Neuropathic pain in endometriosis

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Abstract

Chronic pelvic pain is one of the main symptoms of endometriosis which affects 1 in 10 women of reproductive age. Historically, despite the prevalence of this condition and the impact it has on those affected, endometriosis-associated pain (EAP) has been poorly understood. In this thesis I explore endometriosis-associated pain, specifically the presence of neuropathic pain (pain due to lesion/disease of the somatosensory system). To do this, I use a variety of different techniques including questionnaires, functional magnetic resonance imaging (fMRI) and quantitative sensory testing (QST).

To determine the prevalence of neuropathic pain in a large population is difficult. In this thesis I use an online questionnaire on patient support websites to give an estimation of the proportions of women experiencing neuropathic pain. I investigate differences between those with and without neuropathic pain with respect to pain symptoms, psychological and cognitive function, number of surgeries to the abdomen and duration of pain. I also sub-divided women further based on their sensory symptom profiles. I replicate these findings in a novel cohort and relate them to treatment response.

Central nervous system changes have been demonstrated in people with neuropathic pain. In this thesis I investigate the association between neuropathic pain features and functional connectivity of the brain in women with endometriosis-associated pain.

Sensory testing is an important aspect of neuropathic pain research and in this thesis I use QST to investigate sensory perception. A range of stimuli are used to determine whether there are changes in sensory processing in women with endometriosis-associated pain.

Overall, the aims of the research described in this thesis are to advance knowledge regarding the prevalence of neuropathic pain in endometriosis; how women can be stratified using questionnaires and whether that relates to treatment response; what changes associated with neuropathic pain can be seen in the activity of the brain at rest; and the sensory profiles of women with EAP. Research in the area of EAP is critical to inform clinical practice and improve treatment.

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* publications relevant to this thesis

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Chapter 1: Introduction

Parts are adapted from Coxon, Horne & Vincent 2018 'Pathophysiology of endometriosis-associated pain: A review of pelvic and central nervous system mechanisms'[1]; material produced by me with permission from other authors.

1.1 Thesis Overview

The need for better ways to assess endometriosis-associated pain and to treat it is characterised in this quote from 'Pain and Prejudice A call to arms for women and their bodies'[2] written by journalist and endometriosis sufferer Gabrielle Jackson:

> 'I know about pain, I thought. I've had a broken back, been run over by a train! If anybody knows what ten is on the 1 to 10 pain scale that they ask you about every hour in hospital, it's me! I know what ten is – it's a broken back; it's a broken shoulder, sprained ankle, head injury and bruised hip lying under a train in Mumbai. That's ten. But that pain was focused and intense, and painkillers worked. They gave me relief, even if brief, from the pain and made me happy: grateful to be alive, not paralysed, in possession of all my limbs, able to make jokes and tell good stories. That pain faded, and I felt better.
>
> But this pain – this pain after the laparoscopy and the pain of endometriosis – is nothing like that. This ache, this always-present gnawing, is the pain that makes me feel ***bad***. No painkiller puts me in a happy mood with this pain. No bliss overcomes me. No drug can stop the nausea once it arrives. Only stillness helps. But stillness is so hard. I've felt ten, and I can tell you, this pain is worse.'

This excerpt highlights the unmet need in endometriosis-associated pain, which forms the basis on which this thesis is grounded. Historically endometriosis-associated pain has been poorly defined and thus the understanding of it has been limited. More recent work is trying to rectify this by looking at different ways to measure pain and the different pain types women experience. This research has enabled us to have a better understanding of mechanisms which are giving rise to endometriosis but has focused only on nociceptive pain (pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors). In this thesis, I investigate the presence of neuropathic pain in endometriosis (pain due to lesion/disease of the somatosensory nervous system). In Chapter 3 I explore the prevalence of neuropathic pain in endometriosis in a large cohort using an online survey. I replicate these findings in chronic pelvic pain in Chapter 4, investigating response to treatment with gabapentin in women with neuropathic pain. In Chapters 5 and 6, I study changes in the central nervous system, specifically the resting state connectivity of the brain associated with painDETECT[3] (a screening tool for neuropathic pain). Finally, in Chapter 7 I utilise Quantitative Sensory Testing (QST), an alternative method to assess neuropathic pain, to investigate the sensory phenotype of women with endometriosis-associated pain.

This multi-modal approach to assessing a neuropathic component to endometriosis-associated pain is preceded by background information relevant to this thesis. Firstly, I will define endometriosis and pain, and briefly explain current knowledge. Next, I will discuss what we know about mechanisms which give rise to endometriosis-associated pain, focussing firstly on the periphery and then on the central nervous system. Thirdly, I will define neuropathic pain and briefly describe mechanisms of neuropathic pain, the rationale for hypothesising the presence of neuropathic pain in endometriosis, as well as

the assessment and treatment of neuropathic pain. Finally, I will lay out the aims of this thesis.

1.2 Endometriosis

1.2.1 What is endometriosis?

Endometriosis is a common chronic inflammatory disease which is characterised by the presence of tissue resembling the endometrium outside the uterus (endometriotic lesions)[4]. The main symptom of endometriosis is chronic pelvic pain (i.e. pain perceived to originate in the pelvis lasting for longer than 3 months) as well as sub-fertility[4]. However, the disease is extremely heterogeneous[5] in its presentation, with some women experiencing debilitating pain and others no pain at all; as well as variations in the types of pain experienced (most commonly dysmenorrhea, pain during and after sexual intercourse and non-cyclical pain). Endometriosis is estimated to affect 1 in 10 women of reproductive age, approximately 176 million women worldwide[4,6].

The average time taken to diagnose endometriosis is estimated to be 8 years in the UK (a state-funded health care system), which has not shown improvement in the last two decades[7]. This diagnostic delay adds to the burden on women with endometriosis and may lead to a worsening of their symptoms. Part of the reason for this delay in diagnosis is a lack of awareness in the general population and in health care providers about what endometriosis is and what its symptoms can be. Currently the only way to clinically diagnose endometriosis is through surgery[4]. Availability of both facilities and clinicians to carry out these surgeries therefore determine when and how women receive the diagnosis of endometriosis.

One cause of endometriotic lesions is widely accepted to be retrograde menstruation (the back-ward flux of menstrual debris through the fallopian tubes into the pelvic cavity)[8]. This is supported by the fact that lesions often occur in regions of the pelvic cavity as would be expected due to the force of gravity. However, retrograde menstruation has been shown to occur in 90% of menstruating women with fallopian tubes[9], but only a small proportion develops endometriosis. Current efforts in endometriosis research focus on how the endometrial tissue adheres and forms lesions on ovaries/peritoneal surfaces as well as why this does not happen in all instances of retrograde menstruation[4].

1.2.2 Genetic factors in endometriosis

Genetic 'vulnerability' is one such mechanism which is being investigated currently, with approximately 50% of the risk of developing endometriosis coming from genetic factors[10]. A meta-analysis of 15 Genome Wide Association Studies (GWAS) found 27 genetic loci associated with endometriosis[11]. A sub-phenotypic analysis of data from the UK BioBank (a large-scale biomedical database and research resource) looking at the genetic correlation between endometriosis and nine different pain symptoms found that seven showed a significant positive correlation, suggesting that there is a genetic basis for sensitivity to endometriosis-associated pain[11].

1.2.3 Surgical diagnosis

To clinically diagnose endometriosis, laparoscopic surgery is required during which the surgeon locates and stages endometriosis[4]. Endometriosis can be staged using the revised criteria from the American Fertility Society[12], into four stages and is based on

surgical findings, including lesion size, location and extent of adhesions. There is no correlation between this staging of endometriosis and the severity of symptoms[13,14].

1.2.4 Treatment of endometriosis

The main treatment of endometriosis is the surgical removal of the ectopic tissue and/or, as the growth of endometriotic lesions is oestrogen dependent, hormonal treatment (including oral contraceptives, progestins and gonadotropin-releasing hormone)[4,15]. These treatments are often ineffective in reducing pain symptoms in women suffering with endometriosis-associated pain[16,17] and are associated with unwanted side effects such as mood alterations, menopause-related symptoms and contraception.

The pain experienced as a symptom of endometriosis is often not treated with anything more than over the counter drugs such as paracetamol and ibuprofen, which have limited effectiveness[18].

There is currently a push to provide women access to a multidisciplinary team, including a pain psychologist, gynaecologist and pelvic pain physiotherapist, with the hope such a team would be able to deliver both 'traditional' hormonal and surgical therapies in addition to chronic pain management. Such clinics already exist in a handful of centres and describe both clinical success and high levels of patient satisfaction[19].

1.3 Pain

Before specifically considering endometriosis-associated pain, it is important to understand pain more generally. Pain is defined as "an unpleasant sensory and

emotional experience associated with, or resembling that associated with, actual or potential tissue damage, or described in terms of such damage"[20]. Pain is a very complex, subjective experience and is thus difficult to pin down. However, it can be described both in terms of the mechanism(s) by which it arises and by the location/triggers of the pain. For definitions of pain types see Table 1.

Specific receptors (nociceptors) in the periphery detect noxious stimuli and this message then travels to the brain via the spinal cord (nociception) where the conscious experience of pain is generated. As illustrated in Figure 1, throughout this pathway a variety of different factors can exert an influence leading to either amplification or reduction and thus altering the overall pain perceived. The pain experience is therefore produced from a combination of many different factors, including nociception, context, mood and past experiences and is therefore not necessarily linearly related to the noxious input. This is evident in the brain areas that are active during the perception of pain; areas involved in sensory, discriminatory, affective, emotional, cognitive, motor, decision-making and brainstem modulatory circuits are recruited, but notably in a very flexible manner[21]. There are many mechanisms by which the pain perceived could be amplified or reduced. For example, one pathway that leads to a reduction in the pain perception is endogenous pain inhibition, which arises from cortical and spinal mechanisms.

The combination of brain areas which are involved in the perception of pain have been coined the 'pain neuromatrix'[22]. First thought of as a fixed combination of brain areas which were involved in pain perception in every brain, the idea has since evolved into the 'cerebral signature'[23], a dynamic network of brain regions which varies between individuals reflecting the complexity and individuality of the pain experience. The role

of different brain regions depends on the interaction of many different factors (mood, cognition etc). Some brain areas are very commonly seen to be active in pain perception, whilst other areas appear to be active depending on the circumstances of the pain.

Although ongoing pain can reflect continuing tissue damage, pain can also continue beyond normal tissue healing time. This 'chronic' pain is not simply a continuation of an acute pain but has its own mechanisms[21]. Although we are only beginning to understand the factors that put an individual at risk of developing chronic pain after an acute injury[24,25], the mechanisms maintaining and the changes associated with chronic pain are remarkably consistent no matter where the pain is perceived or what the underlying pathology[26–28]. In fact, this has led to the suggestion that chronic pain should be considered as a disease in its own right[27]. Given its association with chronic pain it is probably therefore not surprising that we are beginning to see many parallels between endometriosis-associated pain and other chronic pain conditions, as I will discuss below.

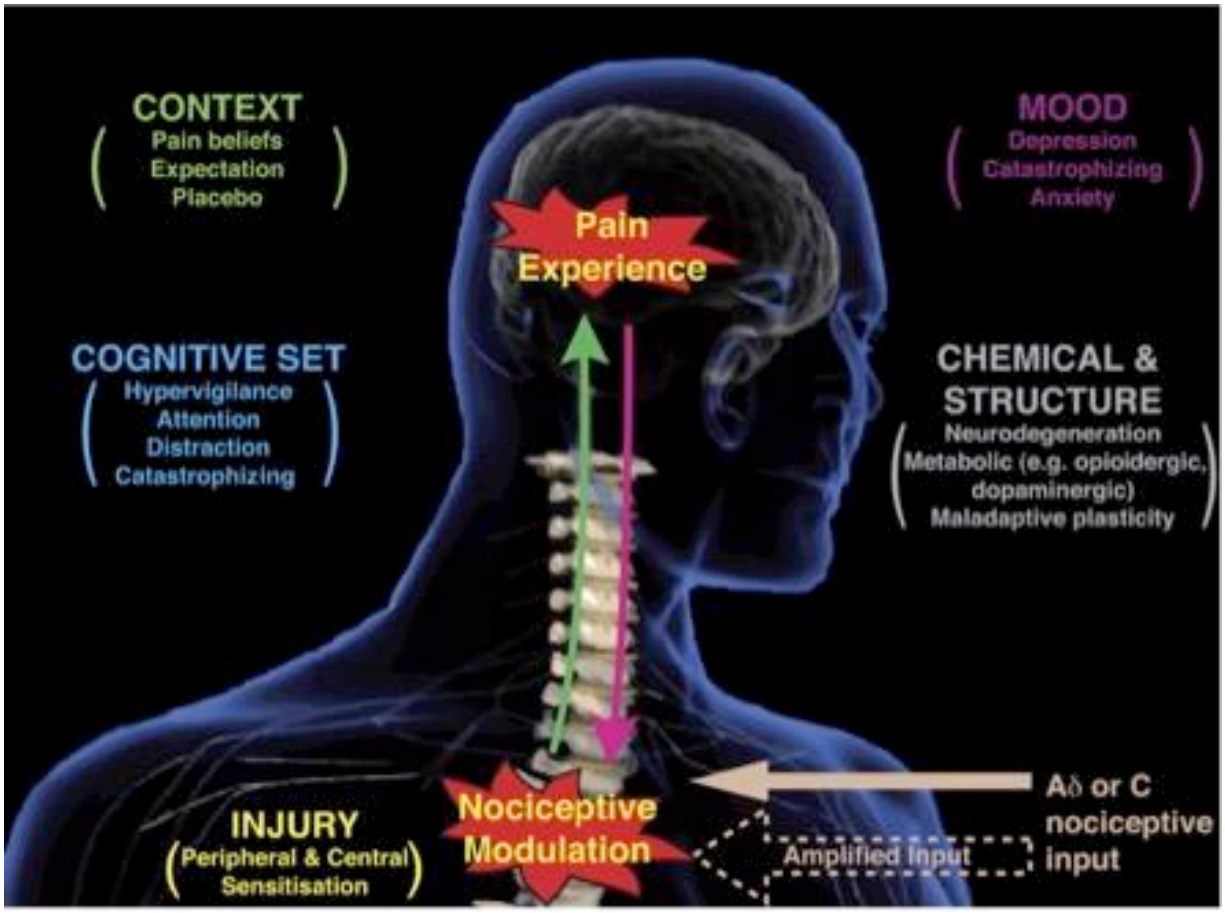

Figure 1: Pain perception can be influenced by a variety of factors. The nociceptive input from the periphery travels to the spinal cord via Aδ or C fibres, this can be amplified by peripheral sensitisation (discussed below). There is both ascending (to brain) and descending (from brain) nociceptive modulation of the pain experience, shown by arrows. This nociceptive modulation can also be altered by peripheral and central sensitisation, often arising from injury. There are many factors that also centrally affect the pain experience: chemical and structure, cognitive set, context and mood. These can be further broken down and can have a variety of effects on the pain experience. Taken from Tracey & Mantyh 2007[23]. Reprinted from Neuron, Vol 55, Issue 3, Tracey & Mantyh, The Cerebral Signature for Pain Perception and Its Modulation, Pages 377-391, Copyright (August 2007), with permission from Elsevier.

Term	Definition	Endometriosis Setting
Nociceptive pain	Pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors (sensory receptor of the peripheral nervous system capable of transducing noxious stimuli). Nociceptive pain can be divided into visceral and somatic depending on the location.	Visceral nociceptive C-fibres activated by noxious stimuli from cells in target organs have been implicated as mediators of noxious stimulus intensity.
Inflammatory pain	Pain associated with active inflammation. It falls in the category of nociceptive pain.	Endometriotic implants cause a local inflammatory reaction, which irritates nerves endings[29]. Nerve fibres also play an active role in the mechanism of inflammatory pain by secreting proinflammatory neuromediators[30]. This is called neurogenic inflammation.
Neuropathic pain	Pain caused by a lesion or disease of the somatosensory nervous system. Neuropathic pain is a clinical description and not a diagnosis which requires a demonstrable lesion or a disease that satisfies established neurological diagnostic criteria.	Recent work suggests that a small proportion of women with endometriosis-associated pain have definite neuropathic pain, however more than half may have a mixed nociceptive-neuropathic picture[31].
Centralized pain	Pain with central nervous system origins of amplification[32]. This does not imply that peripheral nociceptive input is not contributing to the pain experience.	Evidence is beginning to emerge of these phenomena in women with endometriosis-associated pain[33].

Hyperalgesia	Increased pain from a stimulus that normally provokes pain.	Regional hyperalgesia has been observed most commonly in women with current, biopsy-proven endometriosis compared to those with pain only. It was significantly higher in both groups compared with healthy controls. Increased behavioural responses to noxious stimuli at a distant site have been demonstrated in women with chronic pelvic pain and endometriosis, likely reflecting plastic changes in the central nervous system[34].
Allodynia	Pain due to a stimulus that does not normally provoke pain.	In women with chronic pelvic pain, allodynia is detected more often than in healthy controls[34].
Dysmenorrhea	Pain during menstruation.	In 78.7% of women with endometriosis dysmenorrhea was a symptom that led to a diagnosis[35].
Dyschezia	Pain during defaecation.	In 29% of women led to diagnosis[35].
Dysuria	Pain during urination.	Led to diagnosis in 9.9% of women[35].
Dyspareunia	Pain during sexual activity. Can be 'deep' or 'superficial'.	44.9% of women reported this led to diagnosis[35].
Nociplastic pain	Pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion to the somatosensory system causing pain[228].	Nociplastic pain is a relatively new term, which therefore has not been specifically investigated in endometriosis. There is debate around the similarities between this term and Centralized Pain defined above.

Table 1: Definitions of pain terminology and its relevance in the endometriosis setting. Definitions from the Kyoto protocol of IASP Basic Terminology[20,36]. Table adapted and updated from Morotti et al 2017[37].

1.4 Pain in women

It would be remis in a thesis investigating chronic pain in women to not discuss, albeit not in detail, the greater context of the issue of pain in women.

The differences between the sexes in both acute and chronic pain were historically not considered but in recent work this area is starting to become investigated. Whilst it is beyond the scope of this introduction to discuss in any detail these differences, I want to summarise some of the general findings. Firstly, many chronic pain conditions, such as migraine, fibromyalgia, irritable bowel syndrome, osteoarthritis and lower back pain are all more common in women than men[38–40], although exactly why is not fully understood[41] and is likely multi-factorial. There is evidence that the majority of chronic pain patients are women[42–45]. Despite this fact 79% of pain studies, until recent changes in requirements, were conducted on men or male mice[46]. Studies have shown surprising differences in how women's pain is perceived by other people, including health care professionals, with studies showing that men reporting the same symptoms are more likely to receive treatment than women[44,47–50].

The social context of pain in women is also an important consideration for this thesis. Chronic pain, along with other 'invisible' conditions, is the victim of many social prejudices and stigmas, this is especially true for women[2,44,51,52]. This issue is confounded by stigmas associated with discussion of genitals and the pelvic area, meaning that often patients find it difficult to discuss pelvic pain[53], even with their doctors. Adding to this are any psychological or mental health comorbidities women with chronic pelvic pain may experience, which are well documented to be associated with stigmatisation[54–56].

1.5 Assessment of pain in endometriosis-associated pain and the need for stratification

Historically, pain in endometriosis has been assessed using crude "none/mild/moderate/severe" responses, which has given us limited information on the pain experienced by patients. There is now a growing call to assess pain in endometriosis more deeply. The World Endometriosis Research Foundation (WERF) Endometriosis Phenome and Biobanking Harmonisation Project (EPHect) project has aimed to harmonise the data collected on endometriosis and as a part of this a very detailed questionnaire was created[57]. This questionnaire includes numerical rating scale (NRS) 0-10 questions on different pains experienced by patients with endometriosis, including dysmenorrhea, dyspareunia and dysuria. This enables us to identify which pain symptoms are experienced by an individual, which is important due to the heterogeneity of the pain symptoms seen in endometriosis-associated pain. Some women experience severe pain only with periods, others experience moderate pain throughout the month (non-cyclical pain) or others experience pain with sexual intercourse – and some women will experience all of these symptoms in combination. It is important, therefore, to ensure that we are assessing different types of pain experienced separately and do not simply use a global measure of pelvic pain.

More recently, researchers who aim to understand pelvic pain in more detail have begun to combine NRS intensity ratings with other more sophisticated pain questionnaires, such as the Fibromyalgia Scale[58] to assess how widespread the pain experienced is, giving an indication of whether the pain has a centralised component. Moreover, the painDETECT[3] questionnaire has been used, which is a screening tool for neuropathic

pain and allows for the grouping of participants as having probable 'nociceptive', 'neuropathic' or 'mixed' pain.

The use of a variety of different assessment tools allows us to properly phenotype the pain experienced by women with endometriosis-associated pain. This is important when thinking of future work into the treatment of endometriosis-associated pain for many reasons. Firstly, better phenotyping of the pain experienced by women with endometriosis-associated pain can allow us to better design animal models that try to emulate that phenotype. Secondly, it allows us to sub-group/stratify women with endometriosis-associated pain more accurately, which is necessary both in the design of clinical trials and treatment.

The stratification of patients is vital in the search for new treatment options in endometriosis-associated pain. The ability to group women based on their pain symptoms, or the underlying mechanisms giving rise to/maintaining pain can be used in drug discovery efforts. Clinical trials can fail if the population sampled is too heterogeneous as a given treatment may only be effective in a subset of the participants. In a condition such as endometriosis where we know there is large variation in the pain symptoms experienced, and thus likely different mechanisms giving rise to and/or maintaining the pain, it is especially important to try to find a way to group women with endometriosis based on what the underlying cause could be. Investigations into these different mechanisms, therefore, should look at a (relatively) homogenous subset of patients at a time.

So far, research aiming to identify the mechanism(s) underlying pain in endometriosis and develop effective treatments has come up short, leaving many women with very little relief from their pain symptoms. Better assessment of the pain experienced can

only aid our future work and hopefully will help us to find the answers so many women desperately seek.

1.6 Possible peripheral mechanisms underlying EAP

1.6.1 Nerve fibres and the autonomic nervous system

As described above, nerves are responsible for conveying nociceptive signals from the periphery to the brain. Therefore, it is logical to try to determine whether, in patients with pain, there is a change to these nerves that could lead to alterations in the signals the brain is receiving about nociception. Alterations in the structure or function of peripheral nerves has been demonstrated in a variety of conditions associated with chronic pain (e.g. osteoarthritis[59], painful intervertebral discs[60] and interstitial cystitis[61]). When considering this in the context of endometriosis, it is important to realise that endometriotic lesions themselves are innervated, as has been demonstrated for peritoneal disease[62], ovarian endometriomas[63] and deep infiltrating lesions[64]. Interestingly, however, there is a suggestion that ovarian disease is less well innervated than lesions elsewhere in the pelvis[65].

The autonomic nervous system consists of the sympathetic and parasympathetic systems, which have different roles generally, and of relevance here given that endometriosis is an inflammatory disease[4], are part of different stages of inflammation. The sympathetic nervous system is involved in the initial phases of inflammation and gives rise to a proinflammatory environment which can sensitise nerve endings, through adrenoreceptors[66]. In the later stages of inflammation there is an alteration in the balance of proinflammatory and anti-inflammatory nerve fibres. The sympathetic

nervous system has been shown to be involved in both the development of and severity of chronic inflammatory diseases[67]. Changes in the number of sympathetic neurons have been investigated using a rat arthritis model[68]. Arthritis is a well-studied chronic pain condition, which shares a number of features with EAP. In response to injury the sympathetic nerves of distal regions die back, and more sympathetic nerves sprout in the area of the spleen innervated by nerves from the injury site. This shows that sympathetic innervation can change as a response to injury/inflammation. In the following, I will discuss the changes in the nerve fibres and the autonomic nervous system more generally which have been seen in endometriosis specifically in both peritoneal and ovarian endometriosis, and how these findings can help our understanding of endometriosis.

First, I will discuss studies focussing on peritoneal endometriosis. Endometriosis patients with and without pain have been investigated, along with non-endometriosis controls, for nerve fibre density[69]. There was no difference between the group of endometriosis patients and the endometriosis free group in terms of the number of nerve fibres in the peritoneum. Also, there was no difference in the nerve fibres (at histological examination with immunocytochemistry) between the endometriosis with and without pain groups. This suggests that endometriosis-associated pain is not simply due to an increase in the number of nerve fibres sending nociceptive signals to the brain. However, another study found contradicting results. When investigating peritoneal endometriotic lesions compared to healthy peritoneum and samples from endosalpingiosis lesions they found significantly more nerve fibres in the peritoneal endometriotic lesions than the two control groups[62]. Aδ sensory, C sensory, cholinergic and adrenergic nerves all innervated the endometriotic lesions. The different findings of these two studies could be due to the fact that the latter specifically used endometriotic

lesions in the peritoneum, rather than just a peritoneum sample. It is not difficult to believe that changes in nerve fibre density are present at endometriotic lesions but are not the same throughout the peritoneum. Additionally, it is plausible that a subgroup of women with CPP but without macroscopic endometriosis also have altered innervation of their peritoneum. Whether these women will then go on to develop endometriosis that is visible at laparoscopy or if this is a distinct pathology remains unknown. The mechanism by which changes in the innervation of peritoneal lesions occur is starting to be investigated. Mechsner and colleagues have shown that there is an increase in semaphorins and their receptors in peritoneal endometriotic tissue[70]. It has been suggested that semaphorins repel nerves in chronic inflammatory diseases, which could explain the reduced innervation by the sympathetic nervous system[70].

Further studies have investigated the types of nerve fibres innervating lesions, and whether this is seen only in endometriosis. Arnold and colleagues compared the relative sympathetic and sensory innervation of peritoneum in different samples[71]. Overall nerve fibre density in unaffected peritoneum of endometriosis patients was significantly reduced when compared to both endometriotic lesions and to healthy peritoneum. When looking specifically at the sympathetic nerve fibres, healthy peritoneum had the highest density, then unaffected peritoneum of patients, followed by endometriotic lesions. Sensory fibres were significantly higher in areas close to the endometriotic lesion, compared to healthy controls. These results show that there are changes both to the peritoneum which contains the endometriotic lesion, and to the unaffected peritoneum in patients. The mechanisms by which this occurs and how this leads to pain in patients needs to be further studied.

Studies investigating nerve fibres in ovarian endometriosis have demonstrated that the lining of ovarian endometriomas has many more nerve fibres than both normal ovarian tissue from these patients as well as ovarian tissue from women without endometriosis[63]. These nerve fibres were shown to be a mixture of sensory, sympathetic and parasympathetic. This study did not assess dysmenorrhea however, participants were undergoing oophorectomies due to severe dysmenorrhea and other pain symptoms. In contrast to this, McKinnon et al found that lesions in the ovary were not associated with nerve fibres, and that the ovarian endometriosis group reported the lowest level of pain intensity (compared to peritoneal and rectovaginal septum endometriosis)[65]. Further studies should compare these results to patients with endometriosis that have no pain symptoms to determine whether the changes are linked to endometriosis itself or endometriosis-associated pain.

Whilst studies describing alterations in nerve fibres in association with endometriosis are interesting, it is only when they also relate to the pain that a patient experiences that they can help us understand the underlying pain mechanisms and potentially lead to personalised treatments. To date, only a few studies have explored these relationships, but with intriguing results. Mechsner and colleagues demonstrated a relationship between increased nerve growth and severity of pain symptoms[72]. Specifically, they showed that women with higher pain scores for dysmenorrhea and pelvic pain had significantly higher concentrations of neuronal markers (neurofilament (NF) and protein gene product (PGP) 9.5). Similarly, McKinnon and coworkers showed that dysmenorrhea scores are significantly higher when the lesions are innervated[65], whilst Kajitani and colleagues found nerve growth factor (NGF) in the peritoneal fluid to be more frequently elevated in women with severe endometriosis-associated pain than in those with milder pain[73]. In addition to altered nerve density, differences in peripheral

receptors may also be important in generating pain. The transient receptor potential vanilloid 1 (TRPV1) receptor is particularly interesting in this context as it responds to noxious heat and certain chemicals, and has been shown to be of importance in many pain mechanisms including hyperalgesia[74,75]. Not only is the density of nerve fibres containing TRPV1 higher in ectopic endometrial implants compared to control endometrium from women without endometriosis, but this density is also positively correlated positively with the severity of dysmenorrhea in women with endometriosis[76].

Interestingly, the extent of innervation of the lesions appears to vary with the location of the endometriosis and this relates to the pain experienced[65]. Thus, it has been shown that women with deep endometriosis (rectovaginal septum) experience more pain and more often have nerve fibres close to the lesions, although there was not a direct relationship between the two. Also found was a direct relationship between significantly higher menstrual pain and higher endometriosis-associated nerve fibres in peritoneal lesions. Endometriosis lesions on the ovary were the least likely to have a nerve fibre in close proximity (1.5mm) and these women reported the lowest pain.

1.6.2 Pelvic environment

Many studies have explored the difference between the peritoneal fluid of women with and without endometriosis. Although a wide variety of differences have been identified[77–79], as with nerve fibres discussed above, only a very few have actually related these differences to the patient's pain experience. I will first describe two studies relating pain to growth factors in the peritoneal fluid (PF), whilst inflammatory mediators are discussed later in this section.

Of particular interest given the emerging relationships between nerve fibre density and pain symptoms is a study exploring the influence of PF on nerve fibre growth[80]. Chicken dorsal root ganglia (DRG) cultured in the PF of endometriosis patients showed increased neurite growth compared to controls with other gynaecological conditions. Furthermore, this growth was inhibited by nerve growth factor (NGF) inhibitors, proving that the pathway of nerve growth was NGF dependent. A further study by this group[81] explored the relationship between PF-induced nerve growth and pain symptoms. Interestingly, they found no difference in neurite outgrowth between the women with endometriosis-associated pain and those with endometriosis but no pain. Similarly, there was no difference between those with mild pain and those with severe pain, suggesting that the altered nerve growth is a correlate of endometriosis, not of endometriosis-associated pain specifically.

Transforming growth factor β1 (TGF-β1) has been shown to be elevated in the PF of women with endometriosis[82] and subsequently also expressed more in the peritoneum of these women[83]. This increased peritoneal expression correlated with the severity of dysmenorrhea assessed using a visual analogue scale[83].

PF protein concentrations have also been compared to generalised hyperexcitability[84]. Generalised hyperexcitability is considered as an alteration in the processing of the nociceptive signal in the central nervous system leading to the amplification of pain, and is therefore thought to be very important in the induction and maintenance of chronic pain[28]. Higher TNF-α correlated with a higher magnitude of central hyperexcitability (lower pain threshold); higher osteoprotegerin (OPG) concentration was correlated with a greater magnitude of central pain sensitivity; glycodelin positively correlated with reflex receptive field area; as well as other interesting results relating to

other cytokines. Glycodelin and TNF-α have also been shown to be correlated with the level of menstrual pain endometriosis patients experienced, along with RANTES[85]. These studies highlight that peripheral changes measured in the peritoneal fluid can be associated with central changes that have an effect on the pain a patient experiences.

During inflammation, nerves that carry the nociceptive signal to the brain (C fibres), both transduce signals to the central nervous system and release peptides into the local environment. Once these C fibres are activated, they may display on-going electrical activity, even when the inflammation is resolved[86]. The central nervous system can also become sensitised, which is further discussed below. Several of the molecules which play a part in the start of endometriosis and its maintenance are also involved in the sensitisation and activation of peripheral nerves. One example of this is NGF (discussed previously, has been found to be elevated in endometriosis[73]), which is secreted from inflammatory and endometriotic cells, and triggers the release of other factors. These other factors as well as NGF itself are able to activate the nerve endings in the endometrium, as well as sensitise them, meaning that they will be activated by a lower threshold of stimulus.

