (Sockeel et al., 2006). The LARS consists of 33 items, divided into nine domains. The score ranges from -36 to +36, with higher score representing a greater degree of apathy.

5.3.4.3  *Pain intensity:*

Pain was only quantified in PD patients as healthy controls were selected to be pain-free. The Short Form McGill Pain Questionnaire (SFMPQ) (Melzack, 1987) was used for scoring pain intensity. SFMPQ consists of 15 descriptors (11 sensory; 4 affective), which are rated on an intensity scale from 0-3. SFMPQ also includes a present pain intensity index scored from 0-5 and a visual analogue scale (0-10), which are also included to provide an overall pain intensity score.†

5.3.5  **Statistical analysis**

IBM SPSS version 22 was used to analyse the data. All data are expressed as mean ± SEM. Normality of distribution was assessed using histograms and the Shapiro-Wilk test. A repeated measure ANOVA with between subject factor of group (PD vs Controls) and within subject factor of stroking velocity was used. For the purpose of ANOVA a departure from normality of pleasantness ratings at

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† Some participants in this study took part in the previous study reported in chapter 4. The short form McGill pain questionnaires (but not King’s PD pain scales) were repeated for this study to ensure timely pain assessment.
0.3cm/sec was detected in control subjects therefore a square root transformation of all pleasantness ratings was undertaken to meet the assumption of normality. Individual means relating to demographics and other disease characteristics were compared using the student t test and where the data are not normally distributed the Mann-Whitney U test. Correlation between continuous variables was assessed using Pearson’s r coefficient. A p value of less than 0.05 was considered statistically significant. P value was not corrected for multiple comparisons.

5.4 Results

Participants’ characteristics are summarised in table 5-1. There was no significant difference in demographics between PD patients and control subjects. There was a significant positive correlation between perceived pleasantness at 3cm/sec and 30cm/sec and small fibre nerve density measured by both skin biopsies and corneal confocal microscopy in PD patients (p < 0.05) (Figure 5-1). Pain intensity in PD patients correlated positively with ratings of 0.3 cm/sec (Pearson’s r = 0.483, p = 0.02) but not with pleasantness ratings at other velocities (Figure 5-2). When pain scores were broken down to sensory and affective sub-scores similar results were obtained with only the sensory component of pain intensity correlating with pleasantness rating of slow brush strokes (Pearson’s r = 0.497, p = 0.019). No significant relation was found between affective touch ratings and HADS or apathy scores in either PD or
control subjects. Apathy and HADS scores were significantly higher in PD compared to controls (Table 5-1).

Table 5-1 Demographics and clinical characteristics of Parkinson’s disease (PD) patients and healthy controls. Perceived pleasantness to brush stroking marked on a 1-100 visual analogue scale is also provided. Data are presented as Means ± SEM (range). SFMPQ: Short Form McGill Pain Questionnaire. HADS: Hospital and Anxiety Scale. LARS: Lille Apathy Rating Scale.

<table>
<thead>
<tr>
<th></th>
<th>PD patients (n = 24)</th>
<th>Controls (n = 27)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>14 males, 10 females</td>
<td>11 males, 16 females</td>
<td>0.210</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.7 ± 1.6 (51–78)</td>
<td>63.6 ± 1.1 (50–71)</td>
<td>0.938</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>7.7 ± 0.9 years (1.2–18)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hoehn and Yahr Stage.</td>
<td>I = 8, II = 12, III = 4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SFMPQ</td>
<td>23 ± 2.6 (0–51)</td>
<td>8.8 ± 1.1 (0–20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HADS</td>
<td>18.9 ± 1.9 (2–34)</td>
<td>8.8 ± 1.1 (0–20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LARS</td>
<td>-20.4 ± 1.1 (-32–9)</td>
<td>-24 ± 0.8 (-32–13)</td>
<td>0.003</td>
</tr>
<tr>
<td>Pleasntness at 0.3cm/sec</td>
<td>30.3 ± 4.3 (5–77)</td>
<td>16.8 ± 2.8 (0–58)</td>
<td>0.017</td>
</tr>
<tr>
<td>Pleasntness at 3cm/sec</td>
<td>60.3 ± 4.2 (19–92)</td>
<td>55.0 ± 4.0 (19–92)</td>
<td>0.371</td>
</tr>
<tr>
<td>Pleasntness at 30cm/sec</td>
<td>48.3 ± 3.8 (19–87)</td>
<td>41.2 ± 4.6 (1–96)</td>
<td>0.247</td>
</tr>
</tbody>
</table>