1.7 Central changes associated with endometriosis-associated pain

1.7.1 Brain function

There is a very large body of work which shows that there are changes in brain function associated with chronic pain. The most commonly used techniques to investigate brain function in pain are functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) imaging. Both give an indirect measurement of activity as

they measure metabolic activity (which increases in active areas), as opposed to electrical activity. One problem with neuroimaging studies investigating the response to a noxious stimulus is determining what alterations are due to central as opposed to peripheral changes. One way to try to overcome this is to use a distal control site instead of, or as well as, the affected area, broadly enabling the separation of peripheral changes (which will likely only be seen in the affected area) and central changes (which will likely be seen in both areas); whilst this is not always the case it helps to offer some broad breakdown of mechanisms. Although a major focus of pain research in general over recent decades, there are very few studies that investigate brain function in women with pelvic pain specifically. To address this relative paucity, the Multidisciplinary Approach to the Study of Chronic Pelvic Pain (MAPP) project (http://www.mappnetwork.org/) is using a variety of approaches, including neuroimaging techniques, to improve our understanding of urological pelvic pain. Already they have found that women with widespread pain, compared to those with pain localized to the pelvis, showed increased gray matter volume and functional connectivity in the sensorimotor and insular cortices[87]; that brain functional connectivity can predict short term pain reduction[88]; and that early adverse life events alter the brain state in both men and women with urological chronic pelvic pain[89].

Dysmenorrhea is a cardinal symptom of endometriosis but can also occur in isolation. Researchers have shown that women with dysmenorrhea alone (no pain in the pelvis or elsewhere throughout the month, but presence of endometriosis unknown) are significantly more sensitive to a noxious thermal stimulus than dysmenorrhea-free controls (i.e. they require a significantly lower temperature to produce the same pain intensity) throughout the menstrual cycle[90]. Furthermore, this difference was found in response to stimulation of both their arm (control site) and lower abdomen (referral

site). fMRI data showed that despite having less peripheral input (a lower temperature), women with dysmenorrhea have increased activation in response to this input. These findings suggest that long-lasting changes have occurred in the central nervous system (central sensitisation). Abnormal cerebral metabolism in women with dysmenorrhea has also been demonstrated with PET scans[91]. Such findings are concerning given the prevalence of dysmenorrhea, particularly in young women/adolescents, with the majority of adolescents reporting dysmenorrhea[92], with some statistics suggesting 85% suffer[93]. However, an early age of onset may be of particular importance in dysmenorrhea (whether primary or secondary) as the central nervous system is very plastic in adolescence, and thus, changes may occur more readily.

Communication between brain regions (functional connectivity) as well as activity within these regions can also be explored. Functional connectivity is assessed using fMRI data collected when the participant is at rest (resting state). This can then be analysed to look for similarities in the activity of different areas, which suggests that they are functionally connected[94]. It has been used to investigate communication between areas in both healthy participants and in disease states. Resting state can be used to help us understand the intrinsic organization and functioning of the brain, including how the brain processes complex information such as pain. It allows insights both into the strength of networks involved in pain perception but also how brain connectivity at rest may be altered in patients with chronic pain. Women with endometriosis-associated pain have been shown to have greater resting connectivity of the anterior insula (one of the key pain processing regions) to other brain regions than both healthy controls and women with endometriosis but without pain symptoms[33]. Using proton spectroscopy these authors also found, in these women levels of excitatory neurotransmitters in the anterior insula were also measured and found to be higher in

the endometriosis-associated pain than in either the healthy controls or endometriosis/no pain group. Moreover, the level of excitatory neurotransmitters measured was positively correlated to the connectivity between the anterior insula and the medial prefrontal cortex, a key pain modulatory region, which might be a mechanism by which hyperalgesia develops in these women. This was a particularly well-designed study, including a group with endometriosis but no pain, as it allows us to see that these central changes are not due to endometriosis itself, but rather the pain that arises from it. The same group have done many studies in fibromyalgia patients with similar (though subtly different) findings[95], further highlighting the similarities between endometriosis-associated pain and other chronic pain conditions.

There are top-down (from the brain) processes which can alter pain perception. One example of this is endogenous pain inhibition[96]. This phenomenon, which has been shown to underly the analgesic effect of placebos, works to reduce the pain perception in an individual[97]. Endogenous pain inhibition is mediated through a variety of spinal and cortical mechanisms, which form a descending pain modulatory circuit. There are many factors that affect the extent to which these mechanisms are engaged, including genetics[98], psychological state and cognitive factors[99]. An inability to engage these endogenous mechanisms may predispose an individual to the development of chronic pain after an acute painful insult[100]. Endogenous pain modulatory mechanisms can be investigated using heterotopic stimulation (two noxious stimuli in different areas given after one another). This uses the "pain inhibits pain" model which tests the analgesic effect (i.e. in a healthy participant the second stimulus would be perceived as less painful)[96]. For example, in a study with female patients with IBS compared to healthy women, it was found that heterotopic stimulation did not have the same effect on the two groups[101]. In controls heterotopic stimulation decreased the pain reported for the

second stimulus. This is to be expected as the first noxious stimulus activated the descending pain inhibitory pathway, meaning that the second noxious stimulus is perceived as less painful. In the IBS patients however, the rectal pain intensity was increased if there had been a previous stimulus, suggesting abnormal endogenous pain modulation in this population. Further research in this area is needed, especially in an endometriosis setting, to determine if there are such changes in endogenous pain inhibition in patients with endometriosis-associated pain and if such, are these changes a result of chronic pain or do they predispose women to experience chronic pain, or both?

1.7.2 Brain structure

Brain structure can be investigated using magnetic resonance imaging (MRI), which can distinguish between gray matter, white matter and cerebrospinal fluid. Brain structure is known to be different between genders[102] and to change with aging[103]. Alterations in brain structure are well established in chronic neurological conditions such as Alzheimer's disease[104], Parkinson's disease[105] and Multiple Sclerosis[106]. In these conditions, the structural changes likely reflect the underlying pathology (e.g. neurodegeneration). Brain structure in chronic pain has also been widely studied, with alterations in the volume of specific brain regions found[107]. However, the mechanism(s) underlying these findings are not well understood. It is thought that areas whose volume increases reflect an increase in activity of these regions, whilst a reduction in volume may reflect one or more of the following[26,108]: repeated episodes of pain having a neurotoxic effect leading to neuronal atrophy; changes in the metabolic activity or neurotransmitter concentration in neurons; neurodegeneration due to pain related inactivity; an effect of co-morbidities or psychological factors; or a neurotoxic effect of the drugs given to relieve pain. It is important to note that once brain cells have died

(seen as a volumetric decrease), they are not replaced. Thus, where volumetric decreases reflect cell death, they will be irreversible.

As-Sanie and colleagues have investigated brain volume in women with chronic pelvic pain[107]. They employ an elegant design, assessing women with all four combinations of endometriosis/no endometriosis and CPP/no CPP. Compared to healthy controls, women with both endometriosis-associated pelvic pain and pelvic pain without endometriosis had decreased grey matter volume in brain regions involved in pain perception, again suggesting that it is the presence of pain, not endometriosis *per se* that is related to these structural changes. Perhaps more intriguingly, however, not only were these reductions absent in women with endometriosis without pain, but this group of women had an increased volume of the periaqueductal grey (PAG), which was positively correlated with the pressure threshold required to induce pain. The PAG is one of the key regions of the descending pain inhibitory system (an endogenous mechanism of analgesia) and thus this finding may explain why some women with endometriosis do not experience pain. Clearly further work needs to be done to determine whether women with endometriosis with and without pain have the same peripheral pain drivers but differ in their ability to engage descending pain inhibitory pathways.

1.7.3 HPA axis

Chronic pain can be considered as a repeated stressor and thus it is perhaps not surprising that dysfunction in the hypothalamic-pituitary-adrenal (HPA) axis, a central component of the stress response[109], is seen in a wide variety of chronic pain conditions[110,111] in line with other chronic somatic conditions. The mechanisms by which the HPA axis is supressed in these conditions are not, however, well understood.

Certainly, acute stress leads to an activation of the HPA axis and a rise in cortisol levels; however, over time this response will be attenuated – a phenomemon colloquially referred to as burn out. In fact, this may be beneficial for an individual as continued activation of the body's "emergency response" systems could lead to further tissue damage both locally and systemically[112]. However, in the context of pain low levels of cortisol may exacerbate painful symptoms by reducing the endogenous analgesia associated with stress (stress-induced analgesia) that is thought to facilitate the 'fight-flight response'[113].

Reduced levels of serum cortisol in women with dysmenorrhea compared to healthy pain-free controls have previously been demonstrated[90]. Interestingly, cortisol levels were negatively correlated with the duration of dysmenorrhea supporting the burnout hypothesis. However, although endometriosis-associated pain has also been associated with low cortisol levels[114,115], the relationship appears more complex than in dysmenorrhea alone. In one study considering the multiple symptoms with which women with endometriosis may present with, incapacitating pain appeared to be the strongest predictor of cortisol suppression and both frequency of dyspareunia and perceived infertility also influenced salivary cortisol[115].

1.7.4 Pain psychology

Historically, chronic pain was frequently assumed to arise purely from psychological morbidity. Thankfully both clinical opinion and scientific evidence have moved forward in this respect! There is a large library of literature highlighting the comorbidity of chronic pain with mood disorders, such as depression, though it is often hard to disentangle the order in which these symptoms arose. For example, in women with chronic pelvic pain presenting at a tertiary outpatient clinic, more than 25% had

moderate/severe depression and more than 50% had at least moderate anxiety[116]. Moreover, we now know that mood can alter the pain experience by biological mechanisms, in both healthy individuals and those with chronic pain[117–119].

A variety of studies have either exploited normal variation or used robust experimental paradigms to manipulate psychological or cognitive state in healthy volunteers and explore the impact on the processing of acute pain stimuli. Reviewing these studies, it can be seen that depressed mood[120], anxiety[121], catastrophizing[122] and both expectation of[123,124] and attention to pain[125] are all associated with higher ratings of pain intensity. The central mechanisms underlying these relationships vary, however. For some examples, depressed mood induction disrupts the normal emotion regulatory circuitry[120], anxiety amplifies pain via a hippocampal network[121], whilst high catastrophizers appear less able to engage descending inhibitory pathways[122].

Investigations into depression and chronic pain have found associations in certain brain areas. In rheumatoid arthritis studies it has been suggested that the medial prefrontal cortex is an important player in the relationship between depressive symptoms and clinical pain severity[126]. In fibromyalgia it has been shown that the symptoms of depression are associated with the size of neuronal activation in areas of the brain involved in affective pain processing[127]; interestingly the extent of depression had no effect on the sensory-discriminative aspects of pain processing (such as the location of the painful stimulus), highlighting that different pain-processing pathways may be influenced by the presence of depression. Evidence for separate processing systems being influenced by depressive scores has also be seen in relation to the correlation between depression and anxiety scores and subject rating of general health, but not with sensitivity to pain, contradicting previous results[128].

Pain catastrophizing scores in chronic pain patients, have been shown to be correlated with activity in many brain areas, including those involved in the anticipation of pain, attention to pain and emotional aspects of pain[129]. Pain catastrophizing may also have a role in the induction of chronic pain and in recovery. For example, it has been shown that in a population with low back pain, high pain catastrophizing predicts pain at follow up as well as chronic low back pain[130], whilst in spinal cord injury pain catastrophizing was consistently strongly associated with outcome measures[131]. It has been shown that pain catastrophizing seems to mediate the outcome of treatment in active physical treatment, cognitive-behaviour therapy and treatment combining the two[132].

It is highly likely that such interactions between mood and pain experience are of relevance in the context of endometriosis. As with other chronic pain conditions the daily experience of pain frequently impacts on mood and the additional fertility concerns associated with endometriosis may further exacerbate the situation. Some studies suggest rates of depression and anxiety to be approaching 90% in this population[133,134] . Although the proportion of these women meeting clinically relevant diagnostic criteria is much lower than this, as described above, subclinical mood alterations can also augment pain perception. To date, however, the role of psychological factors in amplifying the pain experience in women with endometriosis has received only limited interest. Anticipation and expectation of pain may be of particular interest in this context due to the predictability of the functional pain symptoms associated with endometriosis (dysmenorrhea, dyspareunia, dyschezia), which may differ from other chronic pain conditions where the pain is either less predictable or more constant in nature and severity. Interestingly, women with endometriosis have been shown to have significantly higher pain catastrophizing scores

compared to healthy controls[135] and high levels of pain catastrophizing are associated with lower quality of life scores[136] and predict a worse response to treatment[137] for women with endometriosis.

1.7.5 Autonomic nervous system

Although the autonomic nervous system has been discussed with relevance to the peripheral nervous system above, it is also of relevance due to its central effects and is thus further discussed here. The parasympathetic nervous system is thought to be antinociceptive whereas the sympathetic nervous system is pronociceptive. The balance between the two has been shown to be altered in both fibromyalgia and functional gastrointestinal disorders[138]. For example, in women with IBS, altered autonomic nervous system function includes increased sympathetic and/or decreased parasympathetic activity during sleep[139] and over 24 hours[140]. Recent work by Aziz and colleagues has highlighted the importance of the autonomic nervous system in pain perception[141]. In a healthy cohort they were able to identify two clusters that differed in both baseline measures and their response to painful stimuli. Cluster 1 had high sympathetic and low parasympathetic tone at baseline (in addition to high neuroticism, anxiety and cortisol and the SS/LS genotype of the 5-HTTLPR receptor) and demonstrated sympathetic withdrawal and parasympathetic activation in response to pain (associated with less ability to tolerate or habituate to pain and an elevated cortisol response). Whilst cluster 2 had a low baseline sympathetic tone with a high parasympathetic tone (plus high extroversion, low anxiety, low cortisol and the LL genotype of the 5-HTTLPR receptor) and showed sympathetic activation and parasympathetic withdrawal in response to pain (and were able to tolerate more pain and habituate more). These clusters were also shown to be stable across time. This

group have subsequently shown that these clusters also exist in a cohort of patients with functional chest pain[142] and that the morphology of the brain varies with autonomic nervous system function[143] furthering our understanding of this emerging area of interest. As with mood-pain interactions discussed above, the autonomic nervous system has not really been explored in the context of endometriosis-associated pain. However, one small study (based on self-report questionnaire responses), does suggest there to be significantly higher incidences of autonomic symptoms in patients with chronic pelvic pain than in the healthy controls[144].

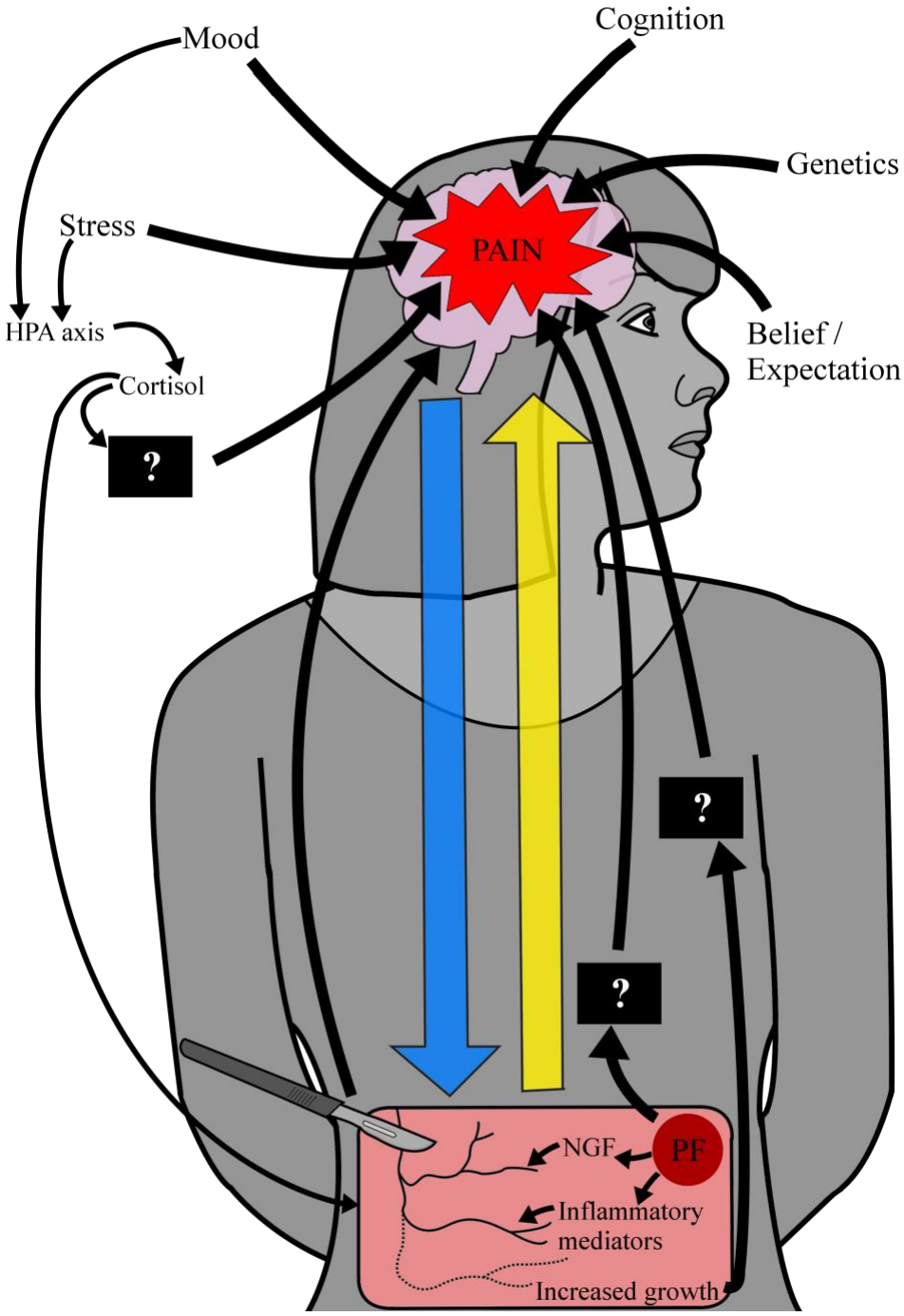

Figure 2: This summary diagram shows some of the many influencers of pain that have been discussed[1]**.** The red box shows processes that occur in the periphery. NGF (nerve growth factor) and other inflammatory mediators are increased in the PF (peritoneal fluid). These have an action on the nerves in the periphery, for example sensitisation. There is also evidence for increase nerve growth in the periphery. The black boxes in the diagram represent an unknown mechanism(s). For example, we do not yet understand how increased growth of neurites in the periphery has an impact on the pain perception, but there is evidence that it does. Similarly, there is an unknown mechanism by which PF influences the pain perception. The surgical knife in the periphery represents the go to method of treatment of endometriosis-associated pain, and also shows how damage to the nerves can occur as a result, which may lead to increased pain. The yellow arrow represents ascending modulation of pain and the blue arrow represents the descending modulation of pain, which have been shown to be disrupted in chronic pain patients. The external factors are represented, such as mood, as well as other biological factors, i.e. genetics, that are known to influence the pain experience. Both mood and stress impact on the HPA axis, which presents in changes in cortisol levels, which may also affect pain perception.

1.7.6 Implications of observed central changes

We have described a number of mechanisms by which the central nervous system may amplify or even generate pain in association with a peripheral pathology such as endometriosis. Additionally, the changes seen in association with endometriosis-associated pain may be responsible for other features commonly described in the endometriosis literature. For example, it is well known that endometriosis co-occurs with other chronic pain conditions[145–147]. Whilst this may be due to genetic or environmental factors that increase the risk of both conditions, additionally, the central changes that occur secondary to repeated episodes of pain may subsequently predispose to the development of other chronic pain conditions. For example, dysfunction in descending pain modulation or HPA axis activity could both lead to acute or chronic pain from what might previously have been an innocuous insult. Furthermore, endometriosis is associated with a variety of other comorbidities including autoimmune and endocrine disorders[145] and these relationships may in part be explained by altered function of the HPA axis, autonomic nervous system and other as yet undescribed changes. It is hoped that future multi-disciplinary studies focussing on comorbid issues of importance to patients will help to unravel these relationships.

1.8 Neuropathic pain

1.8.1 Definition and mechanisms of neuropathic pain

The IASP definition of neuropathic pain is ‘pain caused by disease or lesion to the somatosensory nervous system’[36]; this includes both central and peripheral neurons. There are many different conditions which are considered to be neuropathic pain

conditions, including entrapment neuropathies as well as post-herpetic neuralgia and painful diabetic neuropathy[149], although recently there are many more conditions which have been shown to have a neuropathic component such as osteoarthritis[150] and fibromyalgia[151]. The true prevalence of neuropathic pain is difficult to estimate but screening tools such as painDETECT, Douleur Neuropathique 4 (DN4) and Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) have enabled estimations to be made, with prevalence of chronic pain with neuropathic characteristics estimated to be 7-10%[152,153] and with 40% of chronic pain patients experiencing at least some characteristics of neuropathic pain[154]. We also know that chronic neuropathic pain is more common in women than men[155], although the reason for this is not fully understood.

Neuropathic pain can be split into central and peripheral neuropathic pain. In peripheral neuropathic pain electrical activity in the sensory neurons is changed, which results in the loss of the balance between excitatory and inhibitory signals in the central nervous system, which in turn harms descending control systems[155]. Ultimately, this alters facilitation mechanisms at the spinal cord dorsal horn neurons. Overall, this is characterised as hyperexcitability and over time this state can become chronic. The mechanisms underlying these changes in electrical activity could be any of (or a combination of): changes in ion channel function and expression, alterations in second-order nociceptive neuronal activity and changes in the function of inhibitory interneurons.

Peripheral neuropathic pain usually involves myelinated A fibres (Aß and Aδ fibres) and small unmyelinated C fibres[155]. Peripheral neuropathic pain disorders have different distributions, with some having generalised symmetrical distribution and others having

focal distribution[155]. Focal distributions can present as a 'glove and stocking' distribution in which the extremities are most affected or in a distribution in which the trunk and upper thighs/arms are affected. The latter occurs when the pathology involves sensory ganglia[155]. Some inherited neuropathic pain conditions include channelopathies which can present with very specific distributions, for example inherited erythromelalgia is characterised by pain and reddening in the extremities[156].

Neuroinflammation is one mechanism which can cause alterations in ion channel function and expression, giving rise to and maintaining persistent pain[86]. The inflammatory response involves immune cells as well as chemical mediators. Some of the chemical mediators involved in the process of inflammation can cause stimulation and sensitisation of nerves[157–160]. This inflammatory response can be triggered by damage to nerves itself, or other tissue damage. Unfortunately, the action of these inflammatory mediators can be seen on both damaged or degenerating neurons and the neighbouring uninjured nerves, as they are bathed in the same mediators. These undamaged nerves which have been exposed to inflammatory mediators can exhibit spontaneous pain[161,162], which is a key feature of neuropathic pain.

Various inflammatory mediators have been shown to have a role in hypersensitivity that can be seen in neuropathic pain, and these have been discussed in detail in Ellis & Bennett 2013[86]. Some of these are: TNF-α, which sensitises nociceptors and increases excitatory currents[163]; IL-1ß which is known to sensitise TRPV1 receptors, increase excitatory currents and decrease inhibitory currents[164]; IL-6 sensitises nociceptors and decreases inhibitory currents[165]; bradykinin has direct nociceptive action and modulates the channel kinetics of TRPV1[166]; others, like MCP-1 have indirect nociceptive action, such as activation of microglia[167,168]. Some of the changes brought about by

inflammatory mediators during peripheral sensitisation are short term, such as insertion of channels into the membrane and producing 'irritated nociceptors'[169] by changes in channel thresholds via phosphorylation[86]. Other changes are more long term, such as phosphorylation and upregulation of transcription factors, resulting in increased transcription of channel proteins or neurotransmitters[86].

Another mechanism of neuropathic pain is deafferentation pain which is defined as pain due to loss of sensory input into the central nervous system[170]. This can occur when there is an injury to the dorsal root which means second order neurons cannot receive anatomical/physiological input from the afferent neurons[171].

Second-order nociceptive neuron alterations can generate central sensitisation due to their enhanced excitability and the increased receptive field of afferent neurons, so they excite more second-order neurons[172–174]. This can be seen in phenotypes of allodynia[175].

Central neuropathic pain can be caused by diseases such as multiple sclerosis which affects both the spinal cord and the brain[176], alternatively, the brain can be affected which is the case in post-stroke pain[177]. Changes in inhibitory modulation of pain are also seen in neuropathic pain[178]. This includes dysfunction in descending modulatory control systems, including projections from the cingulate cortex and amygdala to the periaqueductal grey (PAG), the brainstem and spinal signalling[179,180]. This particular network has been associated with the maintenance of ongoing pain and comorbidities associated with neuropathic pain[181–184].

1.8.2 Rationale and evidence for neuropathic pain in endometriosis

The question whether endometriosis-associated pain is (at least in part) of a neuropathic nature has not been properly investigated so far. This is despite the clinical presentation,

with women often describing their pain as ‘stabbing’, ‘tingling’ and ‘like electric shocks’ which are known to be descriptors used in neuropathic pain conditions.

Neuropathic pain could be expected to arise in the context of endometriosis for a number of reasons. Firstly, there is currently no non-invasive test available to establish the diagnosis of endometriosis. As a consequence, all women with a confirmed diagnosis will, by definition, have undergone at least one surgical (usually laparoscopic) procedure, with the associated risk of post-surgical neuropathic pain[185–187]. During this surgery skin, muscle and fat layers (all of which are innervated by the somatosensory nervous system) are cut through, which could lead to the damage of nerves, which could result in neuropathic pain. Usually laparoscopic surgery involves three incisions and it is not uncommon for the surgery to be repeated for recurrence of endometriosis, meaning that women with endometriosis can have had several incisions to their abdomen, each of which could result in nerve damage (and hence neuropathic pain).

Secondly, as already described, endometriotic lesions are highly innervated[62,63]. Surgical procedures excising or ablating the lesions damages these nerve fibres, which in turn could generate neuropathic pain[186].

Thirdly, these nerve fibres express TRPV1 receptors and are bathed in the peritoneal fluid, which has been shown in women with endometriosis to contain high levels of inflammatory mediators such as BDNF and TNF-alpha which sensitise nerve endings[37,76,85,188,189]. Thus, neuropathic pain could develop over time as a result of prolonged exposure to these inflammatory mediators.

A recent study[31] on chronic pelvic pain which included a subset of patients with endometriosis, investigated neuropathic pain using questionnaires and (modified) quantitative sensory testing. Using painDETECT they found that 26% of participants

had neuropathic pain and with their quantitative sensory testing they found no participant with unchanged sensation, with great variation between participants. There was no significant difference between those with endometriosis and those without in classification of neuropathic pain. However, due to the small sample size (n=72 total, n= 32 with endometriosis), findings from this study have to be interpreted with caution[31].

1.8.3 Assessment of neuropathic pain

Neuropathic pain is usually graded by determining the level of certainty with which the pain is neuropathic[149]. Neuropathic pain is described as 'possible' if the patient's history suggests the presence of lesion or disease to the nervous system and screening tools suggest the pain experienced could be related. 'Probable' neuropathic pain requires an assessment of sensory signs of neuropathic pain (such as quantitative sensory testing). Whereas an objective diagnostic test which confirms the lesion/disease of the somatosensory nervous system is required for 'definite' neuropathic pain.

Screening tools, such as painDETECT[3,190], have been developed to help identify neuropathic pain conditions/neuropathic components to chronic pain. painDETECT uses 9 questions to assess the characteristics of pain: one about the time course of pain, one about whether or not the pain radiates and seven about symptoms (i.e. burning, tingling, electric shocks, numbness). However, screening tools cannot completely replace careful examination by a clinician of sensory testing and a thorough history[191]. Whilst these cannot give a 'definite' diagnosis of neuropathic pain, their ease of use can enable them to be used in a variety of studies. Unlike some other screening tools (such as Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) and Douleur Neuropathique en 4 questions (DN4)[192]), painDETECT does not involve clinical

examination, and therefore can be carried out by non-specialists. This enables it to be used in a variety of settings which could not otherwise assess neuropathic pain, both clinically and in research. painDETECT was originally validated in lower back pain patients[3] but has since been used in a broad range of pain conditions, including fibromyalgia[193], osteoarthritis[194,195] and musculoskeletal pain[196,197].

Quantitative sensory testing uses a standardised protocol developed by the German Research Network on Neuropathic Pain (DFNS)[175]. It uses a variety of different test stimuli to assess the sensory profiles and enables the evaluation of any loss and gain of function[198]. These test stimuli include cool and warmth detection, hot and cold pain thresholds, pressure pain threshold, touch and vibration detection and threshold for pinprick and blunt pressures[198]. Noxious stimuli are detected by nociceptors; different nociceptors detect different stimuli, for example the TRPV1 channel detects temperatures over 43°C[199]. Nociceptors then transduce this signal to nerve fibres. These nerve fibres are of different sorts: Aδ nerve fibres are thinly myelinated, Aß nerve fibres are thickly myelinated and finally, C fibres are unmyelinated and are the most common[181]. These nerve fibres enter the spinal cord at the dorsal root ganglion. Once in the central nervous system the signal passes via the spinal cord to the brain. Neuropathic pain can occur due to changes in the activity of nerves in this pathway and as QST assesses peripheral nerve function it is often used in the neuropathic pain setting[200]. The definition of neuropathic pain is pain due to disease/lesion to the somatosensory system[36], so it is not surprising within this definition that there may be some deafferentation (leading to loss of function) as well as sensitisation of nerve fibres (leading to gain of function)[155,172]. QST allows the assessment of these different types of nerve fibres as they are activated by different test stimuli.

1.8.4 Treatment of neuropathic pain

Often in neuropathic pain the cause of the pain cannot be treated (with exception of entrapment neuropathies such as carpel tunnel syndrome[201]). Treatments therefore are primarily focussed on the symptoms of neuropathic pain. It is well known that generally patients do not respond to commonly used analgesics such as paracetamol and ibuprofen[202] (both of which are prescribed for endometriosis-associated pain with varied success).

A systematic review[203] (Finnerup & Attal 2016) found that gabapentin, pregabalin, duloxetine and a variety of tricyclic antidepressants (such as amitriptyline) have strong recommendations for use and are recommended as first-line treatments for both peripheral and central neuropathic pain. However, it is worth noting that pharmacological treatments for chronic neuropathic pain are effective in <50% of patients.

There is also a wide variety of interventional methods which have been shown to be effective in neuropathic pain[204]. Neural blockade and steroid injections are examples for interventions which offers transient relief to patients[205,206]. Spinal cord stimulation is used as a form of neuromodulation and has been shown to have sustained results[207–209]. Therapies targeting the brain have also been studied, including epidural and transcranial cortical neurostimulation[210] which is suggested to activate descending inhibitory pathways, and deep brain stimulation[211] which remains controversial due to safety concerns[212].

Whilst there is evidence supporting physical therapies in neuropathic pain[213,214], this area remains under-researched. Neural mobilisation is one technique used in

neuropathic pain conditions and has been shown to reduce neuro-inflammation[215–218]. Yoga has also been found to reduce pain[219,220], including specifically chronic pelvic pain in women[221], and discussions are ongoing about further exploring this in neuropathic pain soon.