For the ANOVA Mauchly’s test indicated that the assumption of sphericity had been violated, $\chi^2 (2) = 9.41, p = 0.009$, therefore multivariate tests are reported ($\epsilon = 0.85$). Analysis of variance revealed a substantial main effect for velocity with all participants reporting increased pleasantness at 3cm/sec compared to 0.3cm/sec and 30cm/sec (Wilks’ Lambda = 0.26, $F_{2,48} = 66.52, p < 0.001$, partial eta squared= 0.73). There was also a significant main effect of group with PD patients rating brush strokes as more pleasant across all three velocities ($F_{1,49} = 5$,
$p = 0.030$, partial eta squared = 0.093) but this was most notable at 0.3cm/sec (Figure 5-3 & Table 5-1).

**Figure 5-1** Correlation between pleasantness ratings at 3cm/sec and 30cm/sec and small fibre nerve density measured by skin biopsies (A & B) and corneal confocal microscopy (C & D). Pleasantness rating was measured with a 100mm visual analogue scale (VAS). Correlation coefficient and significance are also shown.
Figure 5-2 Correlation between chronic pain intensity measured by the Short Form McGill Pain Questionnaire (SFMPQ) and perceived pleasantness to slow brush stoking at 0.3 cm/sec. Pearson’s correlation was applied.
Figure 5-3 Mean and SEM of affective touch ratings in Parkinson’s disease (PD) and controls using three different velocities of brush strokes. Pleasantness is rated using a 0-100 visual analogue scale (VAS) and square rooted to meet the assumption of normality.

5.5 Discussion

We demonstrate a significant linear relationship between perceived pleasantness of gentle brush stroking and C-fibre density in PD patients. None of our PD patients had significant large fibre neuropathy therefore variation in pleasantness rating at each individual velocity are more likely to be explained by CT denervation rather than concomitant stimulation of Aβ fibres. The pleasantness ratings across the three velocities are consistent with CT afferent response
characteristics with significantly higher pleasantness at optimum stimulation velocity (3cm/sec) compared to velocities that are in the suboptimal range (0.3 cm/sec and 30 cm/sec) (Figure 5-3). Thus, our study further validates the role of CT afferents in affective touch perception but for the first time show a significant positive correlation with C-fibre density. The correlation between small fibre nerve density and pleasantness was demonstrated at optimum (3cm/sec) and fast (30 cm/sec) velocities but not at the very slow velocity of 0.3 cm/sec, which was also recorded as the least pleasant by participants (Figure 5-3). Similar pleasantness ratings have been observed in previous studies (Loken et al., 2009; Macefield et al., 2014) with very slow brush strokes being least effective for producing pleasantness despite microneurography showing equally low firing rate at very slow and very fast brush stroke (Loken et al., 2009). This phenomenon may be related to the differences in skin contact time and vertical force applied between 0.3cm/sec and 30cm/sec.

Small nerve fibre density was measured in two locations: the hairy skin of the dorsa of both feet and both corneas. Skin denervation in PD is likely to involve CT afferents although corneal denervation is not expected to include CT afferents given their specialist role in affective touch. Nevertheless, corneal confocal microscopy is an established technique for measuring C-fibre density, which correlates with skin biopsies (Kass-Iliyya et al., 2015; Quattrini et al., 2007).
Contrary to our hypothesis, PD patients compared to controls reported higher pleasantness to brush stroking across all velocities despite having significantly reduced C afferents. This was particularly noted at the slow speed (0.3cm/sec), which is the least effective for inducing pleasantness. Interestingly this abnormally high rating of 0.3cm/sec also correlated with pain intensity in PD indicating a potentially common pathophysiology relating to abnormal central sensitisation to reward and pain. This is plausible given the significant overlap between brain regions responsible for processing pain and reward (mesolimbic dopamine pathway, prefrontal medial cortex, insular cortex) as well as the common neurochemistry between the two systems (opioid & dopamine) (Leknes and Tracey, 2008; Schultz, 2007; Smith and Berridge, 2007; Wager et al., 2007). Indeed more recent evidence proposes a common neural substrate for chronic pain and addiction resulting in a similar reward seeking behaviour (Elman and Borsook, 2016; Leknes and Bastian, 2014).

Another potential explanation for the higher pleasantness rating in our PD patients is that they did not withdraw dopamine therapy during the experiment. Studies have shown that PD patients with impulse control disorders have higher release of dopamine in the ventral striatum during reward related tasks compared to patients who do not exhibit such behaviour (O'Sullivan et al., 2011; Steeves et al., 2009; Wu et al., 2015). It is possible therefore that an upregulation in central processing of sensory inputs due to exogenous dopamine resulted in enhanced ratings of brush strokes in our PD patients. Curiously this poses the question of
the potential role of dopamine in coding for affective touch perception. Central sensitisation to nociceptive inputs is also thought to be responsible for the increased prevalence and intensity of pain in PD although the exact mechanism is yet to be fully understood (Defazio et al., 2013b).

Although our PD patients had significantly higher scores of apathy and depression compared to controls we did not find a relationship with affective touch ratings at any of the three velocities. This suggests that variation in affective touch perception may not be an important cause for apathy or mood variations and other factors related to PD are more important.

The main limitation of the study is the assessment of affective touch in the “ON” state in PD. Assessing affective touch ratings in the practically defined “OFF” state after withdrawing dopaminergic medications would have been more representative of the influence of dopamine depletion and PD severity on affective touch perception. Nevertheless, we have documented rating patterns in PD that are consistent with CT afferent stimulation and in addition documented a significant correlation with nerve density.
CHAPTER 6

Discussion And Future Work
6.1 Conclusions

Across four studies this thesis explored the characteristics of pain in Parkinson’s disease and related disorders and addressed the role of small fibre neuropathy in PD symptomatology and pain utilising the technique of corneal confocal microscopy. We also assessed the perception of affective touch in PD for the first time and explored its relationship to pain intensity.

We have shown that pain is not only common in PD but is similarly common in MSA and in particular MSA-P. MSA-C patients reported lower pain prevalence compared to PD and MSA-P. Another important observation was that pain is significantly less prevalent in PSP compared to all the other parkinsonian disorders. These variations in pain profiles may have a diagnostic value particularly in the early stages of these conditions when diagnostic clues can be helpful. Currently the presence or absence of pain is not included in the diagnostic criteria of PD, MSA and PSP and this should be an area for future research.

We documented pain prevalence in early PD that is comparable to more advanced disease. In addition we have documented an important role of neuropathic mechanisms in our PD patients.
In clinical practice pain in PD is commonly ascribed to motor factors associated the conditions. However, several findings from this thesis favour a significant central sensitisation for pain in Parkinson’s disease:

1. The lack of relationship between motor disability and pain intensity (Chapter 2): We could not establish a connection between motor disability (as measured by the UPDRS-III) and pain intensity. This strengthens the argument that pain in PD is not a mere function of muscle rigidity, postural abnormalities and other musculoskeletal causes such as arthritis and spondylosis. It is still likely that these factors play a role in pain generation but on their own cannot explain the very high prevalence of musculoskeletal pain in PD. Work done by other groups have supported this finding showing no correlation between pain and UPDRS-III (Tinazzi et al., 2006) although there is evidence of increased pain intensity in advanced disease (Negre-Pages et al., 2008), which may not necessarily be explained by worsening motor disability.