Psychological therapies or talking therapies for chronic pain have in recent years received a lot more attention[222]. Cognitive Behavioural Therapy is one such technique which aims to identify thought processes and actively change these behaviours[223]. Mindfulness is another talking therapy which is used in chronic pain, with many studies looking at its efficacy and the mechanisms by which it has an effect[224,225]. It is worth noting that whilst there is a lot of evidence supporting psychological therapies in chronic pain[222], there is little evidence specific to neuropathic pain, which as discussed presents its own distinct symptoms and difficulties.

A multi-disciplinary approach to care is a collaboration of healthcare professionals from a range of disciplines who together deliver comprehensive patient care that meets the needs of the individual[19,226,227]. Clinically, in many areas of chronic pain management, the need for a multi-disciplinary approach is well established, however this is not always attainable, in part due to the way in which health care (especially in the UK) is set up, with specialities being separated by human anatomy as opposed to symptoms. For example, for the most part we see 'gynaecology' clinics/departments, rather than 'pain' clinics/departments, making cross-fertilisation from different specialisms more difficult. This multi-disciplinary approach is arguably essential, especially in chronic pelvic pain and neuropathic pain, where there is a wide variety of symptoms and patient priorities as well as treatment options.

1.9 Thesis Aims

As detailed in this introduction, endometriosis-associated pain is highly complex and heterogeneous and currently, poorly understood.

In the five results chapters of this thesis, I will:

- Explore the prevalence of neuropathic pain in endometriosis-associated pain
- Explore treatment response to gabapentin in women with chronic pelvic pain with no known pathology
- Investigate the resting connectivity of the brain for mechanisms which could be involved in neuropathic endometriosis-associated pain, based on previous findings in our group
- Investigate whether findings in resting state connectivity in other neuropathic pain conditions can be replicated in endometriosis-associated pain
- Explore results of quantitative sensory testing in women with endometriosis-associated pain

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Chapter 2: Methods

2.1 Questionnaire measures

painDETECT

There are several ways to assess neuropathic pain; the one which I have used in this thesis is painDETECT[1,2]. painDETECT is a questionnaire designed as a screening tool for neuropathic pain, which has been widely used in a variety of pain conditions[3]. It is filled out by the patient/participant themselves and asks questions about the time-course f pain, as well as whether the pain radiates and asks for scores for different sensory pain symptoms (burning, prickling, numbness, pain attacks, thermal hyperalgesia, mechanical allodynia and pressure evoked pain). This tool was originally validated in lower back pain[3,4] but has since been used in several pain conditions, including but not limited to osteoarthritis[5,6], rheumatoid arthritis[7], diabetic neuropathy[8], bladder pain syndrome[9] and fibromyalgia[10–13]. One of the main benefits of this tool is that it does not require clinical assessment, meaning that it can be distributed online to a large sample. Whitaker et al[14] carried out a comparison between different assessments of neuropathic pain in chronic pelvic pain and found that painDETECT had a sensitivity of 85.7% and specificity of 50% and that it was favourably viewed by 93% of subjects, with 90% finding it easy to complete.

This questionnaire consists of nine questions, which assess the severity, course, quality and nature of the patient's pain and specific neuropathic pain symptoms. Intensity ratings are scored between 0 (= never), 1 (= hardly noticed), 2 (= slightly), 3 (= moderately), 4 (= strongly) or 5 (= very strongly). Scores >3 (strongly or very strongly)

are considered clinically relevant. painDETECT standard cut-off scores were used to group participants into those with nociceptive, mixed and neuropathic pain (nociceptive ≤12, mixed 13-18, neuropathic ≥19)[1]. This categorisation has been shown to correspond to clinical diagnoses of neuropathic pain (e.g., neurological or electrophysiological examination, imaging) in various chronic pain populations (e.g., Freynhagen et al., 2006[1]).

Fibromyalgia Symptom Scale (FS)

To assess the involvement of a 'centralised' pain component, I included the Fibromyalgia Symptom Scale (FS)[15] which assesses whether the pain is more widespread or localised, whether patients experience cognitive difficulties (i.e., fatigue, trouble thinking or remembering, and waking up tired; rated between 0= no problem and 3= severe, continuous, life-disturbing problems) and three additional symptoms (pain or cramps in the lower abdomen, depression and headache in the last six months; all rated as 0= not present or 1= present). The total FS score ranges from 0 to 31. Although originally designed for fibromyalgia patients, the FS questionnaire has previously additionally been used in patients with postoperative pain following hysterectomy[16,17] as well as in other pain conditions[18–20].

2.2 Functional magnetic resonance imaging (fMRI)

fMRI analysis looks at the BOLD (blood-oxygen-level-dependent) signal in the brain. At the microscopic level, when a neuron in the brain fires action potentials, there is an increase in blood oxygenation in the area around this neuron. This increase in oxygen supply is greater than the neuron's demand for oxygen, meaning that where there is

greater neuronal activity, there is a greater concentration of oxygen in the blood. BOLD fMRI measures the blood oxygenation level as a proxy for the neuronal activity. This haemodynamic response is a relatively slow process and reaches its peak 5-6 seconds after the start of the neural activity. Whilst BOLD signal is an indirect measure of neuronal activity, it has been used extensively in the neuroscience field to further our understanding of how the brain works.

Briefly, task-based fMRI is used to look at the BOLD signal response in the brain to a certain event or task. In effect, during analysis, the activity of the brain before the task is 'subtracted' from the activity after/during the task to give the activity which is specific to the event/task. This analysis relies on the timing of the events/tasks and has been used extensively in neuroscience research generally, as well as in both acute and chronic pain states[21–25]. In the study used in this chapter, the participant received a punctate stimulus (pinprick) to the lower abdomen, their region of pain. In the analysis discussed here, the brain activity in response to this stimulus was correlated with painDETECT score.

Conversely, resting state functional connectivity analysis, as its name suggests, is a way of assessing connectivity of the brain at rest[26]. Investigating functional connectivity, in a nutshell, is looking at how information is passed from one area of the brain to another and how different brain areas communicate with each other. The way in which this is done is to look at similarities in the BOLD signals from different areas of the brain. The overarching assumption is that if regions are communicating and passing information (are functionally connected) it is likely that they will have similar BOLD signals[26]. Generally, investigations of the connectivity of the brain do so using fMRI scans where no task is given (i.e. resting state scans) as bias could result from the cognitive demand

of a specific task. The typical definition of functional connectivity is "the observed temporal correlation (or other statistical dependencies) between two electro- or neurophysiological measurements from different parts of the brain"[26]. Thus, it is important to highlight that functional connectivity is not typically used to describe any directionality or causality (effective connectivity), nor can it be interpreted as a structural connectivity. Chapters 5 and 6 investigate resting state functional connectivity in women with endometriosis-associated pain.

2.2.1 MRI data acquisition

MRI data was collected on a 3T Siemens scanner (MAGNETOM Prisma syngo MR D13D) with head coil (HEA, HEP). Structural images were acquired using T1 weighted imaging (FoV read 192mm, slice thickness 1mm, TR 1900ms, TE 3.97ms, flip angle 8 degrees, TI 904ms, base resolution 192, phase resolution 100%). Fieldmaps were also obtained (FoV read 192mm, slice thickness 2mm, TR 482ms, TE1 4.92ms, flip angle 46 degrees, base resolution 96, phase resolution 100%, 49 slices). For resting state acquisition BOLD data collection was used (FoV read 192mm, slice thickness 2mm, TR 933ms, TE 33.4ms, multiband acceleration factor 6, flip angle 60 degrees, base resolution 96, phase resolution 100%, 72 slices). Multiband EPI sequences were used for the fMRI scans (accelerator factor of 6). In multiband EPI, data from multiple slices are acquired at once (in this case 6), which allows the reduction of the TR (repetition time). This is beneficial as it allows a wider range of frequencies to be sampled and increased the total number of time points (temporal degrees of freedom), improving the statistical power. The resting state scan was acquired before any tasks to minimise the risk of the participant falling asleep and also to ensure that their background pain state had not been affected by the noxious stimuli used in the tasks.

2.2.2 Resting state fMRI pre-processing

When investigating functional connectivity, it is very important to remove as much structural noise as possible, as it can strongly influence analysis. There are several methods to try and remove such noise, some of which are used generally in fMRI analysis (which I will discuss first) and others which are specific to resting state analysis (discussed at the end of this section).

The sources of structured noise are mainly summarised by two categories: movement and physiological. Movement of the participant in the scanner (head motion specifically) is the most problematic cause of structured noise and thus is a key focus of pre-processing steps. Whilst attempts can be made by the participant themselves, as well as the scan operator (i.e. head padding) to reduce head movement, it is impossible to completely avoid. Another issue with this type of noise is that it varies a lot between participants so can be very problematic, if not controlled for, when making comparisons between participants. Physiological noise includes artefacts from the participants heartbeat, which causes pulsatile motion in large arteries and breathing, which naturally involves movement of the chest and abdomen resulting in small head motion.

All pre-processing was carried out using FMRIB Software Library (FSL)[27–29] tools.

Structural images were brain extracted using BET tool[30] and then visually checked for accuracy. Fieldmaps were available for all bar one participant. Fieldmaps were brain extracted using BET followed by fslmaths -ero to ensure all non-brain tissue is removed as is necessary in the preparation of fieldmaps. The GUI Fsl_prepare_fieldmap was used to combine the phase and magnitude images and create the fieldmap as used in

FEAT[31]. For participant P02, for which no fieldmap was acquired in the scan, an average fieldmap was created from the other participants' fieldmaps.

MCFLIRT[32] was used for motion correction. Spatial smoothing helps to reduce the influence of noise by calculating at each voxel a weighted average over multiple neighbouring voxels. For this analysis, spatial smoothing of 5mm was used to ensure that small structures in the brain stem were able to be examined and as the same smoothing kernel was used in task-based analysis[33]. High-pass temporal filtering of 700 seconds was used to remove any effects of scanner drift. Registration was carried out from the resting state multiband data to a single band reference image which was collected during the resting state scan. Registration was then carried out from the participants' functional space to their structural space using linear registration (FNIRT) and from that to standard MNI-152 (2mm brain extracted) space using non-linear regression (FLIRT[32,34]).

My resting state specific pre-processing included using physiological noise modelling[35–41] and independent component analysis (ICA)[42–44] with manual labelling[45]. Physiological measurements taken during the scan can be used to create regressors that reflect physiological fluctuations. These regressors can then be removed from the data by taking the residuals from a multiple linear regression. Physiological noise regression is especially important when the areas of interest are likely to be affected by physiological fluctuations, as in this analysis investigating the pontine reticular formation (pRF), which is in the brainstem and the insula. Independent component analysis decomposes a whole brain resting state BOLD dataset into a set of spatially structured components. Typically, some components represent neuronal signal and other components represent structured noise. The number of components extracted varies

across participants and is estimated automatically. These components can then be classified as signal or noise, either manually based on inspection or using automated classification methods. This classification can be 'aggressive' or 'non-aggressive'; the 'aggressive' approach leads to the removal of all the variance that can be explained by timeseries of the noise components, whereas the 'non-aggressive' approach only removes the variance which is unique to the noise components. Generally, the non-aggressive approach is preferable as it preserves as much signal as possible.

Firstly, measurements were made during the scan using a heart rate monitor and bellows, which allowed for the collection of data on heart rate and breathing. This supported the use of Physiological Noise Modelling (PNM). The output file of which was used in FEAT in a later step.

To try to reduce any other structured noise, especially head motion, Independent Component Analysis (ICA) was run on each subject using MELODIC. The resulting components were manually labelled, according to Griffanti et al[45]. During manual classification, for every ICA component the spatial map, the time-course and the frequency spectrum are all visually inspected together. Manual labelling was chosen despite the existence of FIX[46,47] because FIX was designed and trained on the labelling of healthy participants. Given that I have a population of female chronic pain patients I decided manual labelling to be more appropriate. Labels were then checked by a colleague (Miriam Szabo) following guidance from the same paper with concordance. A txt file was then created for the noise components using Tmodes and MATLAB.

The noise components identified using PNM and ICA were then simultaneously removed using FEAT[31] for each individual separately. As discussed, MCFLIRT, B0 unwarping with fieldmaps and 5mm spatial smoothing were used. The output file from

PNM was added as the voxelwise confound list and the file from ICA analysis was added as an additional confound EV. The output of FEAT was therefore cleaned data which was subsequently transformed into standard MNI 152 (2mm brain) space.

2.2.3 Resting state fMRI group level analysis

There is no gold standard in functional connectivity analysis and there are many different approaches which can be used. To enable thorough analysis of the dataset I decided to try a variety of different methods which will be discussed in Chapters 5 and 6.

Broadly these are separated into voxel-based methods and node-based methods. Voxel-based methods estimate a value of functional connectivity for every voxel in the brain, which results in a map of the brain containing a value at each voxel. Node-based analysis, however, looks at the connections (edges) between specific brain regions (nodes). As well as these categories, analysis can be hypothesis or data driven. Hypothesis driven analysis discussed in this chapter is the use of regions of interest (ROIs) as seeds/masks for group level analysis. This allows me to investigate specific areas of the brain which I hypothesise will give interesting findings, on the basis of previous studies[25,33,48–50]. The alternative data driven approach is useful as it is able to describe multiple different resting state networks, rather than only being sensitive to the connectivity pattern of the seed.

Voxel-based analysis using ROIs

Both Chapters 5 and 6 use seed-based correlation analysis (SCA). SCA is an example of voxel-based connectivity and gives a whole brain map which describes the functional connectivity of the seed region.

After selection of the seeds, the steps in SCA are extraction of the BOLD timeseries for the chosen seed(s) and then the calculation of the correlation coefficient between the timeseries of the ROI and the timeseries of each voxel in the brain. Specifically, in my analysis these steps were carried out using dual regression[51,52].

First, for each subject, the group-average set of spatial maps is regressed (as spatial regressors in a multiple regression) into the subject's 4D space-time dataset. This results in a set of subject-specific timeseries, one per group-level spatial map. Next, those timeseries are regressed (as temporal regressors, again in a multiple regression) into the same 4D dataset, resulting in a set of subject-specific spatial maps, one per group-level spatial map.

The General Linear Model (GLM)[53] GUI was used to create contrasts testing: group mean connectivity (positive and negative), correlation with demeaned painDETECT score controlling for demeaned background pain score and, in Chapter 5 only, group mean controlling for demeaned pain scores. These contrasts are tested using FSL's randomise[54] permutation-testing tool, with 5000 permutations. The output of this dual regression gives a file for each contrast (i.e. 1 for group mean, 2 for correlation (positive and negative correlations)) which undergoes threshold-free cluster enhancement[55] (this is a new method which finds ‘clusters’ in data without having to define clusters in a binary way) as well as being corrected for multiple comparisons. These corrections are especially important when using voxel-based methods as a large number of comparisons are made (i.e. greater number of comparisons than node-based analysis).

Node-based connectivity using ROIs

In order to investigate specifically the functional connectivity between the ROIs stated above, node-based analysis was also carried out in Chapter 5. As discussed, node-based methods investigate the strength of connections (edges) between ROIs (nodes). For this analysis, the timeseries (fluctuations of the BOLD signal) of each node is extracted. Correlation analysis is then carried out between every possible pair of nodes. Full correlation analysis uses Pearson's correlation coefficient to estimate the similarity between timeseries. However, full correlation analysis has the disadvantage of being sensitive to both direct and indirect relationships between nodes. Whilst partial correlations try to overcome this by only being sensitive to direct relationships, it can result in a greater number of false negatives. Therefore, I ran both full and partial correlation analysis so that any significant results could be compared between the different methods of correlation, with appropriate corrections applied to significant findings. This analysis was carried out using FSLNets in MATLAB. The timeseries of each ROI was taken from the first stage of dual regression, which used a mask with all seeds combined to form one 4D image. FSLNets then loaded the timeseries of each seed separately and ran the analysis.

Data-driven voxel-based analysis

I also carried out data-driven voxel-based analyses. This gives a non-hypothesis driven analysis of the data and is therefore completely data driven. ICA decomposes a multivariate signal into a set of components which represent some structures present in the data. In summary, ICA aims to separate the resting state fMRI data into separate components which were otherwise combined in the BOLD signal. This independent

component analysis was carried out via FSL MELODIC. In this analysis 25 components were specified to aim to achieve optimal fitting. This was selected as it is within the range of the most commonly used dimensionalities[26] for voxel-based analysis, although it is important to note that this is a compromise between having too few components and having too many which would result in networks being split across components. Each resulting component is described by a timeseries (BOLD signal fluctuations) and a spatial map (reflecting where in the brain the BOLD signal is seen). Each component is spatially statistically independent, meaning that there is no statistical relationship between the components. In my analysis I used multi-session temporal concatenation which is recommended in the FSL user guide (fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC) for analysis of resting state fMRI. Then, dual regression was used to obtain and compare subject maps. For this the output of MELODIC was fed into the dual regression with the GLMs for correlation with painDETECT (controlling for pain scores as described above). As explained above, the first stage of dual regression performs a multiple regression with the group ICA maps as spatial regressors and the subject preprocessed data as the input, resulting in a set of time-courses. These then become the model input for the second regression (stage 2), which outputs a set of maps that describe the network structure. The outputs from stage 2 are used for analysis between subjects, as designed in the GLM.

2.3 Quantitative sensory testing (QST)

2.3.1 Data collection

Whilst there are different QST paradigms, my work has used the German Neuropathic Pain Network's (DFNS) protocol[56] (Chapter 7). The DFNS protocol which is used in

Chapter 7 is highly standardised, and training is required to be able to carry it out to ensure that results from different researchers/clinicians are comparable.

All data collection was done strictly following DFNS protocol by myself and a colleague after having completed training in Mannheim, Germany. It is important to follow the same protocol for each participant so that data from other sites involved in TRiPP can be combined with ours. It also ensures that I can make direct comparisons to reference values[56].

For all QST measures standardised instructions were read aloud to aim to prevent the use of biasing language. To ensure that the participant had understood the instructions, verbal confirmation was attained. Participants were lying on an adjustable testing chair for the duration of the tests, to ensure ease of access to the testing sites. The tests were carried out on the control site (dorsum of the right foot) first, then the test site (lower abdomen/pelvis below naval for all participants). After collaboration with the members of DFNS, the dorsum of the foot was selected for the control site as reference data is available for comparison and it was thought, of available options, to be most similar to the lower abdomen/pelvis. All data was collected on the official QST form (Appendix 1) and later manually uploaded to a secure database and data inputting was independently verified.

Thermal testing

The first tests are of thermal sensation. A thermal tester (thermode) was used with specialised computer software which cools the thermode from baseline (32°C) until the participant detected a change (cold detection threshold CDT), the same is then done for warm detection (WDT). Then thermal sensory limen (TSL) was tested, which asked the

participant when they first detected a change in temperature (either cooler, warmer or hotter), the thermode alternated between cooling and warming. However, during the cooling phases, some participants reported "warm" or "hot" sensations, these are classified as paradoxical heat sensations and the number of occurrences is documented. The last thermal testing was pain threshold for cold and heat (CPT and HPT respectively), which as the name suggests, asked participants to report when the sensation became painful, described as "an additional impression of a burning, stinging, drilling or aching sensation". These stimuli test Aδ and C fibre function[57]. The thresholds in thermal testing uses a 'method of limits', meaning that the actual threshold is slightly overrated due to the artefact caused by reaction time.

Thermal testing was carried out using the Thermal Sensory Analyzer (TSA-II) (Medoc, Isreal) with a 9cm^3 thermode and associated software. The TSA-II was used to test cold threshold (detection and pain), warm threshold (detection and pain) and thermal sensory limen[58]. All thresholds were obtained with ramped increasing/decreasing (1°C/s) thermal stimuli, starting at a baseline of 32°C. For threshold tests (CDT, WDT, CPT and HPT) the test was repeated 3 times, with the thermode returning to baseline in between with an interval between stimuli of 6 seconds for CDT and WDT and 10 seconds for CPT and HPT. Cut-off temperatures of the TSA are 0°C and 50°C to avoid tissue damage. Participants were given a handheld button and instructed, for each test, when to use it (e.g., press the button when they first detected the thermode to be warm).

Mechanical detection thresholds

For mechanical detection thresholds, von Frey hairs are used which are very fine filaments of various thicknesses with rounded ends. These are placed on the skin until they bend, giving a standardised pressure (depending on the filament). The participant is

asked to say when they feel a touch sensation. A modified method of levels is used with five series each of ascending and descending stimulus intensities[57] starting with the 16mN von Frey hair then lowering intensity until participant does not feel any touch. The procedure is then reversed with increasing intensity until the participant reports feeling a sensation and then both steps are repeated. The method of levels determines a threshold based on the stimulus intensity at which half the stimuli are detected, which requires a longer testing period and can lead to the development of sensitization. Mechanical detection threshold assesses function in Aß fibres.

Von Frey hairs (Somedic Aesthesiometer II; add manufacturer) at values 0.63mN, 1.37mN, 3.14mN, 16.67mN, 50mN, 81.4mN and 235.36mN were used for MDT. Whilst these are not currently listed in the QST Investigator's brochure, it was agreed through personal correspondence with the DFNS directors board that these are an acceptable alternative as the original merchant is no longer supplying. The procedure starts with the 16.67mN von Frey: if this is felt the next lower intensity is used until it is not felt; if 16.67mN is not felt the next higher stimulus is applied until a touch sensation is felt. The procedure is then reversed and repeated until five sub-threshold and five supra-threshold values are collected. The von Frey hairs are applied to the skin with enough pressure to produce a small bend in the filament. If a participant can feel the lowest intensity stimulus, half that value is reported (0.315mN). Similarly, if a participant cannot feel the highest intensity stimulus, double its value is reported (470.72mN).

Mechanical pain thresholds

Mechanical pain threshold uses the same method of levels for pin pricks, asking when the participant feels the sensation as “blunt” and then “pricking/sharp/stinging”. Mechanical pain threshold helps identify dysfunction of Aδ and C fibres.

The mechanical pain threshold was assessed using needle stimulators (pinpricks) at standardised intensities of 8mN, 16mN, 32mN, 64mN, 128mN, 256mN and 512mN. Pinpricks were purchased from MRC Systems. Pinpricks were pressed against the stimulation site at a 90° angle for 1 second. The participant was asked to classify each stimulus as “sharp” if they perceived any sharp/pricking/stinging sensation and “blunt” if they only perceived a touching sensation. The procedure started with the 8mN pinprick and was followed by sequentially higher intensity pinpricks until the participant said the sensation is “sharp”. Then the next lower intensity stimulus was used until the sensation was classified as “blunt”. This was repeated until, as with MDT, five sub-threshold and five super-threshold values were collected. As for MDT, 4mN is recorded as the threshold if 8mN is classed as sharp. Likewise, 1006mN is recorded if 512mN is classed as blunt.

Stimulus/response function

Stimulus/response (SR) functions looking at mechanical pain sensitivity (MPS) and dynamic mechanical allodynia (DMA) are then assessed. This uses the pinpricks again, alongside a brush, a cotton wisp and a Q-tip. There is a randomised order to apply the stimuli for both the test site and the control site, with the participant asked to give a pain intensity rating out of 100 (with anything “sharp” or at all painful scored above 0). Mechanical pain sensitivity tests the function of C and Aδ fibres, whereas dynamic mechanical allodynia asses the function of Aß fibres.

The SR function procedure used the same pinpricks discussed above, alongside a SENSELab Brush-05, a Q-tip and a cotton wisp (supplied with pinpricks from MRC). The various stimuli were given to the participant in a randomised order, as dictated by the QST CRF form. The participant was asked to rate the sensation out of 100 according to how painful it is (with 0 = no pain, 100 = worst pain imaginable and any sensation which is sharp/stinging/pricking rated above 0). The order of stimuli was different for the test and control sites.

Wind-up ratio

To determine the wind-up ratio (WUR), one of the needle stimulators was used. Following a single stimulus, a series of 10 stimulations was applied at 1Hz. Participants were asked to provide pain intensity ratings between 0 and 100 for the single stimulus and an average score for the series of stimulations. Wind-up is a phenomenon of temporal summation in the spinal cord, which increases the response of wide-dynamic-range neurons after repetitive stimulation of C fibres.

For WUR a fixed intensity pinprick (256mN) is used. First a single stimulation was given to an area, which the participant was asked to rate, as above, out of 100. Then, 10 seconds later, a series of 10 stimulations were given (one every second) within an area approximately $1cm^3$ (close to single stimulus). The participant was then asked for a rating of the average painfulness of the series. The procedure was repeated five times.

Vibration detection threshold

The vibration detection threshold is assessed using a tuning fork, which is placed on a bony prominence at the test and control sites. The participant is asked to report when

they no longer feel a vibration sensation and the test is repeated twice more (these are then averaged in analysis). Vibration detection threshold assessment tests Aß fibres.

A Rydel Seiffer 64Hz tuning fork was used for VDT assessment. The tuning fork was hit and then placed on a bony prominence (navicular bone on dorsum of foot at control site and pubis symphysis at test site). The participant was asked to say when they can no longer feel the vibration and the tuning fork is read using the scale out of 8. This was done a total of three times.

Pressure pain threshold

The pressure pain threshold is the last test in the QST protocol and uses an algometer to apply a steadily increasing pressure to a specific area, until the participant reports it is painful. This test is done on a muscle site and is repeated a total of three times. Pressure pain threshold helps identify dysfunction of Aδ and C fibres.

Finally, PPT uses an algometer (10kg/100N from Wagner Instruments) with a contact area of $1cm^3$ with a flat tip. This test is carried out above muscles (dorsum of foot and lower abdominal muscles). Pressure was applied and increased at approximately $0.5kg/cm^2s$ and participants were asked to report when it becomes painful. This procedure was repeated to give 3 readings.

2.3.2 Data analysis

All data transformation was done in Microsoft Excel. All analysis followed DFNS protocol[56].

For all QST variables except PHS, CPT, HPT and VDT, raw data was log transformed (using $\log_{10}$).

The responses for CDT and WDT were calculated as difference from baseline (32°C). These values were then log-transformed (for CDT the negative was ignored). For CDT, WDT and TSL the values after log-transformation were used to calculate the mean and standard deviation for each individual. For PHS as the raw data are counts, they did not need to be altered on an individual level. For CPT and HPT individual mean and standard deviation values were calculated from the data without log-transformation.

For MDT and MPT the geometric mean was calculated and then log-transformed. The standard deviation was calculated from log-transformed data.

SR function data was collected as scores 0 – 100; 0.1 was added to each value so as not to lose 0 values during log transformation. Mechanical pain sensitivity (MPS) was calculated using the responses from all pinprick stimuli and allodynia (DMA) was assessed using ratings to brush, Q-tip and cotton wisp only. For both MPS and DMA the geometric mean was calculated and log-transformed and the standard deviation of log-transformed values was also calculated.

As above, 0.1 was added to pain ratings for WUR to ensure 0 values were not lost. The data were then log-transformed. The mean and standard deviation of the single stimulus and the series of stimuli were calculated. The mean rating of the series was divided by the mean rating of the single stimuli to calculate the ratio.

The mean and standard deviation for VDT were calculated from the raw data (scored out of 8).

PPT data were collected in kg. These values were transformed into kPa and log-transformed. The mean and standard deviation were calculated from the transformed data.

All data were then z-transformed using reference data. As reference data was available for women split by decade of age, for each participant the relevant reference data was used (i.e. if participant was 32 years old, reference data from women aged 30-40 was used). Reference data for the dorsum of the foot was used to Z transform both control and test site data. A Z score greater than 0 shows a gain of function and a Z score less than 0 shows a loss of function.

The Z transformation equation[59] used is below:

$$Z = \frac{value_{participant} - mean_{reference}}{standard\ deviation_{reference}}$$

Comparisons between the test and control sites for the group were carried out using paired Student's t-tests. Comparisons between control/test sites and healthy control (reference data) were carried out using an alternative t-test (as data is previously Z transformed), this equation is from Magerl et al[59]:

$$t = \frac{mean_{participants} - 0}{\sqrt{standard\ deviation_{participants}^{\ 2} \Big/ (n-1)/n}}$$

Multiple comparison correction was not used for these tests as it is pilot data.

To relate QST measures to painDETECT scores, correlation analyses were carried out. Normality tests were carried out on each painDETECT sensation variable (i.e. those scored out of 5, excluding question on time course and spatial properties of the pain).

For normally distributed variables Pearson's correlation was used; non-normal measures non-parametric Spearman's correlation was used. Each QST variable was correlated against each sensory painDETECT variable.

To determine whether individuals could be categorised into clusters previously described[60], I used a recently developed algorithm[61,62]:

$$F(3):\ p*_{i_{n,m}} = \exp\left(-\left(\frac{\left(x_{i_n}-\mu_{i_m}\right)^2}{2{\sigma_{i_m}}^2}\right)\right)$$

with *i* = one of 13 QST parameters, *n* = the *n*th patient in a set of patients, *m* = one of 4 phenotypes and conclusively, σ_{i_m} being the SD of the *i*th QST parameter for the *m*th phenotype in the defining data set μ_{i_m} being the mean *z*-value of the same QST parameter and phenotype (these values are taken from Vollert et al 2017[62]) and finally x_{i_n} being the *z*-value found in the *n*th patient for the *i*th QST parameter. This gives a probability percentage for each parameter for each phenotype. The percentages for the 13 QST parameters are then averaged, and the individual is classified by the phenotype with the largest probability percentage.

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Chapter 3: Is there a neuropathic component to endometriosis-associated pain? Results from a large cohort questionnaire study

The contents of this chapter are taken and adapted from Coxon et al. 2020 (preprint), with permission from authors.

3.1 Abstract

Endometriosis-associated pain is commonly understood to be nociceptive in nature. I have carried out a large cohort online survey to identify the prevalence of neuropathic-like pain in women with endometriosis-associated pain. Using the painDETECT questionnaire, I have found that 40% of women have neuropathic-like pain, with a further 35% having a mixed neuropathic/nociceptive pain. Those with neuropathic pain had greater pain scores and more psychological distress and alterations in cognitive-affective functioning. Those with a greater number of surgeries to the abdomen were more likely to be classified as neuropathic-like pain. I found two subgroups within the neuropathic and mixed groups, based on sensory symptom profiles. This evidence of neuropathic symptoms in a large number of women with endometriosis-associated pain challenges our current understanding of this type of pain and advocates the exploration of neuropathic adjuncts for treatment.

3.2 Background

As discussed in Chapter 1, my thesis introduction, there are many reasons to suspect that neuropathic pain may be present in some women with endometriosis-associated pain (EAP); however, the prevalence of a neuropathic component has not been properly investigated in this population. In this chapter I discuss my investigation into this.

In clinical practice, women often describe their endometriosis-related pain as 'stabbing' and 'tingling', which are known to be key features of neuropathic pain. Neuropathic pain could be expected to arise in the context of endometriosis for a number of reasons. Firstly, there is currently no non-invasive test available to establish the diagnosis of endometriosis. As a consequence, all women with a confirmed diagnosis will, by definition, have undergone at least one surgical (usually laparoscopic) procedure, with the associated risk of post-surgical neuropathic pain[1–3]. Secondly, endometriotic lesions are innervated by new nerve fibres[4,5]. Surgical procedures excising or ablating the lesions may damage these nerve fibres, which in turn could generate neuropathic pain[1]. Thirdly, these nerve fibres express TRPV1 receptors and are bathed in the peritoneal fluid, known to contain high levels of inflammatory mediators such as BDNF and TNF-alpha in women with endometriosis, potentially sensitising nerve endings[6–10]. Thus, even without further surgery, neuropathic pain could develop over time as a result of prolonged exposure to these inflammatory mediators. In a recent study on chronic pelvic pain which included a subset of patients with endometriosis, over 50% of patients showed clinical features of neuropathic pain. However, due to the small sample size (n= 32 with endometriosis), findings from this study have to be interpreted with caution.