2. The positive relationship between pain and in particular neuropathic pain and motor complications such as dyskinesia, on/off fluctuations that are manifestations of central sensitisation (Chapter 2): Motor fluctuations and dyskinesia are an aspect of PD symptomatology that arises with increasing sensitivity to dopamine therapy. In normal physiological state dopaminergic neurons have a constant rate of tonic activity (Grace and
Bunney, 1984) with occasional increased firing in association with rewards and unexpected stimulus (Suri, 2002). However, in PD there is depletion of intrinsic dopamine and a reduced capacity to buffer variation in exogenous dopamine levels, which results in abnormal fluctuations in dopamine concentration, and increased sensitivity to dopamine. In clinical practice dopamine depletion and sensitisation in PD can be observed as Levodopa induced dyskinesia (involuntary, choreiform purposeless movements induced by dopamine therapy) and impulse control behaviour (pathological gambling, hypersexuality, binge eating and compulsive buying) (Jimenez-Urbieta et al., 2015; Marechal et al., 2015; Sujith and Lane, 2009). The association between pain intensity and motor fluctuations is intriguing and has been replicated by other groups (Lim et al., 2008; Tinazzi et al., 2006). Given this association and available evidence of impaired central processing of nociceptive inputs it is plausible to conclude that central sensitisation is an important mechanism of pain in Parkinson’s disease.

3. The high prevalence of pain in early PD when the motor syndrome is easily treatable and of mild severity (Chapter 3): We have shown that pain is very common not only in advanced PD but also with those of disease duration of less than three years. Pain in early PD has not been studied before. In the early stages of PD, muscle rigidity, postural instability and bradykinesia are often readily treatable with dopaminergic
therapy and most patients can function at a near normal level from a motor perspective. The high prevalence of pain in this patients sub-group is another argument against pain in PD being a mere function of musculoskeletal factors although this finding does not, on its own, prove central sensitisation.

4. The inverse correlation between heat pain threshold and pain intensity in PD indicates that those patients who report higher pain intensity also have lower pain tolerance (Chapter 4). This finding indicates sensitisation to experimental heat pain in painful PD phenotypes. However our data also showed increased heat pain threshold compared to controls. This discrepancy from previous reports may be explained by the sensory denervation in our PD patients. Given that previous evidence indicates impaired central processing of nociceptive inputs in addition to peripheral denervation in PD it is plausible that sensitisation to pain can be mediated either centrally or peripherally. However, in the context of above arguments central sensitisation appears more likely. Interestingly a recent electrophysiological study has supported this showing that the abnormal central processing of nociceptive inputs is driven centrally rather than peripherally (Zambito-Marsala et al., 2016).

5. The correlation between pain intensity and an abnormally high pleasantness rating of skin stimulation (Chapter 5): Contrary to our
expectations in our last study I found an abnormally high pleasantness rating to slow brush stroking in PD patients compared to control subjects. This is despite evidence of peripheral denervation. Interestingly this correlated with reported pain intensity in PD patients. There was also a correlation between peripheral C fibre nerve density and perceived pleasantness to brush stroking supporting their role in affective touch perception. No other group has assessed the perception of affective touch in PD. The enhanced pleasantness in PD despite peripheral denervation and the positive correlation with nerve density all argue for central sensitisation of afferent inputs in PD. However, one cannot draw firm conclusions on the central processing of affective touch in PD without more conclusive methods using functional imaging or neurophysiological studies.

A consistent finding across all studies has been the association of pain with negative affect. This is widely reported in the literature and is not a new finding (Bair et al., 2003; Gureje et al., 2008; Means-Christensen et al., 2008). It is difficult to establish whether the negative affect is a result of the negative experience of pain or whether there is a common underlying pathophysiology. Serotonine and noradrenaline depletion is a feature of PD. These neurotransmitters are implicated in both descending pain suppression and negative affect. As such, reduced level of these monoamines within the brain could explain both pain and depression. Very few studies have assessed the
efficacy of antidepressants as treatment for pain in PD. Interestingly one study found that duloxetine was beneficial for pain in the context of PD (Djaldetti et al., 2007) which calls for more trial of antidepressants as a treatment for pain in PD.

Prior to conducting this research we hypothesised that small fibre neuropathy was a contributory cause for pain in PD. However, when small fibre nerves and pain were quantified we could not establish any relationship between small fibre neuropathy in PD and pain. Therefore one can conclude that the relationship between small nerve fibre density and pain in PD is not linear. Despite the lack of a linear relationship it is still possible that peripheral denervation can cause pain in PD in a way that is similar to diabetic neuropathy where there is causality but no correlation with pain intensity (Sorensen et al., 2006). Nevertheless, central sensitisation to nociceptive inputs is likely to be more important than small fibre neuropathy in PD pain. This has been further supported by a recent neurophysiological study measuring laser evoked potentials (Zambito-Marsala et al., 2016).