Clinically, it is important to investigate the prevalence of neuropathic pain in women with EAP as it is widely accepted that neuropathic pain requires different treatment options than 'traditional' nociceptive pain. One key example of this is neuromodulators,

such as gabapentin and amitriptyline which have been shown to be effective in the treatment of neuropathic pain; whereas classic painkillers such as paracetamol and ibuprofen are known to have little benefit in neuropathic pain. Given that many women with endometriosis-associated pain do not receive adequate pain relief from classic painkillers it is especially important to find new treatment options; if there was evidence that women have a neuropathic component to their pain then neuropathic adjuncts would be a clear choice. It is also critical to consider neuropathic pain when contemplating surgery. There is little to no evidence that surgical intervention helps alleviate neuropathic pain and, in fact, there is a lot of literature showing that surgery can lead to neuropathic pain[2,3,11,12]. Given that surgery is necessary for clinical diagnosis of endometriosis as well as a treatment option, it is easy for surgeries to become routine, the result of which is women having repeated surgeries every few years with the hope that they will help relieve pain symptoms. Surgeons considering the presence of neuropathic pain may therefore reduce the number of surgeries given to women (which may not be successful) and also change post-operative care so as to try to reduce the risk of post-operative neuropathic pain.

There are several ways to assess neuropathic pain; the one which I have used in this chapter is painDETECT[13,14] (see Chapter 2).

Patient support sites for endometriosis provide a support network for women with endometriosis and have a lot of information and resources (such as support groups and information pages). Often members are willing to take part in research where they can, therefore they can provide a valuable resource for researchers. As these support sites often have a large following, the volume of women who respond to posts and surveys can be large. This I think highlights how important such work is and how fervent

women are to improve the understanding and treatment of endometriosis. Whilst targeting women via these sites may lead to a bias in the sample, there is of course a large variety of women on these sites. Although women on these sites may represent those who experience greater impact of symptoms or respond less well to treatment which may make them particularly susceptible to bias, it can be argued that these women are the most in need of research characterising their symptoms. In this chapter I asked patient support groups to share a link to an online survey for women to complete, as it is a good way to try to reach a lot of women in a relatively short time frame.

From reviewing the literature, I identified an evidence gap in knowledge about the prevalence of neuropathic pain in women with endometriosis-associated pain. I therefore designed a study to address this. The aims of this study where:

- To determine the proportion of women with endometriosis-associated pain who, using the painDETECT questionnaire, are classified as having neuropathic-like pain

- To examine the correlation between painDETECT and other commonly used pain assessments: the Fibromyalgia Symptom Scale and Pain Sensitivity Questionnaire

- To establish whether there are differences in pain severity between painDETECT groups, as well as differences in psychological and cognitive measures

- To investigate whether the duration of pain or number of surgeries to the abdomen are different between the painDETECT groups

- To explore whether subgroups can be found in those with a neuropathic component based on their sensory symptom profiles.

In summary, I undertook a study using an online survey to determine the prevalence of neuropathic-like pain in endometriosis-associated pain in a large cohort. To target a large sample size from across the UK, I designed an online survey using painDETECT as a screening tool for a neuropathic component to pain and posted this on patient support group websites. I hypothesised that endometriosis-associated pain would be classified as neuropathic in a subgroup of women (findings from a small study in chronic pelvic pain would suggest over 50%[15]). As in other types of neuropathic pain[16], I hypothesised that these women would score higher on questionnaires assessing depression and anxiety scores. I also expected the proportion of women with neuropathic pain to increase with the time since pain onset and with the number of surgeries to the abdomen. Studies on postherpetic neuralgia, painful diabetic neuropathy and other neuropathic conditions have proposed subtypes of neuropathic pain which differ in their sensory profile as determined based on painDETECT responses in combination with a cluster analysis approach[17,18]. Using a similar strategy, I tested for subgroups amongst those classified as having neuropathic and mixed pain.

3.3 Methods

3.3.1 Survey design

The survey was created using Online Surveys (https://www.onlinesurveys.ac.uk, formerly Bristol Online Surveys) and was designed by combining single questions regarding patients' pain characteristics and other relevant variables with validated

questionnaires. Single questions included: patients' ratings of the maximum pain intensity experienced over the last 12 months for dysmenorrhea, dyspareunia, non-cyclical pain, dyschezia and dysuria using a Numerical Rating Scale (NRS) anchored at 0= no pain and 10= worst imaginable pain; duration of each type of pain since onset of symptoms (in years) and number of surgeries received to the abdomen.

In addition to these single questions, participants completed a set of validated questionnaires. For somatosensory symptoms of neuropathic pain, painDETECT[13,14] was used (discussed in more detail in Chapter 2). To assess the involvement of a 'centralised' pain component, I included the Fibromyalgia Symptom Scale (FS)[19] (see Chapter 2).

For a specific assessment of symptoms of depression and anxiety I included: the Beck Depression Inventory (BDI) which requires participants to rate the occurrence of thoughts, feelings and behaviours associated with depression such as self-dislike, crying, and loss of interest in the last 2 weeks (total score: 0-63)[20–22]; and State Trait Anxiety Inventory, Trait (STAI-T) which assesses how individuals generally describe themselves with regards to anxiety such as "I am nervous and restless" and "I feel inadequate" (total score: 20-80)[23,24].

Finally, the Pain Sensitivity Questionnaire (PSQ) was included as a patient-rated assessment of their pain sensitivity to 17 hypothetical stimuli, such as "imagine you burn your tongue on a very hot drink" (0= not painful at all to 10= worst pain imaginable)[25]. Total scores are calculated as mean score for 14 of these (removing 3 which refer to normally non-painful stimuli). PSQ ratings have been shown to be positively correlated with experimental pain intensity ratings and pain thresholds in healthy volunteers and chronic pain patients[25,26].

The survey was posted on patient support websites (Endometriosis UK, www.endometriosis-uk.org; Endometriosis Association of Ireland, www.endometriosis.ie; and Endometriosis SHE Trust UK, www.facebook.com/EndoSheTrust/). It was open for responses between March and May 2018. Participants could complete the survey at their leisure and in several sessions and were able to withdraw at any time. The survey was beta tested on 9 patients, recruited from clinics at the Oxford University Hospitals Foundation Trust and the survey was modified according to their feedback. Patients were not reimbursed for their participation.

3.3.2 Ethical approval

I received approval for this study from the Central University Research Ethics Committee, University of Oxford, R56567/RE002. Implied consent was attained via tick boxes to ensure the participant was over the age of 18 and that they agreed to take part in study.

3.3.3 Data analysis

All statistical analysis was carried out using SPSS Statistics version 25.

All data was visually inspected for errors. Participants were excluded if they had not received laparoscopic surgery to confirm the diagnosis of endometriosis or if they had not provided the age at which they received the diagnosis. If participants provided a range in response to a question requiring a numerical response, the median of this range was used. The duration of pain symptoms was calculated as the difference between patients' current age and the time-point they first experienced their symptoms. The

maximum duration of pain was defined as the longest duration for either dysmenorrhea, dyspareunia or non-cyclical pain. I computed all scores for validated questionnaires according to their respective manuals, ensuring any participant with missing values was not included. Participants were categorised into painDETECT groups based on standard cut offs (nociceptive ≤12, mixed 13-18, neuropathic ≥19[13,14]).

To explore whether neuropathic pain is related to a higher pain intensity, I compared pain intensity ratings between the three painDETECT groups separately for each pain type (i.e., dysmenorrhea, dyspareunia, non-cyclical pain, dyschezia and dysuria). In addition, I used correlation analyses to explore the relationship between painDETECT scores and pain intensity ratings for each pain type.

To explore the relationship between painDETECT and (i) Fibromyalgia Symptom Score (FS) and (ii) Pain Sensitivity Questionnaire scores, correlation coefficients were calculated. BDI scores for depression and STAI scores for anxiety were compared between the neuropathic, mixed and nociceptive group. Furthermore, full and partial correlations were calculated between painDETECT and BDI and STAI (controlling for pain scores).

The number of surgeries to the abdomen was used as a grouping variable: group 1 had one surgery, group 2 had two surgeries, group 3 had three to four surgeries and group 4 had five or more surgeries. This grouping was chosen with the aim of having similar numbers in each group. To test whether neuropathic pain was more prevalent in those with more surgeries, I compared the proportion of patients classified as having neuropathic pain between groups 1 to 4.

As most variables were not normally distributed (as indicated by significant Kolmogorov-Smirnov tests), I chose to use non-parametric tests throughout. Group

differences were explored using Kruskal-Wallis tests; significant results were followed up with Mann-Whitney U tests. Multiple comparison correction was carried out by multiplying p values by the number of comparisons made (Bonferroni correction). Correlation analyses were performed using Spearman's correlations. In order to also test whether group differences in cognitive and affective processing were driven by differences in pain intensity, I calculated partial correlations controlling for pain intensity. Rho values are interpreted such that rho<0.2 is 'poor'; rho between 0.2 and 0.59 are 'fair'; rho between 0.6 and 0.79 are 'moderate'; and rho>0.8 are 'very strong'[27].

Cluster analysis

Sensory symptom profiles of participants categorised as having mixed and neuropathic pain (as indexed by painDETECT) were used in a two-step cluster analysis. Profiles are based on seven symptoms assessed in the painDETECT questionnaire including spontaneous burning pain, spontaneous paresthesia, dynamic mechanical allodynia, spontaneous pain attacks, thermal hyperalgesia, numbness and deep somatic allodynia. To account for inter-individual differences in pain sensitivity, painDETECT scores for each of these symptoms were recalculated by subtracting the mean across all seven responses from each individual response as described in Baron et al., 2009[18]. This gave an adjusted value for each of the sensory symptoms assessed in painDETECT which controlled for individual differences in the general perception of sensory stimuli. Scores larger than zero thereby indicate a sensation that is more intense than the average individual symptom score. Scores smaller than zero indicate a sensation that is less intense than the average individual symptom score.

Two-step cluster analysis with log-likelihood as distance measure was used with these variables as continuous variables. Note that previous studies had employed a slightly different approach in their cluster analysis. Baron et al., and Mahn et al. used a hierarchical cluster analysis with five clusters[17,18]. In contrast, I opted for a two-step cluster analysis as this is better suited to nominal data and does not assume normal distribution. Two-step cluster analysis automatically determines the optimal number of clusters using the Schwarz Bayesian Criterion (BIC). This method is arguably preferable as it is more objective in the determination of the number of clusters, rather than an arbitrary decision about the number of clusters. In addition to the number of clusters identified, I report the quality of the suggested solution based on the silhouette coefficient (ranging between -1 and 1), which jointly considers cluster cohesion and separation. The quality of the cluster solution is categorised as “poor”, “fair” or “good” (as provided by SPSS). Furthermore, I report the importance of each symptom for cluster formation (predictive importance). I ran cluster analyses on those in the neuropathic and mixed groups as well as on those only in the neuropathic group to see if I found a difference in the cluster profiles, to verify findings were related to a neuropathic component to the pain.

Non-parametric Mann-Whitney tests were used to assess differences between clusters with respect to pain and psychological measures.

For comparability reasons, I also carried out cluster analysis using the same method as Baron et al. and Mahn et al.[17,18]. For this I used a hierarchical WARD-approach with a squared Euclidian distance measure and a cut-off point of five clusters. I ran cluster analyses on both the neuropathic and mixed groups and neuropathic group alone with the number of clusters set to 5 for each.

3.4 Results

3.4.1 Participant demographics and pain intensity ratings

1615 responses were received, with 1417 meeting the inclusion criteria. Participant demographics can be seen in Table 1.

	Median (range) [IQR]
Age (years)	33 (18-59) [27-38]
Dysmenorrhea Numerical Rating Scale Score (0-10)	8 (0-10) [7-9]
Dyspareunia Numerical Rating Scale Score (0-10)	7 (0-10) [5-8]
Non-cyclical Pain Numerical Rating Scale Score (0-10)	8 (0-10) [6-9]
Duration of Pain Symptoms (years)	18 (1-47) [13-24]
Number of surgeries to the abdomen	2 (1-23) [1-4]

Table 1. Demographics of participants. Median values, range and interquartile range (IQR) of responses given as data are not normally distributed.

3.4.2 Prevalence of a neuropathic component to EAP

Of the 1417 participants, 40% (n= 558) were categorised as having neuropathic pain according to their painDETECT scores. Pain was classified as mixed

nociceptive/neuropathic in a further 35% (n= 488) of participants and as nociceptive in the remaining 25% (n=358) of participants (Figure 1).

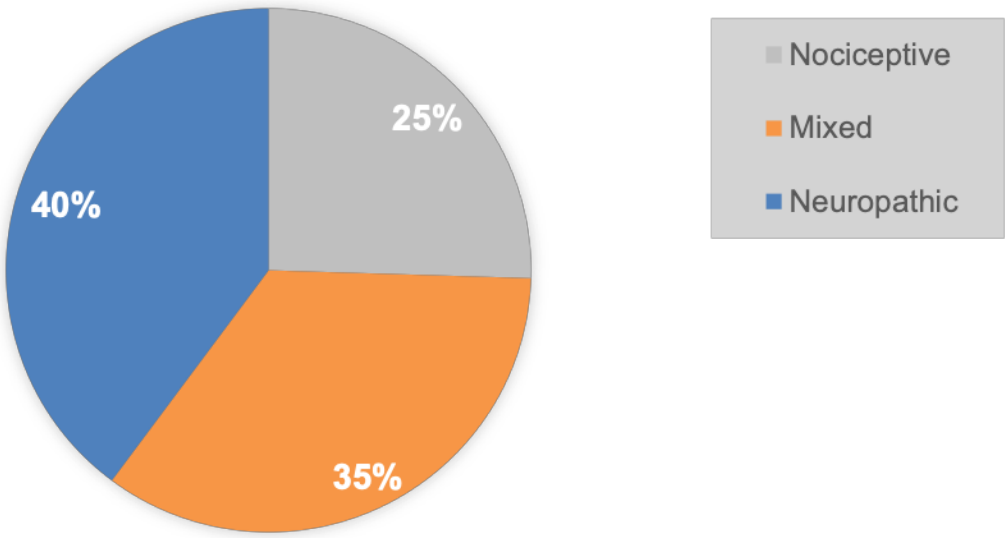

Figure 1. Proportion of participants with neuropathic, mixed and nociceptive pain. These groups were created using standard painDETECT cut off scores: nociceptive ≤12, mixed 13-18, neuropathic ≥19[13,14].

3.4.3 Does neuropathic pain mean more pain?

To test whether the classification of pain as neuropathic was related to more intense pain, I compared intensity ratings for all relevant types of pain between the three groups. These analyses showed significant differences in NRS scores for dysmenorrhea ($\chi2(2)=91.710$, $p<0.001$), dyspareunia ($\chi2(2)=80.191$, $p<0.001$), non-cyclical pain ($\chi2(2)=96.884$, $p<0.001$), dyschezia ($\chi2(2)=70.162$, $p<0.001$) and dysuria ($\chi2(2)=117.853$, $p<0.001$). For all pain types, scores were highest for the neuropathic group followed by the mixed group and the nociceptive group (Table 2). Post hoc tests showed that all pairwise group comparisons reached statistical significance (i.e., $p< 0.001$) with the exception of non-cyclical pain for which the difference between

nociceptive and mixed groups did not withstand multiple comparison correction (p=0.027 uncorrected).

	Neuropathic (median, IQR)	Mixed (median, IQR)	Nociceptive (median, IQR)
Dysmenorrhea	9 (8-10)	8 (7-9)	8 (7-9)
Dyspareunia	7 (6-9)	7 (5-8)	6 (4-8)
Non-cyclical pain	8 (7-10)	8 (6-9)	7 (5-8)
Dyschezia	8 (6-9)	7 (5-9)	6 (4-8)
Dysuria	6 (2-7)	4 (0-6)	0 (0-5)

Table 2. Numerical Rating Scale scores for participants in each painDETECT group (neuropathic, mixed and nociceptive). IQR= Interquartile range

Correlation analyses between painDETECT score and NRS scores for dysmenorrhea, dyspareunia and non-cyclical pain showed a significantly positive relationship between variables, indicating that neuropathic pain is linked to higher pain intensities (rho=0.286 $p<0.001$, rho=0.271 $p<0.001$, rho=0.297 $p<0.001$; Figure 2A-C).

Correlation analysis between painDETECT scores and other commonly used pain questionnaires, FS and PSQ, was also carried out. There was a moderate correlation between painDETECT and FS scores (rho=0.441, $p<0.001$; Figure 2D). The correlation between painDETECT and PSQ scores did not reach significance.

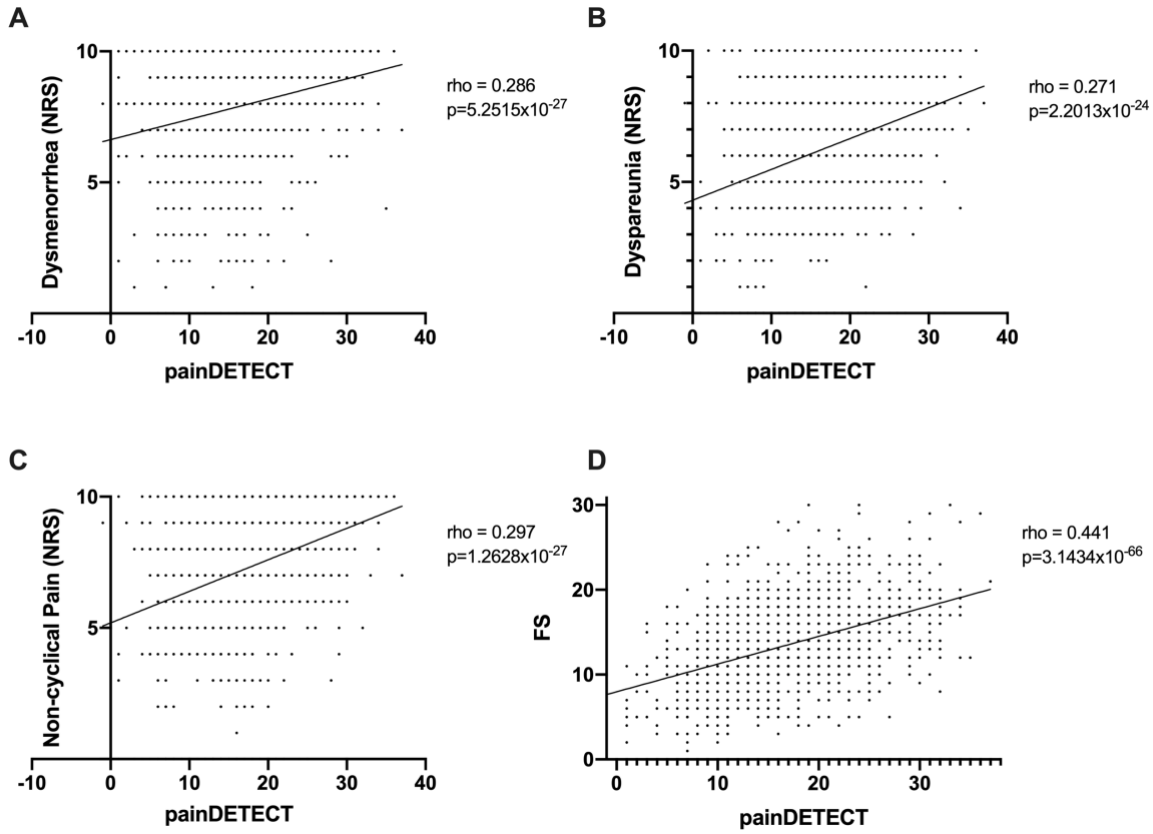

Figure 2: **Spearman correlations (rho) between painDETECT scores and other variables**. (**A**) shows the correlation between painDETECT and numerical rating scale scores for dysmenorrhea, (**B**) dyspareunia and (**C**) non-cyclical pain, (**D**) between painDETECT and Fibromyalgia Scale scores.

3.4.4 Neuropathic pain and emotional and cognitive processing

Scores for depression and anxiety were significantly different between groups (depression $\chi2(2)=130.907$, $p<0.001$; anxiety $\chi2(2)=67.389$, $p<0.001$), with highest scores in the neuropathic pain group for both measures. Similarly, fatigue and trouble thinking/remembering were significantly different between groups (fatigue $\chi2(2)=85.219$, $p<0.001$, trouble thinking/remembering $\chi2(2)=68.349$, $p<0.001$). Additionally, the neuropathic group was more likely to report waking up feeling tired ($\chi2(2)=80.588$, $p<0.001$). Post hoc tests showed significant differences in all pairwise comparisons between groups, with the neuropathic group showing the strongest

impairment followed by the mixed group and the nociceptive pain group ($p<0.05$, corrected for all post hoc comparisons; Table 3).

	Neuropathic (median, IQR)	Mixed (median, IQR)	Nociceptive (median, IQR)
Depression (BDI)	28 (19-38)	24 (16-34)	16 (11-26)
Anxiety (STAI-T)	55 (47-64)	53 (45-61)	48 (38-56)
Fatigue	3 (2-3)	2 (2-3)	2 (2-3)
Trouble thinking / remembering	2 (1-3)	2 (1-2)	2 (1-2)
Waking up feeling tired	3 (2-3)	3 (2-3)	2 (2-3)

Table 3. Scores of cognitive and affective measures for participants in each painDETECT group (neuropathic, mixed and nociceptive). IQR= interquartile range

Standard correlation analyses revealed a significantly positive correlation between painDETECT scores and each of the cognitive-affective variables (see Table 4). To test whether these significant correlations were driven by the higher pain intensity reported in the neuropathic pain group, I also calculated partial correlations controlling for dysmenorrhea, dyspareunia and non-cyclical pain. As shown in Table 4, correlations remained significant even when the influence of pain intensity was corrected for.

	BDI	STAI	fatigue	trouble thinking/remembering	waking up tired
painDETECT (standard correlations)	0.33*	0.24*	0.26*	0.24*	0.26*

painDETECT (partial correlations)	0.27*	0.19*	0.19*	0.18*	0.19*

Table 4: **Spearman correlations between painDETECT score and cognitive-affective variables.** Partial correlations control for Numerical Rating Scale scores for dysmenorrhea, dyspareunia and non-cyclical pain. * = p<0.001; BDI= Beck Depression Inventory, STAI-T= State-Trait Anxiety Inventory.

3.4.5 Does the prevalence of neuropathic pain increase with the number of surgeries?

In our cohort, the median number of surgeries to the abdomen was 2, with a range from 1 to 23 surgeries. Comparing painDETECT scores between groups with increasing numbers of surgical procedures revealed significant group differences (χ 2(3)=19.756, p<0.001) (see also Figure 3). Post hoc tests confirmed that group 4 with five and more surgeries had a significantly higher painDETECT score than group 1 that had undergone one surgery (p<0.001). Other pairwise comparisons did not withstand multiple corrections (see Table 5).

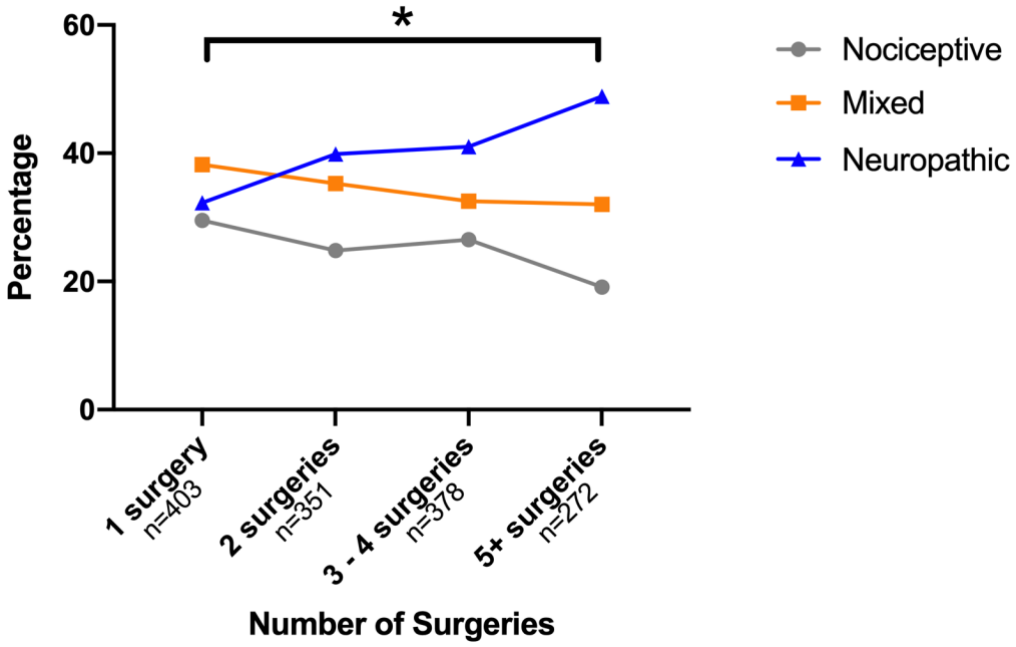

Figure 3: **Relationship between painDETECT scores and number of abdominal surgeries.** Surgeries were grouped so that each group had approximately the same number of participants.

	painDETECT score (median, IQR)
Group 1 (1 surgery)	16 (11-20)
Group 2 (2 surgeries)	17 (13-22)
Group 3 (3-4 surgeries)	17 (12-22)
Group 4 (5+ surgeries)	18 (14-23)
Pairwise comparisons	**p-value (uncorrected)**
Group 1 vs. Group 2	0.071
Group 1 vs. Group 3	0.057
Group 1 vs. Group 4	0.000012*
Group 2 vs. Group 3	0.971
Group 2 vs. Group 4	0.110
Group 3 vs. Group 4	0.011

Table 5. painDETECT scores for participants in each surgery group and p-values for pairwise group comparisons. IQR= interquartile range. $p<0.0083$ was considered significant to account for multiple comparisons, indicated by *.

3.4.6 Is a longer pain duration associated with a higher prevalence of neuropathic pain?

Exploring the relationship between duration of pain and painDETECT group, significant group differences in pain duration were only found for non-cyclical pain ($\chi2(2)=16.806$, $p<0.001$). Post hoc tests showed that patients in the neuropathic group had experienced non-cyclical pain for longer than those in the mixed ($p=0.006$) and nociceptive ($p<0.001$) groups. There was no significant difference between the groups for duration of dysmenorrhea ($\chi2(2)=3.208$, $p=0.201$ uncorrected) or duration of dyspareunia ($\chi2(2)=3.985$, $p=0.136$ uncorrected) nor for the maximum duration of any pain ($\chi2(2)=2.244$, $p=0.326$ uncorrected) (i.e. whichever of the three types of pain was longest in duration for that individual, as described in section 2.2.3).

3.4.7 Sensory symptom profiles

To explore the sensory symptom profile of those categorised as having neuropathic or mixed pain, sensory symptoms rated as part of the painDETECT questionnaire were analysed in more detail. Table 6 shows the proportion of participants that had clinically relevant sensory disturbances (i.e. a score of >3, strongly or very strongly) for each symptom. The presence of painful attacks was by far the most common symptom seen in this cohort, followed by pressure evoked pain.

Sensory symptom	**Proportion of patients affected**
Burning	28.2%
Prickling	25.4%
Mechanical Allodynia	23.3%
Painful Attacks	79.3%

Thermal Hyperalgesia	8.9%
Numbness	14.1%
Pressure Evoked Pain	45.2%

Table 6. Reported symptoms in neuropathic and mixed groups. Proportion of participants in the neuropathic and mixed groups reporting clinically significant symptoms (i.e., a score of >3, strongly or very strongly) in the painDETECT questionnaire.

3.4.8 Subgroups in neuropathic pain

To determine if those with a neuropathic pain component (i.e., patients categorised as having neuropathic or mixed pain) could be divided into further subgroups based on their sensory symptom profiles, I performed a cluster analysis on their ratings of sensory symptoms. The analysis produced two clusters (Figure 4), with a silhouette measure of cohesion and separation of 0.3, indicating that the cluster model was of a 'fair' quality. 56.3% of patients fell into cluster 1 (n= 589), and 43.7% in cluster 2 (n= 457). The symptoms with the greatest predictive importance were 'prickling/tingling' (predictive importance = 1), and 'burning' (predictive importance = 0.77), which were most characteristic for cluster 1. In contrast, 'pressure evoked pain' (predictive importance = 0.48), 'thermal hyperalgesia' (predictive importance = 0.27) and 'mechanical allodynia' (predictive importance = 0.27) were more common in cluster 2. 'Painful attacks' (predictive importance = 0.11) and 'numbness' (predictive importance = 0.05) were least discriminatory.

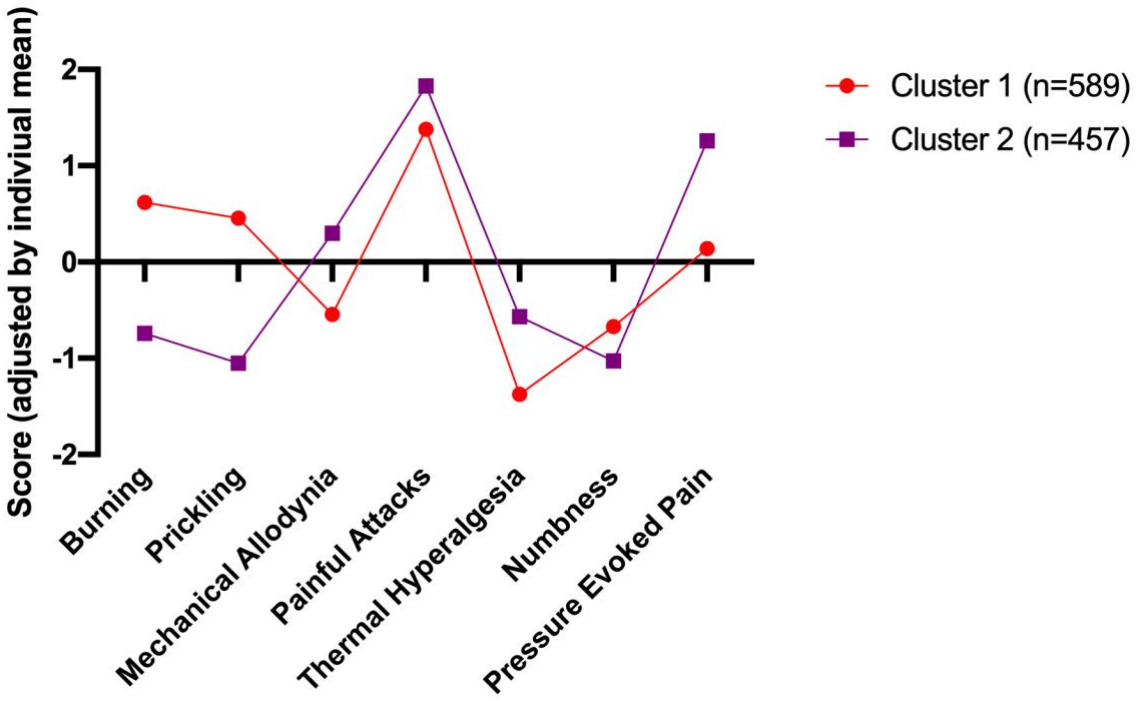

Figure 4. Sensory symptom profile of the two clusters (neuropathic & mixed pain group combined). Scores were individually mean adjusted.

The cluster analysis was also run on those in the neuropathic group only. The analysis produced three clusters (Figure 5), with an average silhouette measure of cohesion and separation of 0.2, indicating that the model was of a 'fair' quality. 38.7% of patients fell into cluster A (n=216), 38.5% into cluster B (n=215) and 22.8% into cluster C (n=127). The symptom with the greatest predictive importance was 'burning' (predictive importance = 1), followed by 'prickling/tingling' (predictive importance = 0.75), which were both most prominent in cluster B. In contrast, 'numbness' (predictive importance = 0.59) was most characteristic for cluster A. 'Thermal hyperalgesia' reached a predictive importance of 0.48 and was least characteristic of cluster B. 'Pressure evoked pain' (predictive importance = 0.44) and 'mechanical allodynia' (predictive importance = 0.42) were typical for cluster C. 'Painful attacks' only reached a predictive importance of 0.14 as they were common in all three clusters. .

Cluster B resembles cluster 1 of the analysis, which included patients of the neuropathic and mixed group whereas cluster C is similar to cluster 2. Cluster A shares features of clusters 1 and 2.

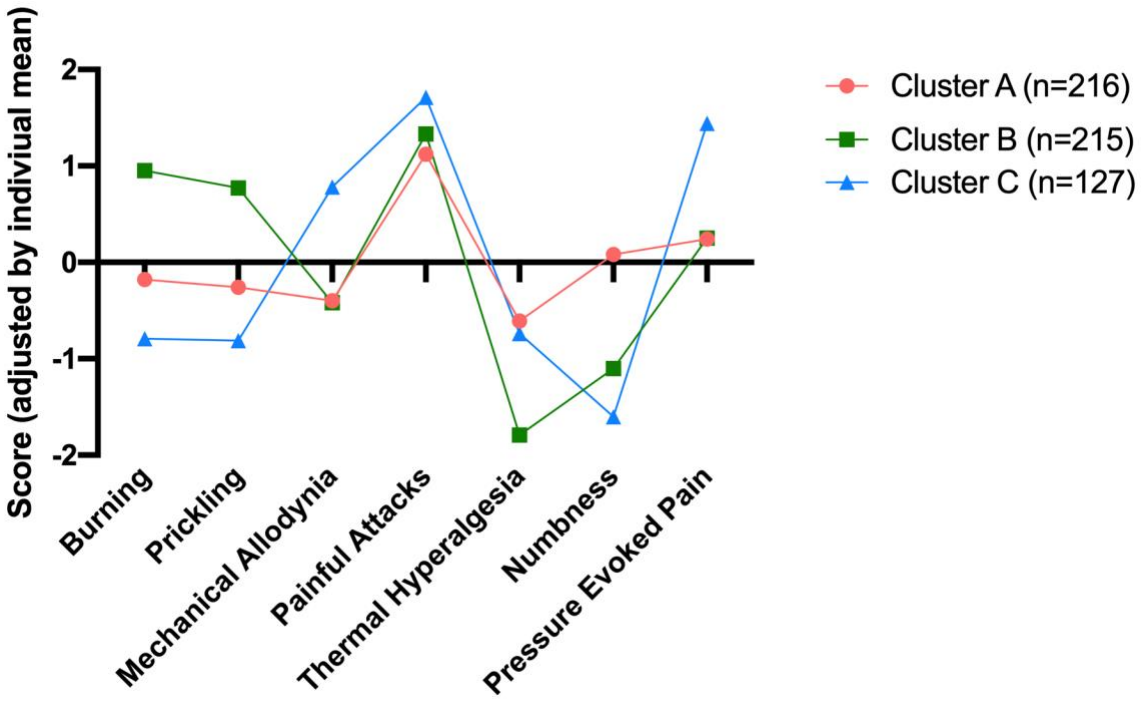

Figure 5. Sensory symptom profile of the three clusters (neuropathic pain group only). Scores were individually mean adjusted.

Cluster analysis as Baron et al. and Mahn et al.

Using the alternative method of cluster analysis, as Baron et al and Mahn et al gives 5 clusters[17,18]. For analysis of those in neuropathic and mixed groups 20% fell into cluster 1 (n= 212), 24% in cluster 2 (n= 247), 13% into cluster 3 (n= 132), 28% into cluster 4 (n=298) and 15% into cluster 5 (n= 157). The differences in sensory symptom profiles between the clusters can be seen in Figure 6.

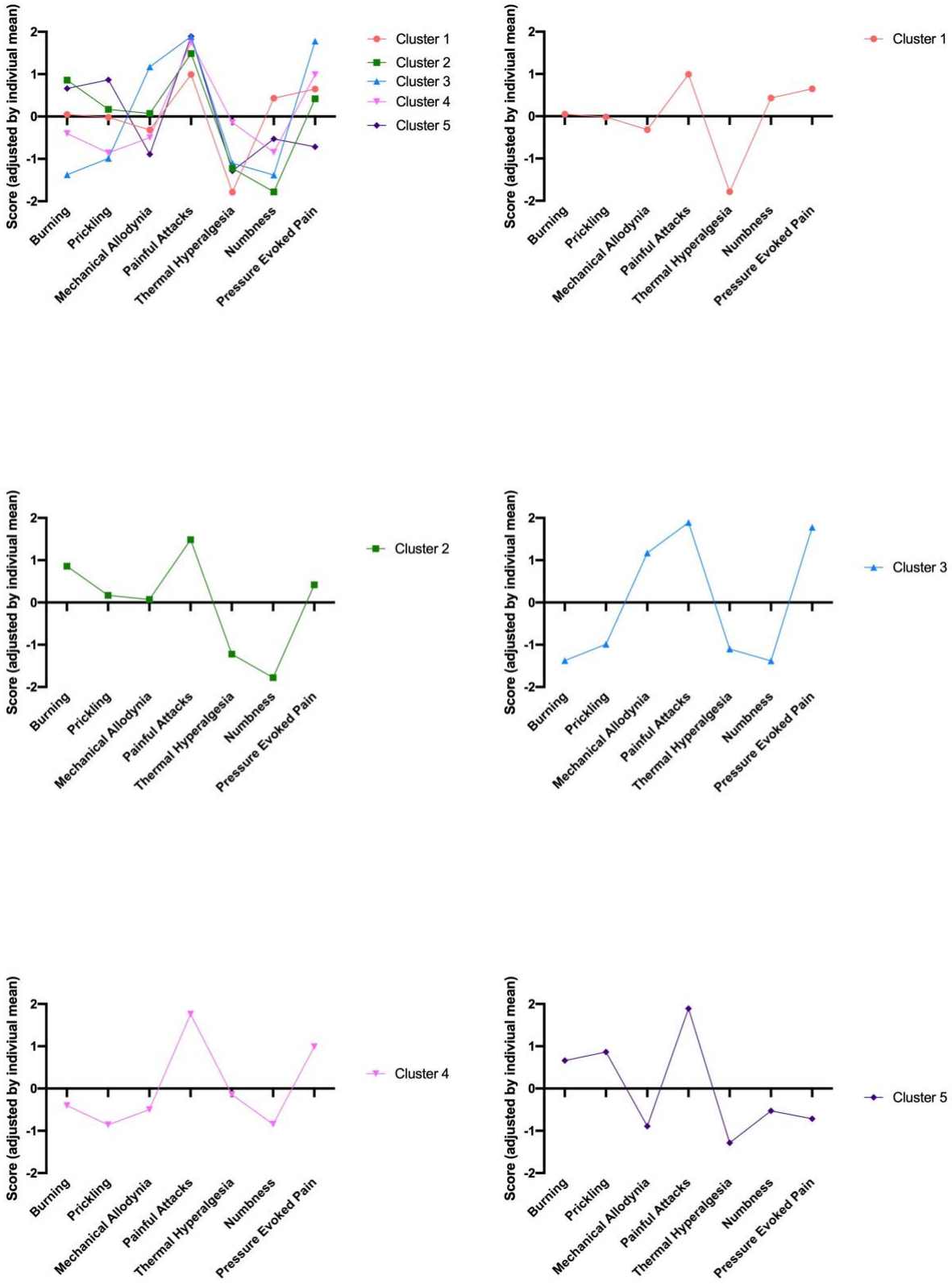

Figure 6. Sensory symptom profile comparison between clusters. Sensory variables derived from painDETECT responses and were individually mean adjusted. Hierarchical cluster to create 5 clusters within neuropathic and mixed groups. This figure shows the symptom profile based on individually mean adjusted responses to painDETECT questions for the 5 clusters (together and separately).

For analysis of those in the neuropathic group only 16% fell into cluster 1 (n= 88), 28% into cluster 2 (n= 154), 16% into cluster 3 (n= 92), 11% into cluster 4 (n= 59) and 30% into cluster 5 (n= 165). The differences in the sensory symptom profiles between clusters can be seen in Figure 7.

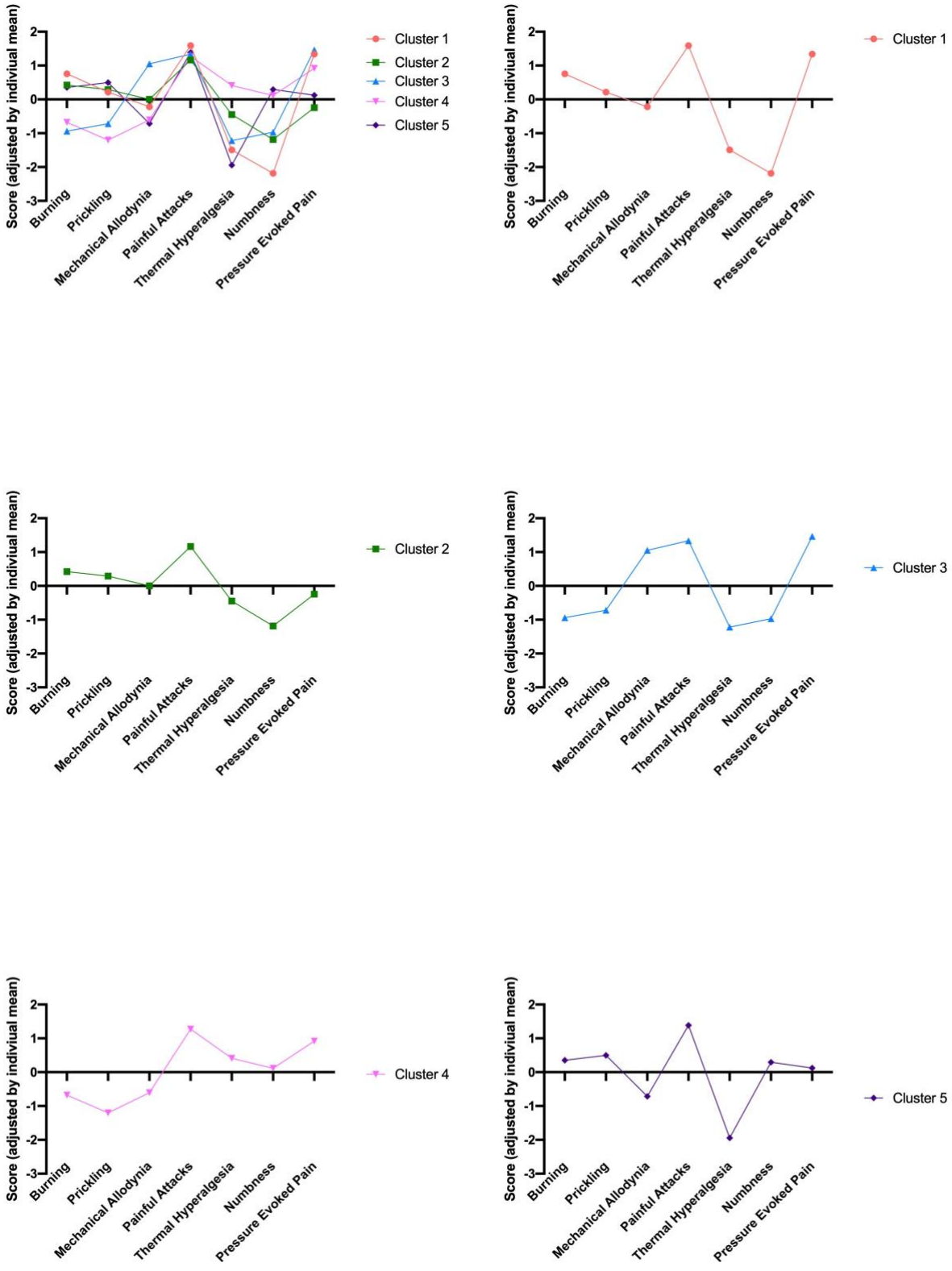

Figure 7. Sensory symptom profile comparison between clusters in neuropathic group. Sensory variables derived from painDETECT responses and were individually mean adjusted. Hierarchical cluster to create 5 clusters within neuropathic group only. This figure shows the symptom profile based on individually mean adjusted responses to painDETECT questions for the 5 clusters (together and separately).

3.4.9 Cluster differences in cognitive-affective and pain measures

There were no significant differences between clusters 1 and 2 or between clusters A, B and C with respect to depression (BDI), anxiety (STAI-T), fatigue, trouble thinking/remembering or waking up feeling tired (all $p>0.05$; Table 7).

	Depression (BDI score	**Anxiety (STAI-T score)**	**Fatigue**	**Trouble thinking/ remembering**	**Waking up feeling tired**
Neuropathic & mixed pain group					
Cluster 1	25 (17-36)	53 (46-63)	3 (2-3)	2 (1-2)	3 (2-3)
Cluster 2	26 (17-36)	55 (46-63)	3 (2-3)	2 (1-2)	3 (2-3)
Cluster 1 vs. cluster 2	p = 0.948	p = 0.486	p = 0.238	p = 0.245	p = 0.245
Neuropathic pain group					
Cluster A	28 (19-39)	56 (47-65)	3 (2-3)	2 (1-3)	3 (2-3)
Cluster B	26 (18-37)	53 (47-63)	3 (2-3)	2 (1.5-3)	3 (2-3)
Cluster C	29 (21-39)	56.5 (48-65)	3 (2-3)	2 (1-2)	3 (2-3)

Cluster A vs. B	p = 0.179	p = 0.252	p = 0.737	p = 0.824	p = 0.879
Cluster A vs. C	p = 0.878	p = 0.754	p = 0.752	p = 0.348	p = 0.492
Cluster B vs. C	p = 0.174	p = 0.172	p = 0.550	p = 0.253	p = 0.582

Table 7. Cognitive-affective scores for participants in each cluster. Median values and interquartile range (IQR) of responses given as data not normally distributed. Comparisons between clusters carried out using Mann-Whitney U tests and reported are effect sizes and p values (corrected).

Clusters 1 and 2 of the combined group (neuropathic & mixed pain) did not differ significantly with respect to the intensity of dysmenorrhea, dyspareunia or non-cyclical pain and PSQ scores but FS scores were significantly higher in cluster 1 (Table 8). Clusters A, B and C of the neuropathic group only did not differ in any of the pain variables (Table 8).

	Dysmenorrhea	**Dyspareunia**	**Non-cyclical pain**	**PSQ**	**FS**
Neuropathic & mixed pain group					
Cluster 1	8 (8-10)	7 (5-9)	8 (7-10)	3 (2-4)	13 (10-16)
Cluster 2	8 (8-9)	7 (5-8)	8 (6-9)	3 (1-4)	12 (9-15)
Cluster 1 vs. cluster 2	p = 0.435	p = 0.504	p = 0.19	p = 0.066	p = 0.00046

Neuropathic pain group					
Cluster A	9 (8-10)	7 (6-9)	9 (7-10)	3 (2-4)	14 (11-17)
Cluster B	9 (8-10)	8 (6-9)	8 (7-10)	3 (2-4)	13 (11-17)
Cluster C	9 (8-10)	7 (6-9)	8 (7-10)	3 (2-4)	13 (10-16)
Cluster A vs. B	p = 0.654	p = 0.971	p = 0.940	p = 0.959	p = 0.360
Cluster A vs. C	p = 0.695	p = 0.700	p = 0.456	p = 0.353	p = 0.066
Cluster B vs. C	p = 0.401	p = 0.743	p = 0.450	p = 0.350	p = 0.294

Table 8. Pain variables for participants in each cluster. IQR= interquartile range

3.5 Discussion

3.5.1 Overview

My data suggests that a considerable proportion of women with endometriosis-associated pain may have a neuropathic component to their pain. Based on painDETECT scores, pain was categorised as neuropathic in 40% of participants and as mixed (i.e., neuropathic and nociceptive pain) in a further 35% of the sample. In line with other chronic pain conditions where a mixture of underlying mechanisms, including neuropathic pain, is found[28–32] women who were classified as having neuropathic pain also report higher pain intensity scores, more psychological distress and alterations in cognitive processing (Table 2 and Table 3). Interestingly when

considering the aetiology of the neuropathic component, we found that women categorised as having neuropathic pain had undergone more surgical procedures and had a longer duration of non-cyclical pain than those in the mixed or nociceptive groups. Based on their sensory profile, those classified as having a neuropathic component can be further divided into two subgroups, suggesting that there may be more than one mechanism underlying neuropathic pain in women with endometriosis.

3.5.2 Features of neuropathic pain in endometriosis

My comparisons between painDETECT groups show that women whose pain is classified as neuropathic have higher pain ratings for dysmenorrhea, dyspareunia, dyschezia, dysuria and non-cyclical pain (see Table 2). Correlation analysis shows that there is a 'fair' but significant correlation between the NRS scores for dysmenorrhea, dyspareunia and non-cyclical pain (see Figure 2).

There is also a significant moderate correlation between painDETECT score and FS scores as seen in Figure 2. This is not surprising as one of the known features of neuropathic pain is that it radiates to other parts of the body and a large part of the FS score is related to the number of body areas affected. The FS score is often used as an indirect measure of centralised pain or central sensitization, which whilst different to neuropathic pain can overlap. The IASP definition of central sensitization is "increased responsiveness of nociceptive neurons in the central nervous system to their normal or subthreshold afferent input". Central neuropathic pain is defined by IASP as "pain caused by lesion or disease of the central somatosensory nervous system". Clearly within these definitions there are symptom profiles which could fit with either/both of these descriptors. It is important however, whilst the correlation is not surprising, to not forget that this correlation is only moderate strength and that these measures are

assessing different aspects of the pain phenotype. There was no significant correlation between PSQ and painDETECT, showing that whilst painDETECT has some measure of hyperalgesia and allodynia, it is not simply measuring individual pain sensitivity across their body. It would be useful to validate this finding with sensory testing of pain sensitivity (for example Quantitative Sensory Testing QST).

The neuropathic pain group also showed the greatest psychological impact, with the highest scores for both depression (BDI) and trait anxiety (STAI-T). Alongside this, the neuropathic group were also significantly more affected by cognitive-affective measures such as fatigue, trouble thinking/remembering and waking up feeling tired. There was a significant correlation between these measures, even when controlling for pain scores, showing that it is not simply the presence of greater pain which is related to these symptoms but specifically the presence of neuropathic pain.

3.5.3 Surgeries and neuropathic pain

Whilst this evidence does not show causation, the finding that the proportion of women in the neuropathic pain group increased with number of surgeries to the abdomen is intriguing.

As discussed, surgery can lead to neuropathic pain as during surgery damage can be done to nerves through the incision or the cutting/lasering of lesions which leads to pain. It may be that the proportion of women with neuropathic pain increases as for each surgery there is a risk of resultant neuropathic pain thus women who have had more surgeries naturally are more likely to have neuropathic pain.

It could also be argued that women who have neuropathic pain who come to clinic are given surgery firstly to diagnose endometriosis and also to try to remove lesions with

the aim of relieving symptoms of pain. If the mechanism of the pain is neuropathic then surgery is unlikely to resolve the symptoms so the women will return to clinic and then sometimes be offered surgery again. This is can ultimately lead to women having several surgeries to the abdomen, an example of which is the 272 women who reported having five or more surgeries to the abdomen in this survey.

3.5.4 Sensory symptom profiles

Sensory symptom profiles were investigated in those who were categorised as having neuropathic or mixed pain. For each symptom, the proportion of women who rated this as greater than 3 out of 5 was calculated, as this is deemed a threshold for a clinically relevant complaint.

The symptom which is most common in this population was pain attacks, with 79.3% of women in the neuropathic or mixed groups experiencing it. Pain attacks is also one of the most common symptoms in painful radiculopathy (RAD) (32%) and post-herpetic neuralgia (PHN) (46%) but not in painful diabetic neuropathy (BPN) (18%) (see Table 9).

As can be seen in Table 9, the relative proportions of those with neuropathic/mixed pain in our cohort experiencing the other clinically relevant symptoms are very comparable to what has been shown in other neuropathic pain conditions. As can be seen in the range of symptoms reported, at a population level, participants experience both gain-of and loss-of-function symptoms (i.e. hyperalgesia and numbness), in comparable proportions to other neuropathic patients.

Overall, whilst there are differences in the proportions of symptoms experienced in each of these conditions, there are similarities between them (for example very similar

proportions of numbness in my findings and painful radiculopathy (RAD) and post-herpetic neuralgia (PHN)) (Table 9). For each condition there is at least 8% experiencing each symptom, despite the wide variety of symptoms assessed, this highlights that individuals with high painDETECT scores do not only exhibit hyperalgesia and/or allodynia but also exhibit loss of function symptoms, such as numbness. It is interesting that pain attacks (like "electric shocks") is much more common in EAP categorised as neuropathic/mixed than in other conditions; this could be related to the mechanisms driving this neuropathic component being different to those seen in other conditions, or alternatively could be related to sex differences in neuropathic pain symptoms (the studies investigating RAD, PHN and DPN have between 49.2% and 60% female participants). Future work should investigate the presence of pain attacks in women with endometriosis-associated pain further as it is clearly a pain symptom which is very commonly clinically relevant.

Sensory symptom	Proportion of patients affected			
	Endometriosis	**Painful radiculopathy**	**Post-hepatic neuralgia**	**Painful diabetic neuropathy**
Burning	28.2%	25%	54%	33%
Prickling	25.4%	26%	38%	35%
Mechanical Allodynia	23.3%	10%	47%	18%
Painful Attacks	79.3%	32%	46%	18%
Thermal Hyperalgesia	8.9%	8%	31%	14%
Numbness	14.1%	16%	14%	30%
Pressure Evoked Pain	45.2%	21%	58%	40%

Table 9. Sensory symptom profiles from this study cohort (Endometriosis) compared with painful radiculopathy, post-hepatic neuralgia and painful diabetic neuropathy[17,18].

3.5.5 Cluster analysis

It has been suggested that distinct subsets of neuropathic pain may exist, which would lead to different sensory phenotypes[17,18] one way to assess whether different subsets exist is with cluster analysis. My cluster analyses look to categorise individuals into groups based on their mean adjusted responses to painDETECT questions.

Interestingly, two-step cluster analysis exploring those categorised as neuropathic or mixed only identified two subgroups in our cohort (as opposed to the five seen in other

conditions), whereas the cluster analysis run on only the neuropathic group gave three clusters. Given that I had 1046 women in my analysis and Baron and colleagues had similar sized groups (for painful diabetic neuropathy n=1623, for postherpetic neuralgia n=498 and for painful radiculopathy n=2094), I do not believe this to be simply a power issue.

Analysis of the neuropathic and mixed groups only gave 2 clusters. Cluster 1 experienced greater 'burning' and 'tingling' sensations, whilst cluster 2 reported more 'pressure evoked pain' suggesting potentially different underlying mechanisms in the two clusters. For example, it has been suggested that pain that is characterised by evoked pain could be due to "irritable nociceptors" and pain characterised by burning/tingling sensations with numbness could be due to deafferentation[33].

A similar pattern emerged when only patients from the neuropathic group were included, although here, a solution with three clusters was suggested (Figure 5). As before, painful attacks was the most common clinically relevant symptom in all three clusters. Cluster A is characterised only by pain attacks. Cluster B was characterised by 'burning' and 'tingling' sensations and therefore similar to cluster 1 in the previous analysis while cluster C showed greater scores for 'pressure evoked pain' similarly to cluster 2 in the analysis for the neuropathic and mixed group.

Given the similarity between the results for the combined group (i.e., neuropathic and mixed group) and the neuropathic group only, it remains to be determined whether differentiating between both groups is clinically meaningful in this context. As there were no significant differences between any of these subgroups in psychological or cognitive measures or in NRS scores for dysmenorrhea, dyspareunia and non-cyclical pain, there is no indication to prioritise any subgroup; however, the difference in

sensory symptom profiles does suggest that the subgroups may respond differently to treatments and this is a further area to be explored in future studies.

Baron et al. cluster analysis method

This analysis was carried out to ensure comparability with previous studies, however in this setting I felt it was not as appropriate so chose to focus on the two-step method. The two-step cluster method automatically selects the number of clusters based on the data which provides a more objective output. I also considered that forcing 5 clusters on our data did not give us clinically significant results (see Figures 6 and 7), and that fewer clusters were more meaningful in this setting. Furthermore, following the same method of cluster analysis did not give clusters which were more comparable to those found in previous work, this is in part due to the fact that pain attacks are far more frequent in our population to those previously studied in this way. This difference in the cluster symptom profile could be due to differences in the generation and maintenance of a neuropathic component to pain, which is to be expected given the clear differences in conditions with regards to location, as well as differences in whole symptom profiles (such as the cyclical nature of some aspects of EAP as well as differences in functional triggers of pain). It is also possible that the differences in clusters seen is related to the fact that my cohort are exclusively female.

3.5.6 Strengths & Limitations

As participants were recruited from patient support groups, my sample might not necessarily be representative of the population of those affected by endometriosis-associated pain. My cohort had experienced pain for a relatively long duration (median 18 years, range 1-47) and had repeatedly undergone surgery (median 2, range 1-23).

However, both characteristics are not unusual amongst this patient group. With an average time of 8.3 years between symptom onset and diagnosis of endometriosis in countries with state-funded healthcare[34], many women will have lived with their symptoms for an extended period of time. Once diagnosis is confirmed, multiple surgical interventions are common for these women[35,36].

Given that data were acquired using an online survey, I was unable to verify the diagnosis of endometriosis for participants. Although women had to confirm that they had received the diagnosis of endometriosis following a laparoscopy to be included in the study, it cannot be ruled out that some respondents actually have chronic pelvic pain without endometriosis.

3.5.7 Future Directions

The data presented in this chapter is the first strong evidence for a neuropathic component to endometriosis-associated pain, therefore naturally there are more aspects of this that need to be investigated in future work than there is room to discuss here, thus I have briefly outlined some key themes that need to, in my opinion, be focused on.

The data presented here do not allow for conclusions regarding molecular mechanisms underlying neuropathic pain in patients with EAP, such as inflammation or innervation of ectopic lesions. Future studies are therefore needed to characterise underlying pathological processes and establish causality between these changes and the patients' clinical presentation. It is also important to determine the mechanisms which give rise to these different subgroups (both in terms of mixed and neuropathic pain and in the clusters described in this chapter). I speculate that these different pain symptoms are the product of different mechanisms giving rise to and maintaining them. A better

understanding of the mechanisms causing pain can aid research into possible treatment options, for example new drug targets.

Moreover, future work should investigate whether painDETECT can be used as a grouping variable to determine treatment response for existing and new treatments. Future studies should investigate whether the nociceptive, mixed and neuropathic groups respond differently to treatments, specifically treatments that have been shown to be effective in other neuropathic pain conditions. Furthermore, any such studies should also investigate whether the different clusters respond differently to treatment.

When taking part in this study all participants had received at least one laparoscopic surgery. A simple but interesting follow-on study would investigate the prevalence of neuropathic pain in women who had not yet undergone surgery but who went on to have endometriosis surgically diagnosed. Alternatively, a longitudinal study exploring neuropathic pain symptoms in women as they first present with symptoms, through their care (and associated surgeries) would be incredibly useful (if unlikely) in determining the relationship between abdominal surgery and neuropathic pain in women with EAP. Perhaps more realistically, the exploration of the relationship between surgery and neuropathic pain specifically in women with EAP may have to 'piggy-back' off studies exploring post-surgical pain in general.

3.6 Conclusion

The data presented here indicate that endometriosis-associated pain includes a neuropathic component in a substantial proportion of women. My findings challenge the current conceptualisation of endometriosis-associated pain as purely nociceptive and

advocates for a new perspective on this type of pain, which is explored further in following chapters.

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Chapter 4: Investigating neuropathic pain in Chronic Pelvic Pain: similarities to findings in endometriosis and gabapentin treatment response

4.1 Abstract

In this chapter I have explored data from a randomised placebo-controlled clinical trial of gabapentin in women with chronic pelvic pain (CPP) with no known pathology. I have replicated my findings (discussed in my previous chapter) of differences in mental health and functioning between different painDETECT groups. Using individual painDETECT responses I have sub-grouped those with a neuropathic component to their pain and found that the sensory symptom profiles of the two sub-groups are very similar to those found in endometriosis-associated pain. painDETECT groups did not differ significantly in their response to gabapentin (or placebo).

4.2 Background

The Gabapentin for Pelvic Pain (GaPP2) trial was carried out to determine whether gabapentin reduces pain in women with chronic pelvic pain (CPP) of unknown origin. This was a multicentre, randomised, placebo-controlled, double-blind study across 39 UK hospitals which recruited between 22nd January 2016 and 6th March 2019. The primary outcomes of the study are already published (Horne et al 2020[1]); in summary, this study aimed to determine the efficacy and safety of gabapentin in women with CPP and no obvious pelvic pathology.

Whilst my thesis has focused on endometriosis-associated pelvic pain specifically, there are similarities between these two populations. Both are both populations of women with chronic pain originating in the pelvis, with overlapping symptoms (i.e., dysmenorrhea, dyspareunia). Therefore, it is useful to draw parallels between these conditions to determine if there are more similarities between symptoms and possible mechanisms giving rise to and maintaining pelvic pain.

In this chapter I aim to replicate my findings in endometriosis-associated pain (discussed in Chapter 3) using painDETECT[2,3], in this cohort of women with CPP. Specifically, I aim to investigate whether neuropathic like pain is associated with changes to physical and mental health. Whilst the GaPP2 study has used different measures to assess fatigue and mental health status, they are comparable when exploring whether scores differ between painDETECT groups. I investigate sensory symptom profiles and compare with endometriosis-associated pain (EAP) the proportions of participants experiencing each symptom. Following on from this I carry out cluster analysis to determine whether subgroups are found, how many and whether the profiles of the subgroups show similarity to those in EAP. Collaborating with the GaPP2 study gives the additional benefit of allowing me to explore whether these findings are clinically meaningful in the context of determining response to treatment.

In addition to pain intensity scores, this trial collected data through questionnaires, including painDETECT[2,3]. Following on from my previous chapter and work with painDETECT, in this chapter I aim to replicate some of my findings. One is to assess differences in painDETECT groups in psychological variables, as well as variables assessing quality of life. Another is to determine whether CPP can be subdivided into

clusters similar to those I found in endometriosis-associated pain on the basis of sensory symptoms assessed in painDETECT (Chapter 3).