There are a number of limitations to each of the studies presented in this work and these are mentioned in the relevant chapters. The body of work presented in this thesis has focused on descriptive studies of pain characteristics and the contribution of peripheral factors. More studies with a focus on the central processing of nociceptive inputs in PD are needed and this will be addressed in
Some of the future work mentioned below. Another limitation is the small sample size in chapter two, four and five. This was largely due to recruiting patients from a single centre. Performing regression analysis requires a large sample size and this is particularly important due to the multifactorial aetiology of pain in PD. The issue of sample size will be addressed when the study presented in chapter three is complete however for the purpose of this thesis sample size remains a limitation.

Successfully treating pain in Parkinson’s disease is an unmet need, which has led to renewed efforts to characterise this complex symptom and understand the mechanisms underlying it.

I hope that the studies presented in this thesis have added to the available pool of knowledge about pain and about Parkinson’s disease in general. The quest will continue to better understand this common non-motor symptom and develop effective treatment and improve the quality of life of PD sufferers.

6.2 Future work

6.2.1 The Parkinson’s pain study

The demographic and detailed clinical information from the ProBaND study are expected to add significant insights into the mechanisms of pain in PD. These data will be obtained in the coming months and will form the basis of a future
study but as seen in chapter 3 preliminary results already suggest a lesser role for peripheral motor factors for pain generation in PD. It is important that any future trial of pharmacological therapy for pain in PD consider central sensitisation as a potential target.

6.2.2 Corneal confocal microscopy in PD

Although we did not prove a relationship between peripheral denervation and pain in PD we have for the first time documented significant corneal denervation in PD patients compared to age matched controls. The prospect of using corneal microscopy in PD to visualise peripheral nerve pathology longitudinally, non-invasively and reiteratively provides a promising opportunity for a potential non-invasive biomarker. A grant application to use corneal confocal microscopy in a longitudinal study in PD patients has been submitted to assess the utility of this technique as a biomarker for Parkinson’s disease.

6.2.3 Affective touch in PD

The perception of the affective touch in PD will be further studied in the “OFF” and “ON” state to ascertain the role of dopamine in this modality of touch and its relation to pain.

6.2.4 Exploring the central processing of pain in PD

Further studies are underway to ascertain whether an altered top down processing of nociceptive inputs is involved in PD. This will be done through investigating the cortical response to pain or the anticipation of pain in PD patients using source localisation EEG. Amino acid depletion in healthy subjects will also be
used to investigate the role of individual neurotransmitters such as dopamine, noradrenaline and serotonin in pain processing. This may need to be further explored with functional imaging that can measure receptor activity such as dopamine sensitivity and pain.
APPENDICES
Appendix A.
The Short Form of McGill Pain Questionnaire is designed to give a numerical value to the intensity of pain with sub-scores for sensory and affective aspects of pain.

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>None (0)</th>
<th>Mild (1)</th>
<th>Moderate (2)</th>
<th>Severe (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throbbing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shooting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stabbing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sharp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cramping</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gnawing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hot-burning</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heavy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splitting</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Tiring/</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Exhausing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sickening</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fearful</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Punishing-Cruel</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Present Pain Intensity:
0 = No pain
1 = Mild
2 = Discomforting
3 = Distressing
4 = Horrible
5 = Excruciating

0 --------------------------------------------------------------------------------------------------------------- 10
No Pain                                   Worst Possible Pain

A score is derived from the sensory dimension of pain experience (descriptors 1-11) and the emotional dimension of pain experience (descriptors 12-15). Each descriptor is ranked on an intensity scale of 0 = none, 1 = mild, 2 = moderate, 3 = severe. The present pain intensity and the visual analogue scale are also included to provide an overall intensity scores.
Appendix B

The LANSS (Leeds Assessment of Neuropathic Symptoms and Signs) is designed to distinguish neuropathic from nociceptive pain reliably. It incorporates a brief examination by gently stroking the skin in the painful area to determine the presence of allodynia (Pain induced by non-painful stimuli) as well as eliciting the presence of altered pinprick sensation by comparing the sensation in the painful and nonpainful area using a small needle. This scale can be administered by non-specialists and it has shown high sensitivity and specificity.