Gabapentin first received marketing approval in the US in 2002 for post-herpetic neuralgia, with a European label change in 2006 to include peripheral neuropathic pain[4]. The mechanism of action of gabapentin is mainly ascribed to its effect on α2δ-type voltage gated calcium channels found both in the peripheral and central nervous system[5]. This alters the release of neurotransmitters and reduces the excitability of neurons[6,7]. Some research also suggests that gabapentin acts by blocking new synapse formation[8]. Gabapentin is normally taken as a tablet at least 3 times daily and is absorbed through a transport system in the proximal small bowel[4]. A recent Cochrane review has shown that gabapentin provides at least 50% reduction in pain for 3 in 10 people, in both post-herpetic neuralgia and diabetic polyneuropathy[4]. Using fMRI to explore the central effects of gabapentin in healthy participants, Iannetti et al[9] found that gabapentin had a measurable antinociceptive effect and an even stronger anti-hyperalgesic effect. Whilst there is the most and strongest evidence for the use of gabapentin in post-herpetic neuralgia and painful diabetic neuropathy, it has been trialled in many other pain conditions, including fibromyalgia and complex regional pain syndrome. However, evidence for efficacy in other pain conditions remains limited.

Recently there has been a movement in clinical trials to characterise the patient group particularly based on symptoms, not only condition. For example, when analysing clinical trials, it is important to determine not only an overall population effect but whether there are subgroups of the participants who respond differently. Significant treatment responses in a specific subset of participants can easily be lost when only

looking at the whole group level. Therefore, following on from whole group level analysis, it was decided that the GaPP2 trial data should be explored further for treatment effect in sub-populations, with participants grouped based on painDETECT responses. As gabapentin has been shown to be effective and is approved for use in neuropathic pain conditions (mainly in post-herpetic neuralgia and diabetic polyneuropathy), I hypothesised that those who had a neuropathic-like pain would show greater treatment response to gabapentin when compared to those with nociceptive pain.

The aims of this chapter were:

- Determine the proportion of women with CPP who, using painDETECT, are classified as having neuropathic-like pain
- To compare mental health and functioning variables between the different painDETECT groups
- To establish whether sub-groups of those with neuropathic or mixed pain can be identified, and whether these are similar to those found in EAP (in Chapter 3)
- To explore whether treatment response to gabapentin and placebo differed between painDETECT groups and/or between any sub-groups.

4.3 Methods

4.3.1 Data collection

I was not involved in data collection. The study was a multicentre, randomised, placebo-controlled trial across 39 UK hospital sites which followed a strict published

protocol[10]. All methodical information is taken from published protocol and results papers[1,10].

The study had ethics approval from UK Coventry and Warwick Research Ethics Committee (REC 15/WM/0036) and clinical trial authorisation from the Medicines and Healthcare Products Regulatory Authority.

The study population were women with chronic pelvic pain (CPP) of unknown origin. Inclusion criteria were: pelvic pain (with or without dysmenorrhea and/or dyspareunia) of more than 3 months duration, age 18-50 years, use/willingness to use contraception to avoid pregnancy, no obvious pelvic pathology at laparoscopy (must have taken place between 2 weeks and 3 years prior). Women were excluded if they only experienced dysmenorrhea, had a malignancy, were currently using gabapentin or pregabalin, had surgery planned in the next six months, had renal impairment, had had previous adverse reaction to gabapentin, were taking gonadotrophin-releasing hormone agonists and unable/unwilling to stop, were breast feeding, pregnant or planned pregnancy in next six months or had pain suspected to be of gastrointestinal origin.

Prior to randomisation into the study, participants were required to give four weekly pain reports using an automated text messaging service, which included Numerical Rating Scale (NRS) scores for their average pain and their worst pain for the week. At least two of the worst NRS scores had to be ≥4 for the participant to be considered for entry into the trial.

This study was a randomised placebo-control study, so participants were assigned to either receive gabapentin or a matched placebo (tablets themselves were in appearance identical). Participants, clinicians and research staff were all unaware of trial group assignment. Participants were randomly assigned to either the gabapentin or matched

placebo group in a 1:1 using minimisation to ensure balance between groups, according to the presence or absence of dysmenorrhea (≥4 out of 10), psychological distress (using General Health Questionnaire, any score ≥2), current use of hormonal contraceptives and by hospital centre. Participants were unblinded once they had provided all outcome data at week 16/17 (or sooner if necessary due to serious adverse event).

Gabapentin was taken orally, with a 4-week titration phase initially starting on one 300mg capsule daily and increased by one capsule every three days until they were gaining adequate pain relief or reported side effects that precluded them from further increases (up to maximum of nine capsules daily). Once they had found their highest tolerated dose, they were asked to maintain it until the end of week 16. Participants were permitted to use other analgesic medications (including opioids and antidepressants) and asked to record use.

The study had dual primary outcome measures of worst and average pain scores (NRS 0-10) at 13-16 weeks post-randomisation. Both the worst and average pain were used following a PPI survey and what was considered a response to treatment. Secondary outcome measures were the proportion of women that had a 30% or 50% reduction in worst and average pain scores from baseline to the end of treatment; global impression of change (assessed by the question "How do you think your health overall changed with the treatment" with a 6-point response scale from very marked worsening to very marked improvement); and questionnaire measures (discussed in more detail below) at 16 weeks post-randomisation.

4.3.2 Data available

Some of the questionnaire measures, taken at both baseline and follow-up included in this trial were: general quality of life (Short Form(SF)-12[11]), pain interference (Brief Pain Inventory[12]), fatigue (Brief Fatigue Inventory (BFI)[13]), psychological distress (General Health Questionnaire[14,15]), pain catastrophizing[16], impairments in paid work/activities (Work and Productivity Activity Impairment Questionnaire (WPAIQ)[17]) and sexual functioning (Sexual Activity Questionnaire (SAQ)[18]). The SF-12 gives a subset of scores, with eight domains of health and two summary scores: physical function (PF), role limitation due to physical problems (RP), role limitation due to emotional problems (RE), social functioning (SF), mental health (MH), energy/vitality (EV), pain (P), general health perception (GHP), with summary scores for physical component (PCS) and mental component (MCS). The SF-12 was developed as a shorter form of the SF-36[19] which assess the same eight domains but required substantially more questions[11]. The Brief Pain Inventory[12] assesses both pain severity, and pain interference, which is the measure focussed on in this chapter. Pain interference items include general activity, mood, walking ability, normal work, relations with other people, sleep and enjoyment of life, which are scored between 0 and 10 with 0=does not interfere, 10=completely interferes. The analysis in this chapter uses a global fatigue score from the BFI[13] questionnaire which averages scores of fatigue and how fatigue interferes with life. A global score for General Health Questionnaire[14,15] is used in this analysis, which assesses mental health, asking how often participants have recently experienced phenomena such as “losing confidence”, “feeling reasonably happy”, “capable of making decisions” and “constantly under strain”. Pain catastrophizing in brief, measures attitude towards pain, specifically rumination, magnification, and helplessness. WPAIQ[17], as the name suggests gives measures of work productivity loss, activity impairment score, absenteeism (time off work) and presenteeism (work

impaired due to condition). The SAQ[18] assesses aspects of sexual activity, but for this study the function section was focused on, such as the importance, satisfaction and pain; overall it gives scores for habit, pleasure and discomfort.

Data shared with myself included: treatment allocation; average pain scores (NRS 0-10) for 4 screening weeks and 4 end of study weeks (weeks 13, 14, 15 and 16); worst pain scores (0-10) for the same weeks; painDETECT responses for each item (baseline and follow-up); SF-12 scores for physical function, role limitation for both physical and emotional problems, social functioning, mental health, energy/vitality, pain score, general health perception, physical component and mental component (baseline and follow-up); pain interference score (baseline and follow-up); BFI global score (baseline and follow-up); WPAIQ scores for absenteeism, presenteeism, work productivity loss and activity impairment (baseline and follow-up); SAQ scores for present sexual activity, pleasure, discomfort and habit (baseline and follow-up); pain catastrophizing score (baseline and follow-up); adverse event information; as well as follow-up information on what treatment participants thought they received, how they thought their overall health changed and how often they took the study medication.

4.3.2 Data analysis

All statistical analysis was carried out in SPSS (version 25).

Firstly, using standard painDETECT cut-offs (nociceptive ≤12, mixed 13-18, neuropathic ≥19[20]) the frequency of the different painDETECT groups was estimated. Then Kruskal-Wallis tests were carried out to determine if there were any significant differences between the painDETECT groups in the baseline scores listed above. Any significant results were followed up with post hoc Mann Whitney tests.

Cluster analysis was carried out on those in the mixed and neuropathic groups as in Chapter 3 to determine whether this population could be subdivided into groups with different sensory symptom profiles. For these analyses, painDETECT scores were individually mean adjusted. Two-step hierarchical cluster analysis was used with BIC determination of the number of clusters. Mann-Whitney tests were carried out between the clusters on baseline variables listed above.

A percentage reduction in pain scores was calculated (baseline worst pain – follow-up worst pain / baseline worst pain (as well as the same for average pain score)). In accordance with IMMPACT recommendations[21], both 30% and 50% reduction in pain score was investigated as cut-offs for treatment response[21]. Kruskal-Wallis tests comparing the three painDETECT groups (at baseline) were used for 30% reduction in worst pain, 30% reduction in average pain, 50% reduction in worst pain and 50% reduction in average pain for both the gabapentin and placebo groups. Mann-Whitney tests for the same variables were carried out to determine if there were any significant differences between the clusters.

General Linear Model (GLM) was used to model follow-up pain scores. painDETECT group, treatment group, baseline pain scores and the interaction between painDETECT group and treatment group were used as factors and covariates in the model. The model design was built with the terms treatment group, painDETECT group, baseline pain score and an interaction term between painDETECT group and treatment group. The GLM used the sum of squares type III, with significance criteria of 0.05. GLM was also run with the different clusters (in place of painDETECT groups).

Multiple comparison correction was carried out by multiplying p values by the number of comparisons made (Bonferroni correction).

4.4 Results

4.4.1 General results

Analysis of primary and secondary outcomes, as discussed in Horne et al 2020[1] found that there were no significant differences between the gabapentin and placebo groups in the worst and average NRS pain scores at follow-up. Relative to baseline, mean NRS scores for worst pain decreased by 1.4 (SD 2.2) in the gabapentin group and 1.2 (SD 2.1) in the placebo group. The mean change in average pain intensity from baseline was -1.1 (SD 2.0) in the gabapentin group and -0.9 (SD 1.8) in the placebo group.

Horne et al. therefore stated that they "confidently conclude that gabapentin is not effective for chronic pelvic pain at a group level"[1].

	Gabapentin (N=153)	**Placebo (N=153)**
Dysmenorrhoea$_1$ *n (%)*	100 (65)	100 (65)
GHQ score for anxiety and depression$_2$ *n (%)*	38 (25)	38 (25)
GHQ total score$_2$ – *Mean (SD, n)*	4·6 (3·7, 153)	4·7 (3·7, 153)
Current use of sex hormones *n (%)*	99 (65)	99 (65)
Patch	2 (2)	0 (-)
Combined oral contraceptive pill	26 (26)	21 (21)
Progesterone only pill	19 (19)	16 (16)
LNG-IUS	38 (38)	45 (45)
Implant	12 (12)	12 (12)
Injection	5 (5)	8 (8)
Age (years) *Mean (SD, n)*	30·5 (7·7, 153)	30·1 (8·6, 153)
Ethnicity – *n (%)*		
White	150 (98)	148 (97)
Black (Caribbean/African/Other)	1 (1)	0 (-)
Asian (Indian, Pakistani, Bangladeshi/Other)	2 (1)	4 (2)
Mixed (Caribbean/African/Asian/Other)	0 (-)	1 (1)
BMI (kg/m²) *Mean (SD, n)*	27·1 (5·7, 151)	27·8 (5·9, 150)
Education$_3$ *n (%)*		
Primary	4 (3)	5 (3)
Secondary	47 (31)	46 (31)
Tertiary	101 (66)	101 (66)
Menstruating *n (%)*	109 (71)	108 (71)
Pain score during periods$_1$ *Mean (SD, n)*	7·7 (1·6, 103)	7·6 (1·7, 103)
PUF symptom score$_4$ *Mean (SD, n)*	9·7 (4·1, 153)	10·0 (4·5, 148)

PUF bother score[4] *Mean (SD, n)*	5·3 (2·6, 153)	5·4 (2·8, 150)
PUF total score[4] *Mean (SD, n)*	15·0 (6·3, 153)	15·5 (7·0, 147)
Rescue medications[5] *n (%)*	114 (75)	112 (73)
NSAIDS	62/114 (54)	66/112 (59)
Opiates	78/114 (68)	68/112 (61)
Other	61/114 (54)	58/112 (52)
Neuropathic pain[6] *n (%)*	32 (21)	33 (22)
Missing	1	4

Table 1. Demographics of study population. Adapted from Horne et al 2020[1]. [1] Dysmenorrhea is defined as a pain intensity score of ≥4 during periods. Pain scores range from 0 to 10, where 0= no pain and 10=worst pain imaginable. [2] General health questionnaire (GHQ) scores range from 0-12, where higher scores represent higher levels of mental distress. A score of 0 or 1 meets the definition of anxiety and depression. [3] Education unknown (gabapentin n=1, placebo n=1). [4] Pelvic Pain and Urinary/Frequency Patient Symptom Scale (PUF) symptom scores range from 0-23, bother scores range from 0-12, and total scores range from 0-35, where low scores are good and high scores are bad. [5] Rescue medication includes NSAIDS, opiates and other. Other includes paracetamol. [6] Neuropathic pain defined as a PainDETECT score≥19.

4.4.2 painDETECT groups

Using the standard painDETECT cut offs (nociceptive (≤12), mixed (13-18) and neuropathic-like (≥19)), I found that n=138 participants had nociceptive pain (46%), n=98 had mixed pain (32.7%) and n=64 had neuropathic pain (21.3%).

Comparisons for various variables between the different painDETECT groups was carried out. The median and IQR values for the different variables for the three painDETECT groups can be seen in Table 2.

Kruskal-Wallis tests between these painDETECT groups for baseline average and worst NRS pain intensity scores showed a significant difference between the groups ($\chi2(2)=23.0$, $p<0.001$; $\chi2(2)=18.5$, $p=0.0023$ respectively). Post hoc Mann-Whitney tests revealed significant differences between the nociceptive and neuropathic groups, and between the nociceptive and mixed groups in both average ($p<0.001$, $p=0.024$ respectively) and worst pain ($p=0.001$, $p=0.006$ respectively), with the nociceptive group having the lowest scores. The comparison between the mixed and neuropathic

groups found no significant difference in worst pain and the difference in average pain did not withstand multiple comparison correction.

There were also significant differences in baseline fatigue scores (BFI) ($\chi2(2)=45.7$, $p<0.001$), psychological health (GHQ) ($\chi2(2)=26.5$, $p<0.001$), pain catastrophising ($\chi2(2)=36.3$, $p<0.001$) and pain interference scores ($\chi2(2)=29.1$, $p<0.001$). Post hoc Mann-Whitney tests for fatigue (BFI) showed significant differences for all group comparisons ($p<0.05$), with the neuropathic group having the greatest scores and nociceptive the lowest. For psychological health (GHQ) again all comparisons were significant ($p<0.05$), with the neuropathic group having highest scores and nociceptive the lowest. Pain catastrophizing showed the same results with the neuropathic group having the highest scores and nociceptive group the lowest (for all comparisons $p<0.05$). For pain interference there was a significant difference between the nociceptive and neuropathic groups and between the mixed and neuropathic groups ($p<0.05$), with the neuropathic group having the greatest scores; the comparison between nociceptive and mixed groups did not withstand multiple comparison correction.

For baseline sexual activity (SAQ) I found no significant difference in sexual activity at present, pleasure score or habit score. There was a significant difference in sexual discomfort score ($\chi2(2)=26.2$, $p<0.001$). Post hoc tests showed there was a significant difference between the nociceptive and mixed groups and the nociceptive and neuropathic groups ($p<0.05$), with the nociceptive group having the highest scores.

The baseline quality of life (SF-12) variables were all significantly different between the painDETECT groups: physical function ($\chi2(2)=25.7$, $p<0.001$), role limitation due to physical problems ($\chi2(2)=33.2$, $p<0.001$), role limitation due to emotional problems

($\chi2(2)=24.7$, $p<0.001$), social functioning ($\chi2(2)=34.7$, $p<0.001$), mental health score ($\chi2(2)=19.1$, $p=0.0019$), energy/vitality ($\chi2(2)=12.9$, $p=0.048$), pain score ($\chi2(2)=20.7$, $p<0.001$), general health perception ($\chi2(2)=21.2$, $p<0.001$), physical component ($\chi2(2)=28.9$, $p<0.001$) and mental component ($\chi2(2)=22.7$, $p<0.001$). Post hoc Mann-Whitney tests showed significant differences for all measures between the nociceptive and neuropathic groups ($p<0.05$), with the nociceptive group having greatest scores (for SF-12 higher scores indicate better health status). For comparison between the neuropathic and mixed groups, no comparisons withstood multiple comparison corrections. Comparisons between the mixed and nociceptive group showed significant differences ($p<0.05$) in physical function, role limitation due to physical problems, social functioning, mental health, general health perception and summary physical component and mental component scores; again, the nociceptive group had higher scores.

For work/activity/productivity (WPAIQ) at baseline, absenteeism was not significantly different between the groups. However, presenteeism, work productivity loss and activity impairment were significantly different between the groups ($\chi2(2)=16.9$, $p<0.001$; $\chi2(2)=24.1$, $p<0.001$ respectively). Post hoc Mann-Whitney tests showed a significant difference between the nociceptive and mixed group in activity impairment ($p<0.05$), with the mixed group having greater scores; all other comparisons between the mixed and nociceptive group did not withstand multiple comparison correction. Comparisons between the mixed and neuropathic group found presenteeism to be significantly different between the groups ($p<0.05$), with the neuropathic group having the greater scores; all other comparisons between the mixed and neuropathic groups did not withstand multiple comparison correction. Between the neuropathic and nociceptive groups presenteeism, work productivity loss and activity impairment were significantly different ($p<0.05$), with the neuropathic group having the greater scores.

	NEUROPATHIC (MEDIAN, IQR)	MIXED (MEDIAN, IQR)	NOCICEPTIVE (MEDIAN, IQR)
FATIGUE (BFI)	6.7 (2.7)	5.7 (3.4)	3.7 (3.1)
PAIN INTERFERENCE (BPI)	6.4 (2.5)	5.4 (3.3)	3.3 (4.6)
PSYCHOLOGICAL HEALTH (GHQ)	6 (7)	3 (4)	2 (5)
AVERAGE NRS PAIN SCORE	6.5 (2)	5.5 (1.9)	5 (2.5)
WORST NRS PAIN SCORE	9 (2)	9 (1)	8 (2)
SEXUAL ACTIVITY AT PRESENT (SAQ)	2 (0)	2 (0)	2 (0)
SEXUAL PLEASURE (SAQ)	9 (7)	9.5 (6)	10.5 (7)
SEXUAL DISCOMFORT (SAQ)	2 (3)	3 (3)	4 (2)
SEXUAL HABIT (SAQ)	0 (1)	1 (1)	1 (1)
PHYSICAL FUNCTION (SF-12)	50 (25)	50 (31)	75 (50)
ROLE LIMITATION DUE TO PHYSICAL PROBLEMS (SF-12)	37.5 (25)	50 (37.5)	75 (37.5)
ROLE LIMITATION DUE TO EMOTIONAL PROBLEMS (SF-12)	50 (43.8)	75 (50)	75 (40.6)
SOCIAL FUNCTIONING (SF-12)	50 (25)	50 (50)	75 (50)
MENTAL HEALTH (SF-12)	50 (25)	50 (25)	62.5 (25)
ENERGY/VITALITY (SF-12)	25 (38)	25 (25)	50 (31)
PAIN (SF-12)	25 (25)	25 (25)	50 (50)
GENERAL HEALTH PERCEPTION (SF-12)	60 (35)	60 (41)	60 (25)
PHYSICAL COMPONENT SCORE (SF-12)	35.8 (11.3)	39.0 (12.2)	43.3 (13.4)
MENTAL COMPONENT SCORE (SF-12)	38.6 (16.1)	42.4 (12.6)	46.1 (13.5)
ABSENTEEISM (WPAIQ)	0 (10.2)	0 (15.3)	0 (0)
PRESENTEEISM (WPAIQ)	60 (25)	50 (30)	30 (40)
WORK PRODUCTIVITY LOSS (WPAIQ)	60 (29)	53.2 (30)	40.3 (50)
ACTIVITY IMPAIRMENT (WPAIQ)	60 (25)	50 (30)	40 (40)
PAIN CATASTROPHISING (PCQ)	33 (22)	27.5 (15)	19 (18)

Table 2. Descriptors by painDETECT groups. Scores for questionnaire variables for each painDETECT group (neuropathic, mixed and nociceptive). IQR= interquartile range.

4.4.3 Sensory symptom profiles

The sensory symptom profiles across those in the neuropathic and mixed groups were assessed, using symptoms rated as part of painDETECT. Table 2 shows the percentages of participants who had clinically relevant sensory disturbances (scores >3, strongly or very strongly) for each symptom. The most common symptom was painful attacks.

SENSORY SYMPTOM	PROPORTION OF PATIENTS AFFECTED
BURNING	20.4%
PRICKLING	22.2%
MECHANICAL ALLODYNIA	18.5%
PAINFUL ATTACKS	79%
THERMAL HYPERALGESIA	9.9%
NUMBNESS	19.8%
PRESSURE EVOKED PAIN	49.4%

Table 3. Reported symptoms in neuropathic and mixed groups. Proportion of participants in the neuropathic and mixed groups reporting clinically significant symptoms (i.e., a score of >3, strongly or very strongly) in the painDETECT questionnaire.

4.4.4 Cluster analysis on sensory symptom profiles

I carried out cluster analysis, as in Chapter 3. Analysis found two distinct clusters, with a silhouette measure of cohesion and separation of 0.3, showing the model was of 'fair' quality. 38.3% (n=62) fell into cluster 1 and 61.7% (n=100) fell into cluster 2. The symptom with the greatest predictive importance was 'prickling/tingling' (predictive

importance = 1), which was most characteristic for cluster 2, as was 'burning' (predictive importance = 0.72). On the other hand, 'pressure evoked pain' (predictive importance = 0.9) and 'thermal hyperalgesia' (predictive importance = 0.84) were more characteristic of cluster 1. 'Painful attacks' (predictive importance 0.14), 'mechanical allodynia' (predictive importance 0.16) and 'numbness' (predictive importance = 0.13) were the least discriminatory. The sensory profile of the clusters is very similar to that discussed in Chapter 3, see Figure 1 for a comparison. The clusters I have found in this chapter and Chapter 3 differ from those found in other neuropathic pain conditions (see Figure 2).

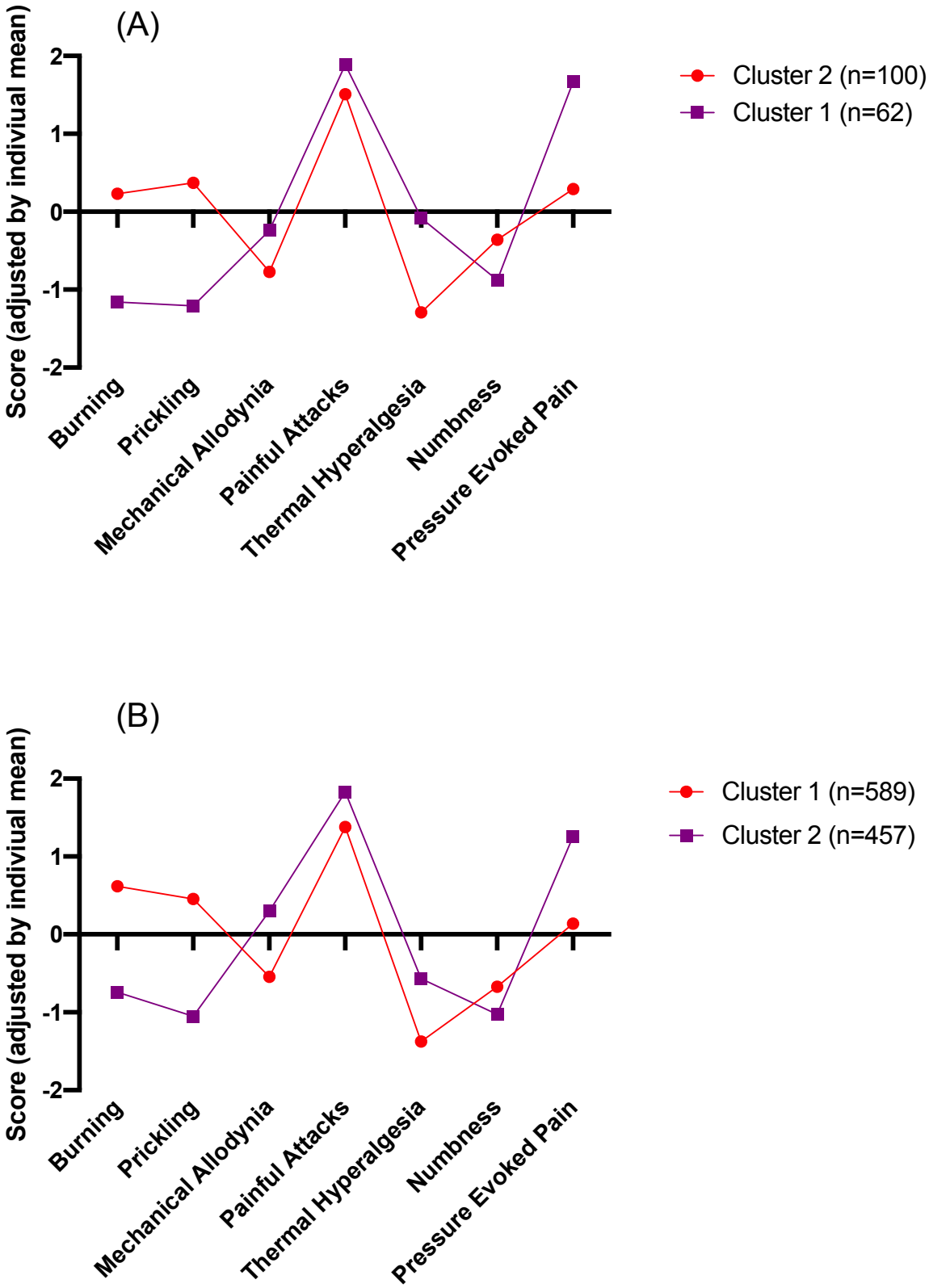

Figure 1. Sensory symptom profile of the two clusters. Scores are individually mean adjusted. (A) shows the clusters found in analysis of GaPP2 data, as discussed in this chapter. (B) shows the clusters as seen in Chapter 3 from the questionnaire study in endometriosis-associated pain for comparison.

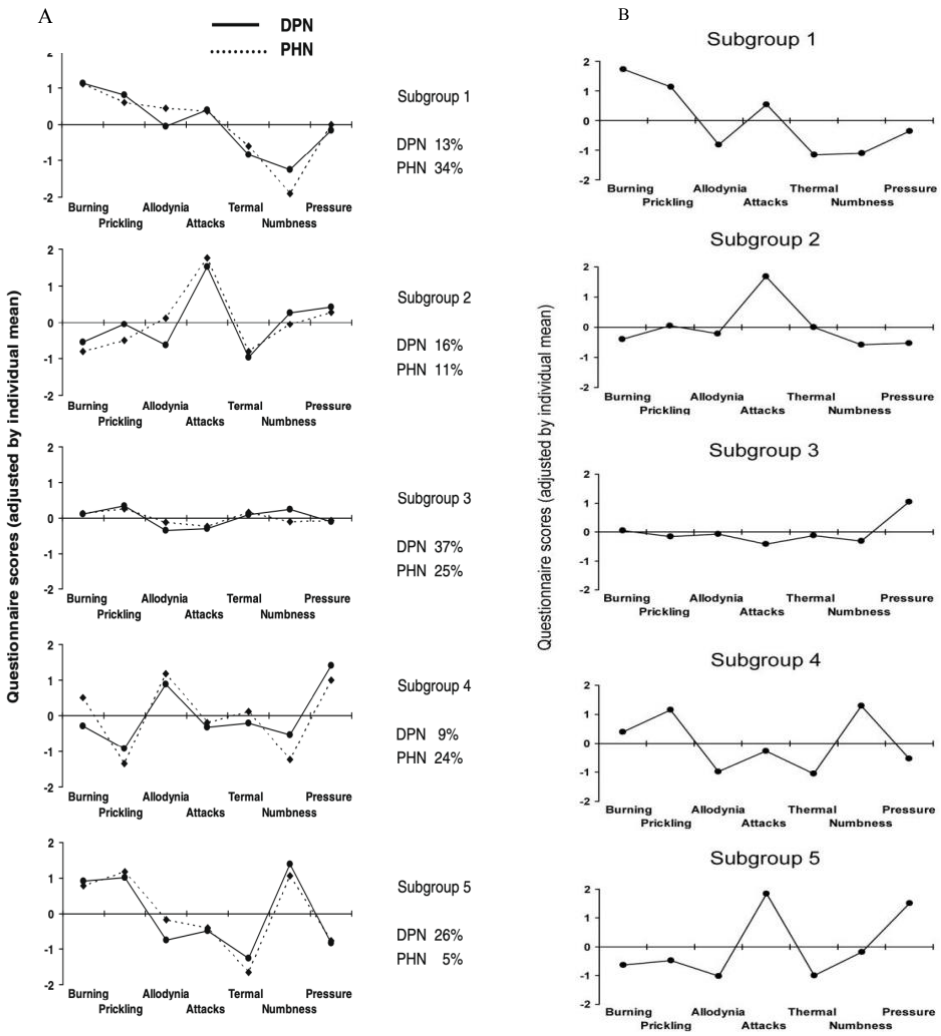

Figure 2. Sensory symptom profiles from Baron et al (A)[22] and Mahn et al (B)[23]. Scores are adjusted for individual mean symptom scores. (A) shows painful diabetic neuropathy (DPN) and postherpetic neuralgia (PHN). (B) shows results from painful radiculopathy.

Mann-Whitney tests to determine if there were significant differences between the clusters found no differences in the majority of measures (fatigue (BFI) score, pain interference score, psychological health (GHQ) score, baseline average pain NRS score, SAQ present sexual activity, SAQ pleasure, SAQ discomfort, SAQ habit, quality of life (SF-12) variables (PF, RP, RE, SF, MH, EV, P, GHP, MCS), absenteeism, presenteeism, work productivity loss, activity impairment, or baseline painDETECT score). There was a significant difference in worst pain intensity and pain catastrophizing score at baseline, but these did not withstand multiple comparison correction (p=0.01 and p=0.012 respectively).

4.4.5 Treatment response

There was no significant difference between the three painDETECT groups in 30% reduction in worst or average pain. There was also no significant difference between the painDETECT groups for a 50% reduction in worst or average pain.

There was no significant difference between the clusters in 30% reduction in worst or average pain or 50% reduction in worst or average pain scores.

GLM analysis

GLM analysis with the worst pain scores (at follow-up) as the dependent variable, with worst pain score at baseline as a covariate, found that there was no statistically significant interaction between painDETECT group and treatment group (for interaction term F=1.944, p=0.146). The model explained 16.2% of the variance of the outcome measure (adjusted R squared=0.162; for visualisation of the results see Figure 3). Similar results were seen with the average pain scores (for interaction term F=1.432, p=0.241, adjusted R squared=0.32; Figure 4). GLM analyses with additional combinations of variables (pain catastrophising, SF-12 variables etc) as covariates did not improve the variance explained by the model (adjusted R squared; results not reported here).