The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) Pain Scale

A. PAIN QUESTIONNAIRE

Think about how your pain has felt over the last week. Please say whether any of the descriptions match your pain exactly.

1. Does your pain feel like strange, unpleasant sensations in your skin? Words like pricking, tingling, pins and needles might describe these sensations.
   a) NO – My pain doesn’t really feel like this……………………………….. (0)
   b) YES – I get these sensations quite often……………………………..(5)

2. Does your pain make the skin in the painful area look different from normal? Words like mottled or looking more red or pink might describe the appearance.
   a) NO – My pain doesn’t affect the colour of my skin……………………......... (0)
   b) YES – The pain does make my skin look different from normal ………..(5)

3. Does your pain make the affected skin abnormally sensitive to touch? Getting unpleasant sensations when lightly stroking the skin, or getting pain when wearing tight clothes might describe the abnormal sensitivity.
   a) NO – My pain doesn’t make my skin abnormally sensitive in that area (9)
   b) YES – My skin seems abnormally sensitive to touch in that area..............(3)

4. Does your pain come on suddenly and in bursts for no apparent reason when you’re still? Words like electric shocks, jumping and bursting describe these sensations.
   a) NO – My pain doesn’t really feel like this………………………………….. (0)
   b) YES – I get these sensations quite often…………………………………..(2)

5. Does your pain feel as if the skin temperature in the painful area has changed abnormally? Words like hot and burning describe these sensations.
   a) NO – I don’t really get these sensations………………………………….. (0)
   b) YES – I get these sensations quite often………………………………….. (1)

B. SENSORY TESTING

Skin sensitivity can be examined by comparing the painful area with a contralateral or adjacent non-painful area for the presence of allodynia and an altered pinprick threshold (PPT).

1. Allodynia

Examine the response to lightly stroking cotton wool across the non-painful area and then the painful area. If normal sensations are experienced in the non-painful site, but pain or unpleasant sensations (tingling, nausea) are experienced in the painful area when stroking, allodynia is present.
   a) NO – Normal sensations in both areas …………………………………….. (0)
   b) YES – Allodynia in painful area only …………………………………….. (5)

2. Altered pinprick threshold

Determine the pinprick threshold by comparing the response to a 23-gauge (blue) needle mounted inside a 2ml syringe barrel placed gently onto the skin in non-painful and then painful areas. If a sharp pinprick is felt in the non-painful area, but a different sensation is experienced in the painful area, eg. none/ blunt only (raised PPT) or a very painful sensation (lowered PPT), an altered PPT is present.

If a pinprick is not felt in either area, mount the syringe onto the needle to increase the weight and repeat.
   a) NO – Equal sensation in both areas …………………………………….. (0)
   b) YES – Altered PPT in painful area………………………………….. (3)

SCORING:

Add values in parentheses for sensory description and examination findings to obtain overall score.

TOTAL SCORE: _________ (maximum 24)

If score < 12, neuropathic mechanisms are unlikely to be contributing to the patient’s pain.
If score ≥ 12, neuropathic mechanisms are likely to be contributing to the patient’s pain.
Appendix C

Patients are asked to locate their pain on a body map.
Appendix D

The Hospital Anxiety and Depression Scale

**Hospital Anxiety and Depression Scale (HADS)**

Tick the box beside the reply that is closest to how you have been feeling in the past week. **Don't take too long over your replies; your immediate is best.**