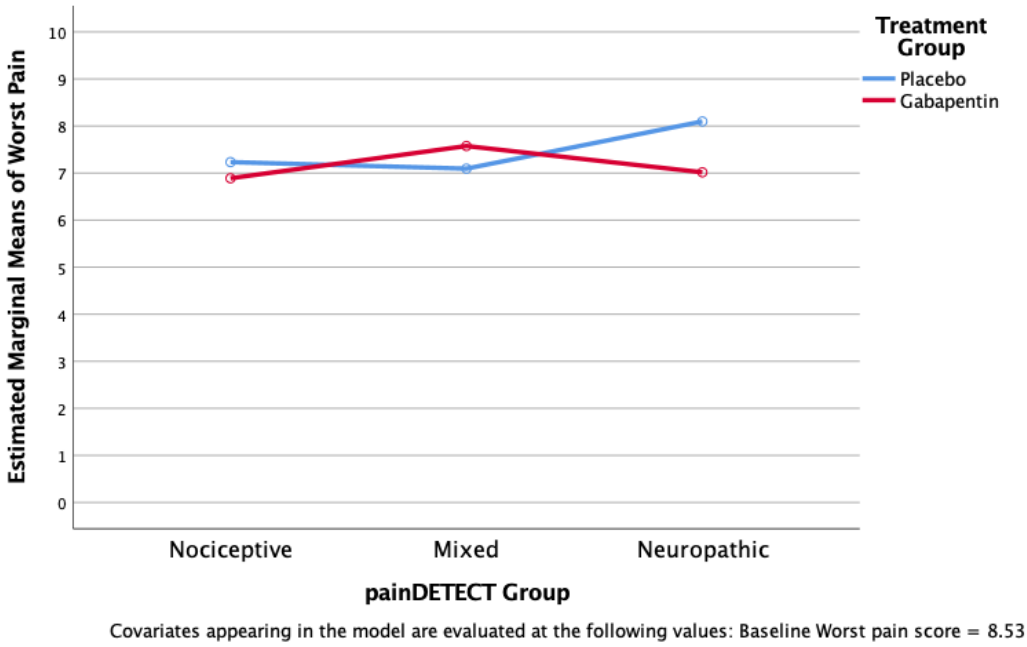

Figure 3 The estimated marginal means of the follow-up scores for worst pain controlled for baseline worst pain. This is shown against painDETECT group and split between those in the placebo and gabapentin treatment groups.

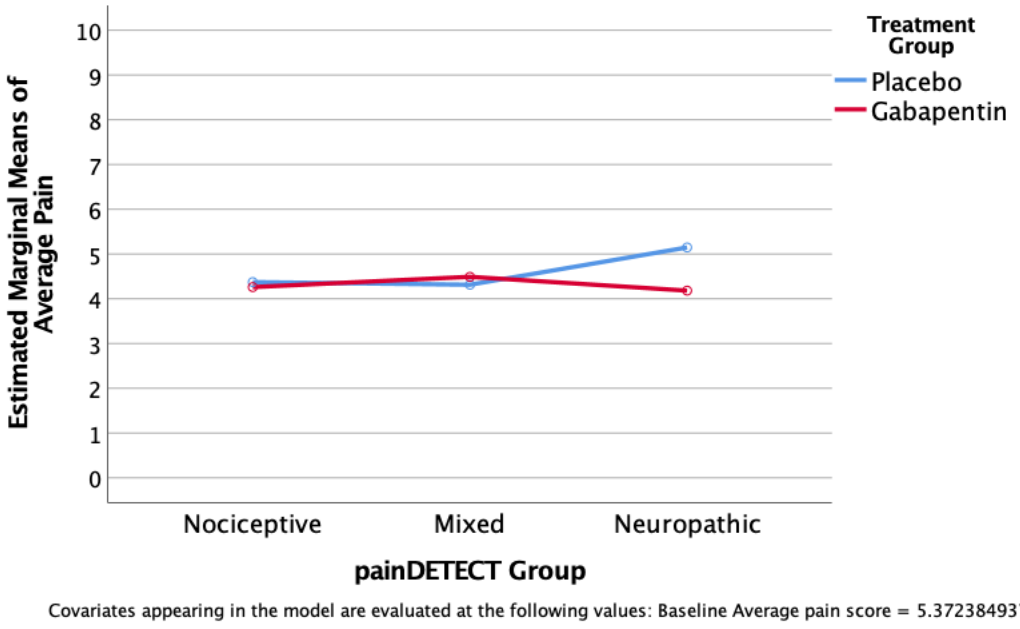

Figure 4 The estimated marginal means of the follow-up scores for average pain controlled for baseline average pain. This is shown against painDETECT group and split between those in the placebo and gabapentin treatment groups.

GLM analysis of the difference in treatment response in the two clusters for both the worst pain scores and average pain scores at follow-up, controlling for baseline scores, found no significant difference in the interaction term (interaction of cluster and

treatment groups) (F 0.001, p=0.971 for worst pain scores and F=0.167, p=0.684 for average pain scores) (see Figures 5 and 6).

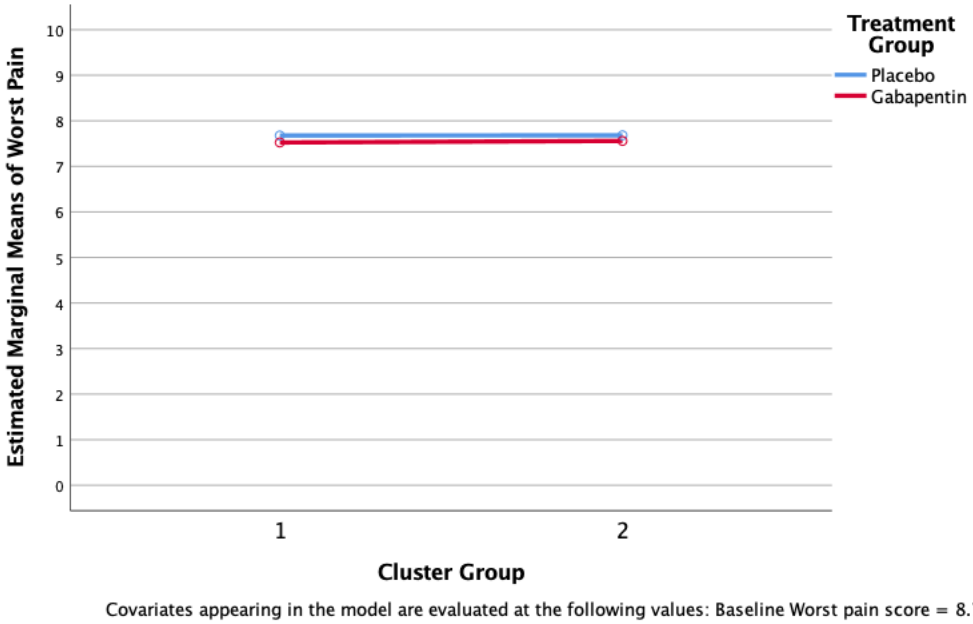

Figure 5 The estimated marginal means of the follow-up scores for worst pain controlled for baseline worst pain. This is shown against the two different cluster groups and split between those in the placebo and gabapentin treatment groups.

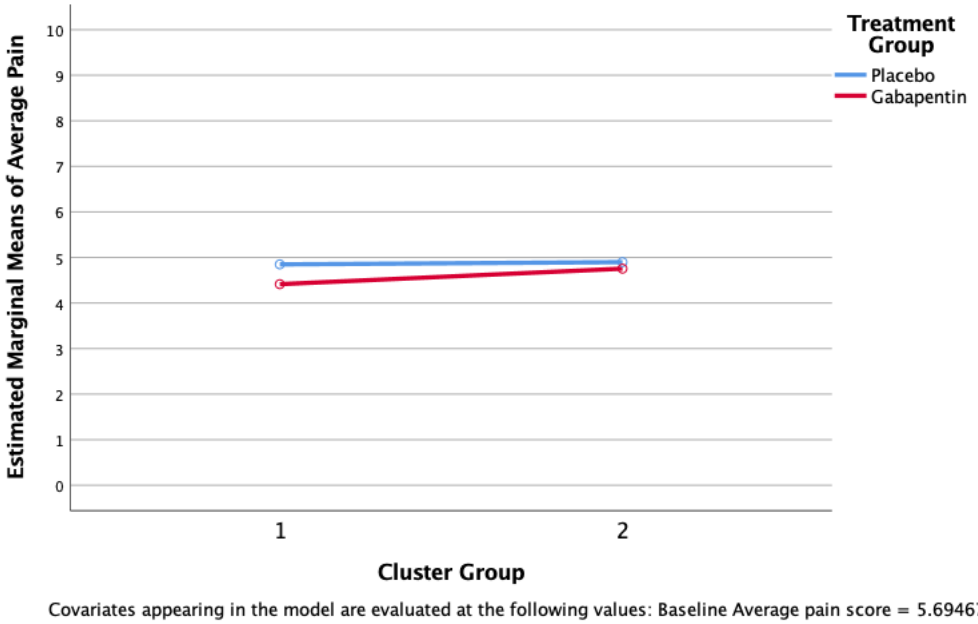

Figure 6 The estimated marginal means of the follow-up scores for average pain controlled for baseline average pain. This is shown against the two different cluster groups and split between those in the placebo and gabapentin treatment groups.

4.5 Discussion

4.5.1 Overview

This chapter aims to investigate differences between painDETECT groups in a variety of measures as well as determine whether painDETECT groups show different treatment response to gabapentin and placebo. Whilst the study population for this chapter is different to those in Chapter 3, there are indisputable similarities. painDETECT shows that there is a subset of women in this cohort who experience neuropathic pain (21%), with a further 33% having mixed neuropathic/nociceptive pain. Within those with neuropathic or mixed pain, there are two distinct subgroups which have incredibly similar sensory symptom profiles to women with endometriosis-associated pain, discussed in Chapter 3. Whilst I did not find a significant difference between painDETECT groups in their response to gabapentin, my results suggest the need for further exploration of the efficacy of gabapentin and placebo in those with neuropathic-like pain.

4.5.2 Comparison between painDETECT groups

I have found that 21.3% of the women in this cohort were classified as having neuropathic pain, with a further 32.7% having mixed neuropathic/nociceptive pain. This is an important finding as CPP is not traditionally thought of as having a neuropathic component.

The results show that the variables investigated show significant differences between the painDETECT groups. When taken together it is clear that the presence of neuropathic pain is negatively associated with many aspects of quality of life.

At baseline, the neuropathic and mixed groups had significantly greater pain intensity than those in the nociceptive group. Similarly, the neuropathic group reported the highest level of pain catastrophising and pain interference. For mental health and fatigue I again found the neuropathic group were most affected. General health perception, social functioning, role limitations, energy/vitality and physical function, all of which aim to quantify aspects of quality of life, were lower in the neuropathic group compared to the nociceptive group. Together, this suggests that those with neuropathic pain have the greatest burden of pain and pain-related symptoms.

4.5.3 Sensory symptom profiles

The sensory symptom profiles of those in the neuropathic and mixed groups for the symptoms assessed in painDETECT show similar prevalence to those seen in endometriosis-associated pain (EAP) (discussed in Chapter 3). Overall, these proportions are very similar in the two cohorts, with painful attacks being by far the most common symptom. As is discussed in Chapter 3, there are similarities with other neuropathic pain conditions, although other conditions have not reported as great a prevalence of painful attacks.

4.5.4 Comparison with findings of Chapter 3

There are many similarities in the results of this chapter and those of Chapter 3, despite the different clinical populations.

This data presented in 4.5.2 suggests, mirroring findings of Chapter 3, that those in the neuropathic group have greater symptom burden and lower quality of life as those categorised as having nociceptive pain.

The clusters found in this cohort (Figure 1) are strikingly similar to those found in EAP (discussed in Chapter 3). These clusters differ to those seen in other studies[22,23] (Figure 2). This could be due to a variety of reasons, as discussed in Chapter 3.

Whilst this chapter and Chapter 3 have different cohorts with different chronic pain conditions the similar findings suggest that there may be underlying similarities in the mechanisms generating and maintaining pain in those with neuropathic and mixed pain. The fact that these do not match with reports from other conditions suggests that these mechanisms may be specific to pelvic pain, other shared features of CPP and EAP (e.g. history of surgery or local inflammation), or to the female population. There is a clear need for further work to elucidate the mechanisms giving rise to pain in these populations and suggests that symptom-focussed research, rather than condition focussed may help in understanding chronic pelvic pain in women, and importantly may aid in the search for successful treatment options.

4.5.5 Differences in treatment response between groups

Across the entire study cohort, the GaPP2 trial did not find a significant treatment effect of gabapentin on pain[1]. Studies investigating other treatments including surgery[24] and tapentadol[25] have demonstrated that treatment effects can differ between painDETECT groups. My aim was therefore to investigate whether those with nociceptive, mixed and neuropathic pain show a different response to gabapentin (or placebo). Whilst the GaPP2 study was not specifically powered to answer this question, data from this study was used for a secondary (exploratory) analysis to explore potential sub-group differences.

Whilst statistical tests show that there is no significant interaction between the painDETECT group and the treatment response, Figures 3 and 4 suggest that there may be a difference in outcome scores between the treatment groups in the neuropathic group. It may be that this is not statistically significant due to the limited sample size when investigating subgroups (n=46 in neuropathic group, n=76 in mixed group and n=117 for nociceptive group for this analysis). The models for average and worst pain explained differing proportions of the variance in the outcome scores (32% for average pain and 16.2% for worst pain), which may relate to differences in the underlying mechanisms of these pains.

The GLM investigating whether there was an interaction between clusters and treatment group found the interaction was not significant. Again, one problem with this analysis is the very limited sample size (n=46 in cluster 1, n=76 in cluster 2).

When investigating pain scores as an outcome measure in clinical trials in order to determine whether there is a significant difference, there needs to be a large sample size, in part due to that fact that drugs always have a Number Needed to Treat (NNT) of above 1. For gabapentin the NNT is 6.3[26]; simply, this means that the drug does not work for everyone, specifically for gabapentin 6.3 patients have to be treated for 1 person to get a 50% reduction in pain. Sample size calculations are therefore carried out to determine the number of participants needed to be included to ensure that results are reliable. For the GaPP2 trial such calculations were carried out on the basis of there being two groups (gabapentin and placebo), not six (gabapentin and placebo in each of the three painDETECT groups). Therefore, the results from this chapter on treatment response can only be taken as pilot data and cannot be used to draw solid conclusions.

4.5.6 Strengths & Limitations

The main limitation to these results is that as this is a secondary analysis and as such was not powered sufficiently. Follow-up studies need to optimise their sample size in order to better answer these questions.

A strength of this study is that the large sample of n=306 women met strict inclusion criteria, such as pelvic pain other than dysmenorrhea alone, two NRS pain scores for their worst pain ≥4 in the 4 screening weeks and no obvious pelvic pathology at laparoscopy; this helps to eliminate confounding factors, however it can mean results cannot be extrapolate to the whole population. The data set was collected by several sites across Britain so that findings would not be limited to one centre for care, which helps to overcome bias in the sample.

4.5.7 Future Directions

Future work should further investigate the mechanism generating and maintaining neuropathic pain in this population and look for similarities in mechanisms between chronic pelvic pain with no obvious pathology and endometriosis-associated pain. I would suspect that the similarities seen in the sensory symptom profiles and cluster analysis are underpinned by shared biological mechanisms.

The cluster analysis should be run in other pain conditions to try to elucidate the reasons the clusters I have found are different to those found in other conditions, specifically investigating whether the difference is due to the location of pain or the entirely female population by running similar analysis in new populations (such as men with chronic pelvic pain and women with other neuropathies).

The results in this chapter cannot answer whether the different painDETECT groups (or different cluster) respond differently to other types of treatments. More studies are

needed to determine this and they need to be appropriately powered to ensure that the results are valid. The findings here about the proportions of women falling into each painDETECT group and each cluster can aid these power calculations for future studies.

4.6 Conclusion

These findings show that there is a subset of women with chronic pelvic pain with no obvious pathology whose pain is neuropathic in nature. In line with evidence from other pain syndromes[27], CPP patients with neuropathic pain experience greater symptom burden. Those grouped as having neuropathic or mixed pain can be subdivided into two clusters with sensory symptom profiles which are very similar to those found in Chapter 3. Overall, there was no evidence for a differential response to gabapentin or placebo in the three painDETECT groups but further research in larger cohorts is needed to assess treatment response in different painDETECT groups for this and other treatments.

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Chapter 5: Multiple analysis strategies fail to identify any relationship between painDETECT and resting connectivity of the brain in women with endometriosis-associated pain

5.1 Abstract

In this chapter I investigate static functional connectivity in women with endometriosis-associated pain and its relation to the presence of neuropathic pain. Firstly, I use seeds selected from previous findings in this cohort and determine both the group mean connectivity of these areas as well as if connectivity correlated with painDETECT score. I then examine whether there is significant connectivity between these regions. Finally, using a data-driven voxel-based approach I determine whether there is connectivity across the whole brain which correlates with painDETECT score. Despite the thorough analysis of this data I find no correlation between functional connectivity and painDETECT in this cohort.

5.2 Background

As I have discussed in previous chapters, there is growing evidence that there is a neuropathic component to endometriosis-associated pain, which could be the result of peripheral or central changes in the nervous system. My work presented in this chapter aims to combine fMRI and behavioural measures from women with endometriosis-associated pain; to search for a significant correlation between painDETECT scores and activity in brain regions where activity in response to punctate stimuli correlates with painDETECT. This approach is useful when trying to determine the central mechanisms

of this neuropathic pain component. As will be discussed again in a later chapter, neuroimaging has been used in a variety of different pain conditions, including neuropathic pain and pelvic pain. It allows us to explore the changes to the central nervous system in these conditions and can be approached in many different ways. In this chapter I discuss work by Katy Vincent using task-based fMRI and my own work using resting state fMRI as these enable us to look at brain activity; there are other methods of MRI which are not discussed in this thesis which allow investigation of different aspects of the brain, such as different assessments of the structure.

Resting-state fMRI measures spontaneous fluctuations in blood oxygenation level (BOLD) in participants who are not carrying out a specific task. This contrasts with task-based fMRI in which BOLD signals in response to a given task/stimulus are investigated. Task-based fMRI is limited by the fact that specially designed tasks are needed to look at specific areas of the brain. The benefit of resting state analysis is that it can be used to investigate many networks at once. Resting state fMRI can tell us about the inherent organisation and functioning of the brain[1], which in turn can aid us in understanding how the brain processes complex information[2]. There is also ongoing work investigating whether resting state networks can be used as biomarker[3] for pain[4] and analgesia[5].

One of the criticisms of resting state fMRI is that it can be difficult to interpret what findings mean as the thought processes of participants are uncontrolled and therefore there could be various processes occurring in different participants; for example, one participant may be think about how they are going to travel home, whilst another may be remembering a dream they had last night. However, the same functional connectivity networks have been consistently found across different studies which suggests that there

are at least some aspects of brain activity which are present regardless of the participant's cognitive state[2,6–11].

As-Sanie et al 2016[12] also investigated resting state connectivity in endometriosis-associated pain. They found that in women with endometriosis-associated chronic pelvic, in the anterior insula had increased connectivity to both the medial prefrontal cortex and the occipital cortex compared to healthy controls. Interestingly, they also looked at connectivity of the insula in women with endometriosis without chronic pelvic pain and did not find any significant differences when compared to healthy controls. This shows that it is the chronic pain experienced by women which is leading to changes in the central nervous system, not the presence of endometriosis per se. To further explore this finding, it is useful not only to differentiate between endometriosis with and without pain but also look at characteristics of the pain experienced and how that relates to central changes.

More broadly in chronic pelvic pain, the MAPP research network which focusses on urological chronic pelvic pain syndrome has investigated functional connectivity in their cohort (a relatively large sample of 45 female patients and 45 age-match female healthy controls)[13]. They found that the posterior cingulate cortex and the left precuneus had altered functional connectivity in patients, which was associated with clinical and behavioural measures.

EndoPain2 Study

EndoPain2 is a study investigating endometriosis-associated pain in women with surgically confirmed endometriosis who were recruited prior to repeated surgery for endometriosis-associated symptoms. The data discussed in this chapter is from this

study cohort. The primary aim of this study was to investigate in women with endometriosis-associated pain, whether brainstem activity in response to noxious stimulation related to the experience of neuropathic pain.

The task-based fMRI analysis of this study by Katy Vincent, investigating brain activity in response to a punctate stimulus identified regions where the evoked brain activity was correlated with painDETECT scores[14]. Analyses followed standard pipelines. She found that painDETECT scores correlated negatively with activity in the pontine reticular formation (pRF) in response to punctate stimulation (i.e. greater activity in those with lower painDETECT scores) (brainstem masked; $Z>3.1$, $p<0.05$ peak voxel $Z=4.14$, $x=4$, $y=-36$, $z=-36$). A whole brain analysis also revealed significant negative correlations with left thalamus, left insula, midcingulate cortex and periaqueductal gray (PAG) at a lower threshold ($Z>2.3$, $p<0.05$). I have taken these brain areas and investigated, using resting state data, their functional connectivity. I hypothesise that the regions identified will have connectivity which correlates with painDETECT score.

I have also carried out a data-driven approach, as this enables us to investigate the whole brain without assumptions, to determine whether there is functional connectivity which correlates with the presence of neuropathic pain (assessed by painDETECT). This provides a broader investigation of the data, which ensures a thorough examination of the data.

My aims in this chapter are to:

- To determine whether there is significant functional connectivity between the pontine reticular formation (pRF), left thalamus, left insula, midcingulate cortex and periaqueductal gray (PAG) at a group level and whether this correlates with painDETECT scores

- Investigate the whole-brain static functional connectivity of these regions at a group level

- To determine whether the connectivity of these areas at the whole brain level correlates with painDETECT scores

- To use a data-driven whole brain approach (Independent Component Analysis ICA) to investigate if painDETECT scores correlated with connectivity of identified networks

5.3 Methods

5.3.1 Data collection

Data was collected by a member of the group before my DPhil commenced, therefore whilst I describe data collection, I was not involved. Ethical approval was obtained (reference: Oxford University Hospitals NHS Foundation Trust Research and Development 15/SC/0372, IRAS No: 163399).

Women with confirmed endometriosis (within the last 5 years) were recruited prior to repeat surgery for endometriosis-associated pain. All women reported non-menstrual pelvic pain of an intensity ≥ 4/10 occurring on at least 7 days per calendar month, for at least 6 months. Women were excluded if pregnant, contraindicated for MRI scan or they had used centrally acting drugs in the last 3 months (e.g. anti-epileptics, anti-depressants, anxiolytics, gabapentin, pregabalin, duloxetine). Women who used standard analgesics including opiates were not excluded but asked to refrain from taking such drugs for 8 hours prior to their brain scan if possible.

On the first visit participants were asked to complete questionnaires which included detailed demographic as well as clinical data. Several validated questionnaires were included, such as painDETECT[15,16], to assess a neuropathic pain component, pain catastrophizing[17] and measures of anxiety[18,19] alongside questions asking for ratings of their pain (Numerical Rating Scale), including their pain "now, at this moment" which was used in analysis.

The MRI section of the visit included collecting data about brain structure and brain activity at rest as well as in response to a standardised noxious stimulus at the site where endometriosis-associated pain is experienced (on their lower abdomen, below naval) and at a control site (left inner arm). For the resting state scan, participants were asked to relax with eyes open, but not fall asleep. Pinprick stimuli were chosen for the stimulus to the lower abdomen as they have been previously shown to give a robust activation in brain areas related to hyperalgesic states (both experimental[20–22] and clinical[23]). For the control site, thermal stimuli were applied as they have previously been used to identify altered central processing in patients with dysmenorrhea[24] and other pain states[25,26], without site hyperalgesia, however these data will not be discussed in this thesis. After each stimulus, online pain ratings were collected using Visual Analogue Scale.

5.3.2 Data analysis

Pre-processing was carried out as discussed in Chapter 2. Group level analysis was carried out using both seed-based hypothesis driven and whole-brain data-driven methods.

The ROIs identified by Katy Vincent in the punctate task fMRI[14] were used as masks for seed-based correlation analysis (SCA); these were the pontine reticular formation (pRF), left thalamus, left insula, midcingulate cortex and periaqueductal grey (PAG). These seeds were created from the spatial maps of task-based fMRI at a threshold of $Z>3.1$ for pRF and $Z>2.3$ for the other seeds.

Specific analysis steps are described in more detail in Chapter 2.

FSLNets was used to determine connectivity between these seeds (controlling for background pain) both without any covariates and correlating with painDETECT score.

Whole-brain seed-based connectivity analysis using dual regression was carried out for these seeds looking at general connectivity (group mean, controlling for background pain), as well as connectivity with correlation to painDETECT using different GLM contrasts. As well as these, for the pRF only, extra GLM designs were used to ensure all control variables had been considered; correlation with painDETECT controlling for each of the following: pain catastrophizing, age and anxiety scores.

All p values given are family-wise error corrected values.

Spearman's correlation was carried out on behavioural measures to ensure they were not significantly correlated ($p<0.05$).

5.4 Results

5.4.1 General results

28 women were recruited: 26 provided good quality data for resting state analysis (2 participants did not have resting state data aquired). painDETECT scores ranged from 1

to 28, which is a good spread as the painDETECT scale ranges from -1 to 32 (see Table 1). There was no correlation between painDETECT score and psychological measures (anxiety and pain catastrophising) (rho=-0.049, p=0.813; rho=0.173, p=0.398 respectively).

For the 26 participants included in the resting state analysis, some information on analysis-relevant variables can be found in Table 1.

	MEDIAN (RANGE) [IQR]
AGE	36.5 (20-47) [29.25-44]
PAINDETECT SCORE	15 (1-28) [12.25-18]
PAIN CATASTROPHIZING SCORE	19.5 (7-41) [12.25-28.5]
STATE ANXIETY SCORE	40.5 (24-63) [33.25-47.75]
BACKGROUND PAIN SCORE (0-10 NRS)	4 (0-8) [3-6]

Table 1: Distribution of pain relevant variables in the participants used for resting state analysis.

5.4.2 Connectivity between ROIs

Firstly, I used FSLNets to investigate the group mean connectivity between the ROIs in my cohort, controlling for pain scores. I found that at group level, these regions were

not significantly connected at rest (p=0.9986 between pRF and left insula and between the pRF and PAG, otherwise all other p=0.9998 when family wise error corrected).

Using FSLNets allowed me to investigate the correlation between painDETECT (controlling for pain scores) and the functional connectivity between ROIs previously discussed. There was no significant positive or negative correlation between the connectivity of these areas and painDETECT scores.

5.4.3 Whole brain seed-based connectivity of ROIs

Whole-brain seed based dual regression was run for each of the ROIs discussed. This was done for two design GLMs, one which gave a group mean connectivity, controlling for pain scores, and another which investigated connectivity which correlated with painDETECT scores, controlling for pain scores.

Pontine reticular formation (pRF)

The group mean connectivity of the pRF showed only significant connectivity ($p<0.05$) to itself (Figure 1-A). Connectivity with other brain areas did not reach statistical significance.

As we were investigating whether connectivity related to measures of neuropathic pain, I also looked at whole brain connectivity to the pRF which correlated with painDETECT score. This analysis showed no significant connectivity to any brain regions when using the standard threshold of $p<0.05$. When the threshold was lowered to $p<0.1$ a positive correlation was found between painDETECT and the connectivity of the pRF and the right thalamus and connectivity of the pRF to the premotor/somatosensory cortex, as seen in Figure 2.

Left thalamus

Group mean connectivity of this area again showed only significant ($p<0.05$) connectivity to itself (Figure 1-B).

When looking at the connectivity of the left thalamus that correlated with painDETECT score, I found no significant results ($p<0.05$), even when the threshold was lowered to $p<0.1$.

Left insula

Group mean connectivity analysis identified several regions which showed significant connectivity with the left insula. At a threshold of $p<0.05$, the left insula exhibited significantly increased functional connectivity with the right putamen and right insula as well as the left putamen, left Broca area and left secondary somatosensory cortex (Figure 1-C).

When investigating connectivity of the left insula which correlated with painDETECT score, none of the results reached the $p<0.05$ threshold. At the lower $p<0.1$ threshold, painDETECT scores were positively correlated with connectivity between the left insula and left secondary somatosensory cortex.

Midcingulate cortex

As shown in Figure 1-D, group mean analysis of the midcingulate cortex showed no significant connectivity to other brain areas (only significant connectivity to itself).

Analysis investigating the correlation between painDETECT and the connectivity of the midcingulate cortex found no significant result (even at the lower threshold of $p<0.1$).

PAG

Again, group mean connectivity of the PAG showed only significant connectivity to itself, as can be seen in Figure 1-E.

Furthermore, the analysis that aimed to identify brain regions in which functional connectivity with the PAG scaled with painDETECT scores did not reveal any significant findings at a threshold of $p<0.05$. At a more lenient threshold of $p<0.1$, connectivity between PAG and the occipital pole were positively correlated with painDETECT score.