<table>
<thead>
<tr>
<th>D</th>
<th>A</th>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Most of the time</td>
<td>3</td>
<td>Nearly all the time</td>
</tr>
<tr>
<td>2</td>
<td>A lot of the time</td>
<td>2</td>
<td>Very often</td>
</tr>
<tr>
<td>1</td>
<td>From time to time, occasionally</td>
<td>1</td>
<td>Sometimes</td>
</tr>
<tr>
<td>0</td>
<td>Not at all</td>
<td>0</td>
<td>Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>A</th>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I feel tense or 'wound up':</td>
<td>0</td>
<td>I feel as if I am slowed down:</td>
</tr>
<tr>
<td>1</td>
<td>Not quite so much</td>
<td>1</td>
<td>Occasionally</td>
</tr>
<tr>
<td>2</td>
<td>Only a little</td>
<td>2</td>
<td>Quite Often</td>
</tr>
<tr>
<td>3</td>
<td>Hardly at all</td>
<td>3</td>
<td>Very Often</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>A</th>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I get a sort of frightened feeling as if something awful is about to happen:</td>
<td>0</td>
<td>I have lost interest in my appearance:</td>
</tr>
<tr>
<td>1</td>
<td>Very definitely and quite badly</td>
<td>1</td>
<td>Definitely</td>
</tr>
<tr>
<td>2</td>
<td>Yes, but not too badly</td>
<td>2</td>
<td>I don't take as much care as I should</td>
</tr>
<tr>
<td>3</td>
<td>A little, but it doesn't worry me</td>
<td>3</td>
<td>I may not take quite as much care</td>
</tr>
<tr>
<td>4</td>
<td>Not at all</td>
<td>4</td>
<td>I take just as much care as ever</td>
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<th>D</th>
<th>A</th>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>As much as I always could</td>
<td>0</td>
<td>Very much indeed</td>
</tr>
<tr>
<td>1</td>
<td>Not quite so much now</td>
<td>1</td>
<td>Not very much</td>
</tr>
<tr>
<td>2</td>
<td>Definitely not so much now</td>
<td>2</td>
<td>Not at all</td>
</tr>
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<table>
<thead>
<tr>
<th>D</th>
<th>A</th>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Worrying thoughts go through my mind:</td>
<td>0</td>
<td>I look forward with enjoyment to things;</td>
</tr>
<tr>
<td>1</td>
<td>A great deal of the time</td>
<td>1</td>
<td>As much as I ever did</td>
</tr>
<tr>
<td>2</td>
<td>A lot of the time</td>
<td>2</td>
<td>Rather less than I used to</td>
</tr>
<tr>
<td>3</td>
<td>From time to time, but not too often</td>
<td>3</td>
<td>Definitely less than I used to</td>
</tr>
<tr>
<td>4</td>
<td>Only occasionally</td>
<td>4</td>
<td>Hardly at all</td>
</tr>
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<tr>
<th>D</th>
<th>A</th>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I feel cheerful:</td>
<td>0</td>
<td>I get sudden feelings of panic:</td>
</tr>
<tr>
<td>1</td>
<td>Not all</td>
<td>1</td>
<td>Very often indeed</td>
</tr>
<tr>
<td>2</td>
<td>Not often</td>
<td>2</td>
<td>Quite often</td>
</tr>
<tr>
<td>3</td>
<td>Sometimes</td>
<td>3</td>
<td>Not very often</td>
</tr>
<tr>
<td>4</td>
<td>Most of the time</td>
<td>4</td>
<td>Not at all</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D</th>
<th>A</th>
<th>D</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>I can sit at ease and feel relaxed:</td>
<td>0</td>
<td>I can enjoy a good book or radio or TV program:</td>
</tr>
<tr>
<td>1</td>
<td>Not at all</td>
<td>1</td>
<td>Oftentimes</td>
</tr>
<tr>
<td>2</td>
<td>Usually</td>
<td>2</td>
<td>Not often</td>
</tr>
<tr>
<td>3</td>
<td>Not Often</td>
<td>3</td>
<td>Very seldom</td>
</tr>
</tbody>
</table>

**Scoring:**

- **Total score:** Depression (D) _______ Anxiety (A) _______
- 0-7 = Normal
- 8-10 = Borderline abnormal (borderline case)
- 11-21 = Abnormal (case)
Appendix E
The scale for outcomes in Parkinson’s disease - autonomic symptoms

SCOPA-AUT

By means of this questionnaire, we would like to find out to what extent in the past month you have had problems with various bodily functions, such as difficulty passing urine, or excessive sweating. Answer the questions by placing a cross in the box which best reflects your situation. If you wish to change an answer, fill in the ‘wrong’ box and place a cross in the correct one. If you have used medication in the past month in relation to one or more of the problems mentioned, then the question refers to how you were while taking this medication. You can note the use of medication on the last page.

1. In the past month, have you had difficulty swallowing or have you choked?
   □ never □ sometimes □ regularly □ often

2. In the past month, has saliva dribbled out of your mouth?
   □ never □ sometimes □ regularly □ often

3. In the past month, has food ever become stuck in your throat?
   □ never □ sometimes □ regularly □ often

4. In the past month, did you ever have the feeling during a meal that you were full very quickly?
   □ never □ sometimes □ regularly □ often

5. Constipation is a blockage of the bowel, a condition in which someone has a bowel movement twice a week or less.
   In the past month, have you had problems with constipation?
   □ never □ sometimes □ regularly □ often

6. In the past month, did you have to strain hard to pass stools?
   □ never □ sometimes □ regularly □ often
7. In the past month, have you had involuntary loss of stools?
   never  sometimes  regularly  often

8. In the past month, have you had difficulty retaining urine?
   never  sometimes  regularly  often  use catheter

9. In the past month, have you had involuntary loss of urine?
   never  sometimes  regularly  often  use catheter

10. In the past month, have you had the feeling that after passing urine your bladder was not completely empty?
    never  sometimes  regularly  often  use catheter

11. In the past month, has the stream of urine been weak?
    never  sometimes  regularly  often  use catheter

12. In the past month, have you had to pass urine again within 2 hours of the previous time?
    never  sometimes  regularly  often  use catheter

13. In the past month, have you had to pass urine at night?
    never  sometimes  regularly  often  use catheter
14. In the past month, when standing up have you had the feeling of either becoming lightheaded, or no longer being able to see properly, or no longer being able to think clearly?

never  sometimes  regularly  often

15. In the past month, did you become light-headed after standing for some time?

never  sometimes  regularly  often

16. Have you fainted in the past 6 months?

never  sometimes  regularly  often

17. In the past month, have you ever perspired excessively during the day?

never  sometimes  regularly  often

18. In the past month, have you ever perspired excessively during the night?

never  sometimes  regularly  often

19. In the past month, have your eyes ever been over-sensitive to bright light?

never  sometimes  regularly  often

20. In the past month, how often have you had trouble tolerating cold?

never  sometimes  regularly  often

21. In the past month, how often have you had trouble tolerating heat?

never  sometimes  regularly  often
The following questions are about sexuality. Although we are aware that sexuality is a highly intimate subject, we would still like you to answer these questions. For the questions on sexual activity, consider every form of sexual contact with a partner or masturbation (self-gratification). An extra response option has been added to these questions. Here you can indicate that the situation described has not been applicable to you in the past month, for example because you have not been sexually active. Questions 22 and 23 are intended specifically for men, 24 and 25 for women.

The following 3 questions are only for men

22. In the past month, have you been impotent (unable to have or maintain an erection)?
   
   never    sometimes    regularly    often    not applicable

23. In the past month, how often have you been unable to ejaculate?
   
   never    sometimes    regularly    often    not applicable

23a. In the past month, have you taken medication for an erection disorder? (If so, which medication?)
   
   no    yes: ______________________

Proceed with question 26

The following 2 questions are only for women

24. In the past month, was your vagina too dry during sexual activity?
   
   never    sometimes    regularly    often    not applicable

25. In the past month, have you had difficulty reaching an orgasm?
   
   never    sometimes    regularly    often    not applicable
The following questions are for everyone

The questions below are about the use of medication for which you may have or have not needed a doctor’s prescription. If you use medication, also give the name of the substance.

26. In the past month, have you used medication for:
   
a. constipation? [ ] [ ]
   
d. urinary problems? [ ] [ ]
   
   no   yes: __________________________
   
e. blood pressure? [ ] [ ]
   
   no   yes: __________________________
   
f. other symptoms (not symptoms related to Parkinson’s disease) [ ] [ ]
   
   no   yes: __________________________
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