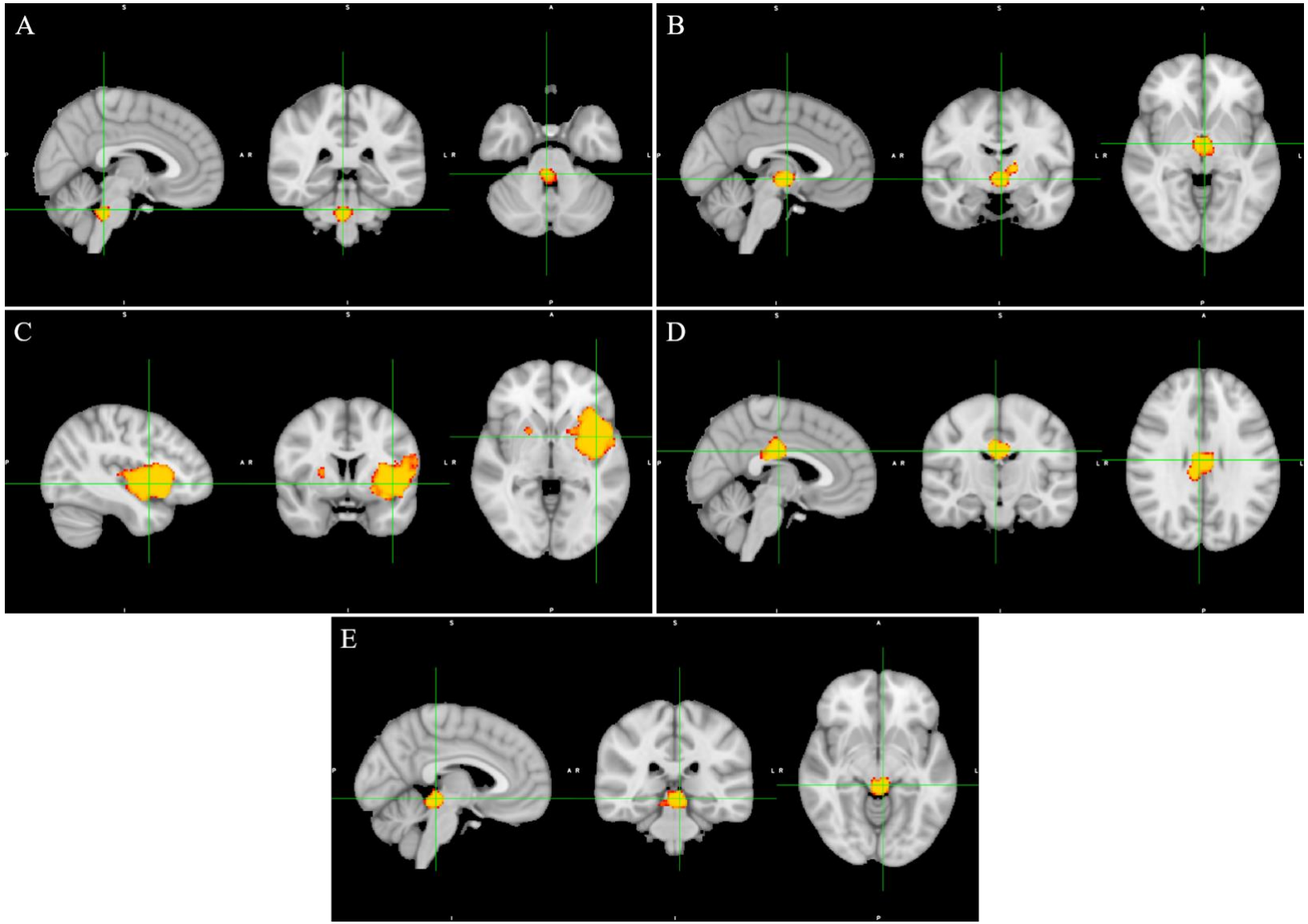

Figure 1: Group mean connectivity of (A) pRF, (B) left thalamus, (C) left insula, (D) midcingulate cortex and (E) PAG at threshold $p<0.0$

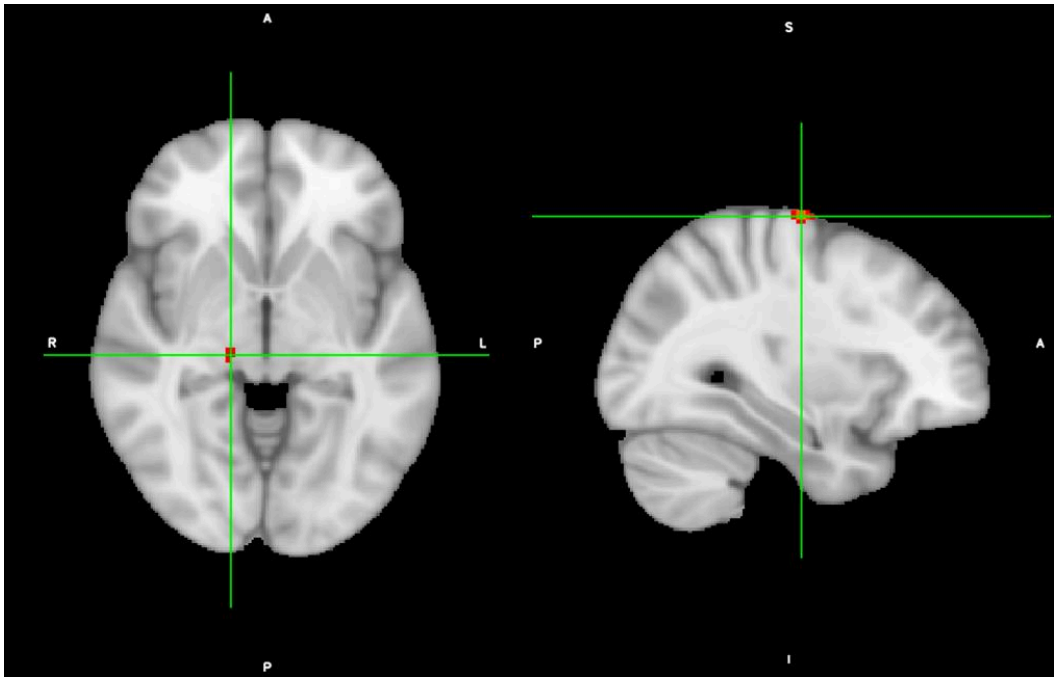

Figure 2: Functional connectivity of pRF correlated with painDETECT score at p<0.1. Cross hairs on right thalamus (left) and premotor area (right).

Seed	**Group mean connectivity**	**Connectivity correlated with painDETECT**	**Neurosynth.org look up**
pRF	Itself only	Positive correlation at p<0.1: right thalamus and premotor/somatosensory cortex	Itself only
Left thalamus	Itself only	None	Itself only
Left insula	Right and left putamen, right insula, Broca area and secondary somatosensory cortex	Positive correlation at p<0.1: secondary somatosensory cortex	Right insula, cingulate/paracingulate gyrus, supramarginal gyrus and parietal operculum
Midcingulate cortex	Itself only	None	Itself and right and left precuneous
PAG	Itself only	Positive correlation at p<0.1: occipital pole	Itself only

Table 2: Comparison of group mean connectivity, connectivity correlated with painDETECT score and findings in healthy controls from coordinates on neurosynth.org of five seeds. pRF (4,-36,-32), left thalamus (-32,20,-4), left insula (-40, 4, -2), midcingulate cortex (4, -16, 28) and PAG (-4,-32,-8). Neurosyth.org correlation coefficient threshold of 0.4 was used.

5.4.4 Data-driven voxel-based analysis

Analysis using FSL MELODIC gave 25 ICA components (discussed in Chapter 2). Dual regression with randomise was used to correlate connectivity with painDETECT scores (with contrasts for both negative and positive correlation). Visual inspection of results was used and components which were classed as artefact/noise were not reported.

Since I had 25 components with 2 contrasts, further correction of the output p value was necessary, meaning $p<0.001$ would be significant for my results. This analysis revealed no significant correlations between functional connectivity and painDETECT score (even before more stringent correction for multiple comparisons).

5.5 Discussion

5.5.1 Overview

Whilst there is evidence for the usefulness of painDETECT as a stratifier in endometriosis-associated pain, my data presented here suggests that the variation in these scores is not reflected in resting state connectivity. This is contrary to what has been seen in other conditions with a neuropathic component[32, 33, 34, 35]. In response to provoked pain, painDETECT is negatively correlated with activity in regions known to be associated with descending modulation of pain[14]. However, despite my thorough analysis of the resting state data using a variety of different methods, I can find no evidence that painDETECT relates to altered resting state connectivity and thus may be a measure of, or a pointer towards, the underlying mechanism of unprovoked pain in endometriosis.

5.5.2 Seed-based analysis

It is generally understood that areas of the brain do not work in isolation. It is expected when looking at activity in one area of the brain one would see synchronous activity in another area, which we interpret as those areas being functionally connected. This sort of communication between brain areas is useful to study as it can help give a more complete picture of brain activity in networks than looking at areas independently. This connectivity can be altered, for example by disease or training, which is why it is interesting to look at in relation to endometriosis-associated pain.

One key aspect of seed-based analysis is that it is hypothesis-driven, in this instance the seeds selected have been shown to have activation in response to a punctate stimulus which scales with painDETECT score[14].

Connectivity between the seeds

I investigated the connectivity between these regions of interest related to patients' painDETECT scores and without entering any covariate of interest. Neither of the two analyses revealed any significant findings.

Whole brain analysis

I carried out whole brain searches for increased or decreased functional connectivity of brain regions identified during painful stimulation. My findings from the five seeds investigated, including comparisons to neurosynth.org are summarised in Table 2.

Surprisingly, when using a seed for the pRF, I found no significant connectivity with other parts of the brain at a group mean level. I also investigated whether the

connectivity of the pRF to any areas of the brain was correlated with painDETECT score to determine whether the presence of a more neuropathic-like pain changed the connectivity of the pRF. Again, this was not statistically significant. This is interesting as the pRF was identified as an area whose activity was correlated with painDETECT score when using a punctate stimulus[14].

A similar pattern was observed in the left thalamus, midcingulate cortex and PAG.

Therefore, my findings seem to potentially relate to the difference between evoked pain and spontaneous background pain in terms of the role these regions (pRF, left thalamus, midcingulate cortex and PAG) have in these processes. Evidently, when experiencing an evoked pain sensation, these areas show altered activity that scales with the extent to which the participant's endometriosis-associated pain is categorised as neuropathic. Interestingly, the same does not apply to when patients are scanned at rest.

The left insula on the other hand, does display statistically significant positive connectivity with other regions at rest across the group, namely with the right insula, left and right putamen, the left primary somatosensory cortex (SI) and left Broca's area. The insula, putamen and primary somatosensory cortices are known to be active in response to a painful stimulus and are part of what has been coined the 'cerebral signature' of pain[27]. This differs from what is seen in healthy participants on neurosynth.org which shows connectivity to the right insula, cingulate/paracingulate gyrus, supramarginal gyrus and parietal operculum. Of note, I had also found increased functional connectivity between the left insula and SI related to painDETECT score (albeit at a lower threshold of $p<0.1$), which suggests that resting state connectivity between both regions is greater the stronger the neuropathic component is in the individual.

Overall however, my data indicate that brain regions which had shown altered activity in response to a punctate stimulus that scaled with painDETECT scores[14] display no changes in functional connectivity during rest.

Comparison of connectivity of seeds to healthy control database

Neurosynth.org[28] is an online platform for large-scale, automated synthesis of fMRI data. I have utilised this tool as it is possible to look-up the coordinates of a given location in the brain, and it will provide functional connectivity results for this area. This comes from analysis of 1,000 healthy participants' resting state data, which were collected as part of the Brain Genomics Superstruct Project[29–31]. I have looked up coordinates of my seeds on this database to determine whether there appears to be differences between healthy controls and those with endometriosis-associated pain in my cohort. For this I used a coordinate in the centre of each seed and thresholded the neruosynth.org output to r=0.4 so as to filter to areas with at least moderately strong correlation to the given coordinate. Table 2 shows a comparison of my findings to those on neurosynth.org.

Briefly, when the pRF (x=4, y=-36, z=-32), PAG (x=-4, y=-32, z=-8) and the left thalamus (x=-2, y=-8, z=-4) are looked at, neurosynth.org showed similar connectivity, in that they only had significant connectivity to themselves in healthy participants. However, for left insula and midcingulate cortex show different connectivity in healthy controls to my findings in endometriosis-associated pain (see Table 2).

5.5.3 Data-driven whole brain analysis

While seed-based analyses can aid understanding of areas with a known function, or, as in this chapter, to further explore findings from task-based fMRI, they have a more restricted approach, which can mean that interesting findings are missed. Whilst hypothesis-driven approaches are a key aspect of research, data-driven approaches are very valuable, especially in contexts such as these were there is limited literature. In the next analysis step, I therefore used a data-driven approach based on an independent component analysis, for a more exploratory analysis. This gave no functional connectivity which correlated with painDETECT score when controlling for background pain score. This shows that whilst these women seem to have a neuropathic pain component to their pain (with n=6 classified as falling in the neuropathic pain group with painDETECT, and a further n=13 in the mixed group), this does not present in altered functional connectivity when they are experiencing their background pain.

This may also be an indication that the different pains experienced by women with endometriosis may be maintained by different mechanisms. For example, it may be that for some women the mechanism of evoked pain is neuropathic in nature (this evoked pain is perceived due to damage to the somatosensory nervous system) whereas their background pain is maintained by different mechanisms all together. Hyperalgesia and allodynia as seen in some women with endometriosis-associated pain are key symptoms of neuropathic pain, however descriptors such as dull/aching pain are not. More research is needed to determine whether this is the case in endometriosis-associated pain. Given the variety of symptoms seen both between and within women with endometriosis-associated pain it would not be surprising if several different pain maintaining mechanisms were involved in creating their pain phenotype.

5.5.4 Strengths & Limitations

This is the first study to examine the relationship between painDETECT scores and resting state connectivity of the brain in women with endometriosis-associated pain.

It is possible that I have not found statistically significant results because of our sample size. However, n=26 is not an unusual size for such studies. Other studies investigating the correlation between resting-state connectivity and painDETECT have used similar sample sizes (n=24 in osteoarthritis[32], n=20 in hereditary and diabetic neuropathies[33], n=30 in diabetic neuropathy[34], n=31 in multiple sclerosis pain[35]) as has the previously discussed study in endometriosis (n=36 across 3 groups in As-Sanie et al[12]). Task-based fMRI data from the same cohort also found significant findings[14]. Therefore, whilst sample size cannot be ruled out, I do not believe it to underpin my null findings.

I have carried out a variety of different techniques to investigate the resting state of this cohort. Firstly, I carried out hypothesis driven methods using seeds, then also used a data-driven approach. These different methods allowed me to ensure that the data had been thoroughly investigated. There is no gold standard for the best technique for resting state analysis and there have been many recent developments in the field since previous relevant work, therefore, it was important to ensure that different techniques were used to make sure no findings were missed. Whilst this increases the risk of false positives, I do not believe this to be an issue with our findings, and instead helps us to be confident in our negative findings.

5.5.5 Future Directions

These findings suggest that when investigating the mechanisms of neuropathic pain in endometriosis it is important to differentiate between evoked and spontaneous pain. Further work is needed to better understand these mechanisms. Evoked pain, in those

with neuropathic pain, seems to involve activation of certain areas of the brain, including the pRF (Vincent et al, in preparation), whilst I could find no significant connectivity of this area at rest. In order to determine whether this finding is true to all evoked pain, it is important to try to replicate these findings using a range of different stimuli, to help elucidate whether it is an evoked pain specific mechanism or, more specifically, a mechanism only for punctate stimulation. For example, different results may be seen if stimuli which activate a different type of nociceptors are used, such as thermal stimuli or pressure stimuli. As well as classical noxious stimuli, it would be interesting to contrast such sensations with non-noxious stimuli such as auditory or visual stimuli to help determine whether changes in perception is limited to painful experiences. It is also important to relate these evoked painful stimuli to sensations that women report in their day-to-day lives, especially those that are bothersome. For example, stimuli should be found which have a clear comparison to real life experiences of pain such as heating thermode relating to pain they experience when having a warm bath/shower. This would enable us to determine whether the activity seen in response to these reflect that seen with punctate, and also help us in understanding how this can relate to 'real-life' noxious stimuli.

Further work investigating the neural underpinnings of background pain is needed in this population. Women with endometriosis-associated pain experience very varied symptoms and the nature of their background pain may be very different. It would be very useful to try to determine the characteristics of their background pain and investigate how resting state connectivity differs between different types of spontaneous pain. I would expect that fMRI data collected when women are experiencing their 'normal' background pain would differ from that collected whilst they were having a flare/pain attack (i.e., described as sudden stabbing pain).

In order to better understand the different mechanisms involved in both evoked and spontaneous pain it is vital to better explore brain activity during different types of pain. Women with endometriosis often use very different descriptors to describe different types of pain they experience. For example, their menstrual pain may be aching, whilst pain with sex may be a burning sensation, and sudden pain attacks may feel like pins and needles. If women are perceiving these pains so differently, it would be surprising if their brain activity during these did not differ.

Resting state analysis is, as the name suggests, usually carried out in people who are at rest, not doing anything. Whilst this is not the first study to investigate resting state in a chronic pain population, it is important to note that this is not 'resting' per se. Arguably, as women in this study had an average background pain score of 4 out of 10 (ranging from 0 to 8) they are not 'at rest' but rather in a permanent pain state. Therefore, comparisons between chronic pain patients and pain-free controls needs to be carefully considered. Potentially, future work should investigate whether inducing a steady pain state in otherwise pain-free controls would be better for comparisons with a chronic pain population, as this would enable a more direct comparison of changes in networks due to a chronic pain state. Currently, it remains difficult to find a dataset which is a good comparison for chronic pain populations.

5.6 Conclusion

My results, when compared to task-based findings, highlight the difference between evoked and spontaneous pain. I have investigated the connectivity of brain areas shown to have, in response to a punctate stimulus, activity which was correlated with painDETECT scores[14]. However, despite my thorough analysis of the resting state data,

using a variety of different methods, I found no evidence for altered resting state connectivity related to the degree of neuropathic pain.

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Chapter 6: Resting state analysis based on neuropathic pain literature

6.1 Abstract

In this chapter I have investigated changes in functional connectivity of resting state networks in relation to painDETECT scores. I have found using seed-based analysis that the changes associated with painDETECT in the functional connectivity of the default mode network, salience network and descending pain modulatory network, which have been seen in studies in different patient cohorts, are not found in this cohort of women.

6.2 Background

As I introduced in the previous chapter, resting state connectivity can be a useful tool when investigating the central mechanisms which give rise to and maintain pain. There is growing evidence of central changes in neuropathic pain, including in resting state functional connectivity. This has been explored in a range of different chronic pain conditions, however there are still many gaps in knowledge. In my previous chapter I have taken regions of interest from stimulus evoked activity analysis to investigate the functional connectivity of these regions at rest.

For the purposes of this study, I have chosen to focus on seeds of known resting state networks, which have been shown to have an association with painDETECT. As resting state acquisition does not involve tasks or stimulus presentation, acquisition paradigms are often more similar and thus it is comparatively easier to replicate other's findings than in task-based fMRI. Therefore, I decided to use seeds which have been identified

in other studies of resting state functional connectivity for the analysis of different rest state networks.

Resting State Networks

A resting state network is a set of brain areas which when fMRI data is collected at rest, have similar BOLD timeseries. Whilst currently we do not have a full understanding of the resting brain's structure of networks, there are some networks which have been identified in the literature[1–6]. The default mode network (DMN)[1,7–9] is probably the most well-known of these.

The DMN consists of brain regions including the posterior cingulate cortex, precuneus, medial prefrontal cortex, inferior parietal lobe and lateral temporal cortex[1,7–9]. These areas reliably show a decrease in activity when performing a task compared to at rest (i.e. these areas show deactivations when performing a task). Recently, evidence has shown that increased activity in this network is seen during tasks involving mind wandering, auto-biographical, future thinking and episodic and semantic memory[10]. There has been found to be altered connectivity of the DMN in chronic pain[11], as well as, depression, Alzheimer's disease, attention deficit and hyperactivity disorder, drug addiction and epilepsy[10]. Changes in the connectivity of the DMN has been seen in both chronic and acute pain, leading some researchers to argue it is the experience of pain which alters connectivity, rather than changes being specific only to long term pain, in which there is more time for plasticity changes. It has been suggested that the changes seen in the DMN may reflect a theorised function of 'pain monitoring' as well as underpin the phenomenon of pain leading to a reduction in the ability to attend to particular tasks[11].

The salience network is another resting state network which has been investigated in relation to chronic pain. Whilst the essential functions of the salience network are uncertain, it is understood to be involved in information processing and how both internal and external stimuli are represented and their emotional weight[12]. The salience network includes brain regions including the cingulate, medial prefrontal cortex, anterior insula and cerebellum[13]. A number of neuroimaging studies have reported changes in the salience network in individuals with chronic pain[14] which may explain lack of habituation seen in the context of chronic pain[15,16]. Evidence has led researcher David Borsook to state "chronic pain may be considered, at least in part, as a condition of altered responsive salience"[16].

There are many areas of the brain which have been shown to have involvement in pain processing. This is not surprising considering the variety of processes which are involved in pain perception (attentional, emotional, somatosensory etc.). The 'cerebral signature of pain'[17] broadly covers areas involved in both bottom-up and top-down processing. This top-down processing, the descending pain modulatory system, includes the periaqueductal gray (PAG), prefrontal cortex, anterior cingulate cortex (ACC), insula, amygdala, hypothalamus and rostral ventromedial medulla (RVM). These areas have been shown to be involved in both the placebo and nocebo effects because of their role in the descending endogenous pain networks, with some of these known to be key regions involved in opioid release during placebo analgesia[18].

When investigating resting state networks in chronic pain conditions these networks are of special interest since, as discussed, their nodes' associated functions cover many facets of pain processing, including somatosensory, attentional, affective and self-monitoring aspects.

Investigations of these networks in neuropathic pain

Whilst there are many papers investigating these networks in chronic pain, I have chosen to highlight four papers which have investigated these networks in neuropathic pain.

Segerdahl et al 2018[19] investigated resting state connectivity of the ventrolateral PAG (vlPAG), an area within the descending pain modulatory system (DPMS), in patients with diabetic neuropathy. They recruited a group of patients with painful diabetic neuropathy and a control group of patients with diabetic neuropathy but no pain. Due to its role in the DPMS, they hypothesised that vlPAG would have altered functional connectivity in patients with painful diabetic polyneuropathy compared to the control group. One of their key findings was that there was enhanced vlPAG functional connectivity to several areas in those with neuropathic pain when compared to the painless group as a function of the background pain. These areas included other regions within the DPMS, such as the hypothalamus, nucleus accumbens (NAc), caudate, thalamus, and rostral anterior cingulate cortex (rACC). They used whole-brain seed-based analysis for this study, with the vlPAG seed from Ezra et al. 2015 and Faull and Pattinson 2017.

Soni and colleagues also investigated the descending pain modulatory network, within a cohort of patients with knee osteoarthritis[20], which like endometriosis is not traditionally thought of as a neuropathic pain condition. They investigated the static functional connectivity of the rostral anterior cingulate cortex (rACC) and rostral ventromedial medulla (RVM), both of which are within the DPMS; they also investigated the static functional connectivity between the RVM and the nucleus accumbens (NAc) (part of the reward system) as there is evidence that abnormal

connectivity in the reward system can contribute to chronic pain[21–23]. Whilst whole-brain seed-based methods did not find any significant differences between nociceptive and neuropathic groups, analysis of the static functional connectivity between the seeds found that differences between the RVM and rACC as well as between the RVM and NAc were greater in the nociceptive group than the neuropathic group.

Bosma et al 2018[24] investigated functional connectivity between the three networks in a cohort of participants with multiple sclerosis (MS) and healthy controls. They used seeds for each of the three networks above as well as the ascending nociceptive pathway and investigated the connectivity between these seeds as a measure of the functional connectivity between these functional networks. For the salience network they used the right temporoparietal junction (rTPJ); for the DMN they use both the posterior cingulate cortex (PCC) and the medial prefrontal cortex (mPFC); for the descending pain modulatory network they use the PAG; and for the ascending nociceptive pathway they use the postcentral gyrus (S1). They used painDETECT to subgroup the MS group into those with nociceptive pain and those with mixed/neuropathic pain using the standard cut offs. They looked at both static and dynamic functional connectivity between these networks. Static functional connectivity has been most commonly used in the resting state literature but there has been recently an emergence of dynamic functional connectivity which reflects the idea that brain communication is not always constant (static) and can fluctuate between different states[25–30]. They found that there was a significant difference in the static functional connectivity between the salience network and the default mode network when comparing the MS group to the healthy controls, which was primarily driven by the mixed/neuropathic subgroup.

Sag et al 2019[31] also investigated the connectivity between networks using several seeds of known regions of interest. They compared groups of patients with different neuropathies to determine whether clinical differences were reflected in the static functional connectivity of these networks. They used 12 seeds for this analysis: left cingulate cortex, right cingulate cortex, left anterior cingulate cortex, right anterior cingulate cortex, left parahippocampal cortex, right parahippocampal cortex, medial prefrontal cortex, posterior cingulate cortex/precuneus, left insular cortex and right insular cortex. They found significant differences in the functional connectivity between patients and healthy controls, as well as differences between those with painful diabetic neuropathy and those with hereditary neuropathies.

My aims of this chapter are to investigate the association between painDETECT score and the static functional connectivity of the salience network, DMN and DPMS. I will do this using seeds for the relevant networks, as have been described in the literature in neuropathic pain.

6.3 Methods

6.3.1 Data collection and pre-processing

The study data discussed in this chapter, is from the same cohort as the previous chapter. Data collection and pre-processing stages described in Chapters 2 and 3 also apply for this chapter.

6.3.2 Resting state fMRI group level analysis

As previously discussed in Chapter 2 there are several approaches to resting state functional connectivity analysis. I have in this chapter focused on seed-based analysis, carrying out both whole-brain and between- seed analysis (see Table 1 for seeds used).

Due to the wide range of painDETECT scores and moderate sample size in this cohort, my analyses focus on correlating with painDETECT as a linear scale, rather than grouping participants into nociceptive/mixed/neuropathic painDETECT groups.

Seeds within DPMS

When replicating the work of Segerdahl et al I have used their seed for the vlPAG (obtained through personal correspondence, transformed into 2mm 3T space). With this seed I carried out whole-brain seed-based analysis using dual regression with a GLM design matrix with painDETECT as a covariate and background pain scores as a covariate of no interest (more information on dual regression and GLMs can be found in Chapter 2).

Using seeds from Soni et al 2019 for RVM, left nucleus accumbens and rACC I investigated both whole-brain static functional connectivity and seed-to-seed connectivity. I obtained these seeds through personal correspondence with the authors. I ran dual regression on these seeds to investigate whole-brain connectivity of these regions correlating with painDETECT score. I also ran analysis with FSLNets to determine the connectivity between the regions of interest, again correlating with painDETECT score.

Cross network static functional connectivity

I created seeds for the rTPJ, PCC, mPFC, S1 and PAG with 8mm spheres (2mm sphere for PAG) centred at given coordinates as described in Bosma et al[24] (see Table 1). Timeseries for the seeds for each participant were extracted using dual regression. The next steps of analysis were carried out in MATLAB as per Bosma et al. For each participant, rho correlation coefficients and p values were then calculated for each pair of interest (rTPJ & PCC, rTPJ & mPFC, rTPJ & S1 and rTPJ & PAG) and rho values were subsequently z-transformed. As in Bosma et al, the connectivity of the rTPJ to the PCC and mPFC were averaged to give one score for the connectivity between the DMN and salience network. I calculated the correlation between the connectivity of the networks and painDETECT scores (Pearson's correlation).

I created 12 seeds from the description in Sag et al's paper, with 6mm spheres around the reported coordinate (left cingulate cortex, right cingulate cortex, left anterior cingulate cortex, right anterior cingulate cortex, left parahippocampal cortex, right parahippocampal cortex, medial prefrontal cortex, posterior cingulate cortex/precuneus, left insular cortex and right insular cortex), as shown in Table 1. These were then used in a dual regression to extract the timeseries of each seed, which was in turn entered into FSLNets to determine the connectivity between the seeds. p values reported are corrected unless otherwise stated.

A summary of the relevant seeds from these studies can be found in Table 1.

NETWORKS	AUTHORS, PAIN CONDITION, PARTICIPANTS (MALE:FEMALE)	SEED(S) (MNI X,Y,Z COORDINATES OF CENTRE)
DPMS	Segerdahl et al. Diabetic neuropathy (painful vs painless) n=26 (20:6)	Ventrolateral periaqueductal gray (x=1, y=-30, z=-10)
DMN, SALIENCE & DPMS	Sag et al. Painful diabetic neuropathy, diabetic controls, hereditary neuropathy and healthy controls Pain patients n=20 (11:9); Healthy controls n=18 (age and gender matched); Diabetic controls n=7 (3:4)	Left cingulate cortex (x=-13, y=-55, z=9) Right cingulate cortex (x=17, y=-55, z=8) Left anterior cingulate cortex (x=-4, y=19, z=18) Right anterior cingulate cortex (x=7, y=19, z=18) Left parahippocampal cortex (x=-32, y=-27, z=-24) Right parahippocampal cortex (x=33, y=-27, z=-25) Left dorsolateral prefrontal cortex (x=-49, y=41, z=10) Right dorsolateral prefrontal cortex (x=54, y=42, z=8) Medial prefrontal cortex (x=0, y=48,z=-4) Posterior cingulate cortex/precuneus (x=-6, y=-52, z=40) Left insular cortex (x=-40, y=-5, z=7) Right insular cortex (x=43, y=-5, z=5)
DMN & SALIENCE	Bosma et al. Multiple sclerosis pain and healthy controls Patients n=31 (20:11); Healthy controls n=31 (age and gender matched)	Right temporoparietal junction (x=62, y=-36, z=28) Posterior cingulate cortex (x=-8, y=-50, z=28) Medial prefrontal cortex (x=-2, y=56, z=16) Postcentral gyrus (S1) (x=-1, y=-40, z=62) Periaqueductal gray (x=0, y=-32, z=-10)
DPMS	Soni et al. Knee osteoarthritis (nociceptive (painDETECT score <13) vs neuropathic) Nociceptive n=10 (7:3); Neuropathic n=14 (6:8)	Rostral ventromedial medulla (x=0, y=-35, z=-46) Rostral anterior cingulate cortex (x=0, y=46, z=2) Nucleus accumbens (x=-10, y=10, z=7)

Table 1: Brief description of samples and seeds regions of the four studies replicated in this chapter.

6.4 Results

6.4.1 General results

Features of the cohort such as descriptions of pain relevant variables have been reported in Chapter 3 (C3.3.1). When painDETECT is used as a grouping variable, the cohort n=26 is split into the nociceptive group n=7, mixed n=13 and neuropathic n=6. Therefore, analysis has focused on using painDETECT as a linear scale rather than a grouping variable.

6.4.2 DPMS

Dual regression analysis showed no significant connectivity of the vlPAG[19] which correlated with painDETECT score at a whole brain level.

Whole-brain analysis using dual regression also found no connectivity correlated with painDETECT score for the RVM, rACC or nucelus accumbens[20]. FSLNets showed no connectivity between the regions of interest which was significantly correlated with painDETECT score (see Table 2).

	FULL CORRELATION	**PARTIAL CORRELATION**
RACC-RVM	Z=0.8838, (p=0.7354)	Z=-0.2386, (0.9328)
RVM-NAC	Z=5.2014, (p=0.905)	Z=5.4193, (p=0.9372),

Table 2: Results from FSLNets analysis of connectivity between rostral anterior cingulate cortex (rACC), rostral ventromedial medulla (RVM) and nucleus accumbens (NAc).

6.4.3 Connectivity between salience network, DMN and DPMS

Using seeds for the rTPJ, mPFC, PCC and S1[24] I found no cross-network connectivity which was significantly correlated with painDETECT score. For connectivity of the DMN and salience network (rTPJ and mPFC/PCC): rho=-0.0729, p=0.7234. For the connectivity of the DMN to the ascending nociceptive pathway (rTPJ to S1) rho=-0.3567, p=0.0737. For the connectivity of the DMN to the descending modulatory pathway (rTPJ to PAG) rho=-0.1716, p=0.4020.

I found that static functional connectivity between the right ACC and the right parahippocampal cortex was negatively correlated with painDETECT score (full correlation gave Z=4.3096, p=0.0366). I found no other connectivity between the 12 Sag et al[31] seeds which correlated with painDETECT score.

6.5 Discussion

6.5.1 Overview

Overall, I found that the patterns of altered functional connectivity of the DPMS, DMN and salience networks identified in patient groups with different types of neuropathic pain could not be replicated in my sample of patients with endometriosis-associated pain.

6.5.2 DPMS

My analyses show that there was no significant connectivity of the vlPAG that correlated with painDETECT score. This contrasts with the findings of the paper by

Segerdahl et al[19] that found, in patients with neuropathic pain, the vlPAG had increased connectivity to several areas known to be involved in pain processing. Their control group consisted of individuals with a neuropathy but no pain. They therefore compared neuropathy patients with and without additional pain, whereas in my sample all participants experienced pain but the degree to which their pain was neuropathic in nature varied between them.

My whole-brain analyses of functional connectivity of the RVM, rACC or left accumbens found no significant correlations with painDETECT scores. Similarly, connectivity between these regions did not scale with painDETECT scores at a significant level. Whilst my whole-brain analysis mirrors the findings of Soni et al[20], they found a significant difference in the connectivity between RVM and rACC and between RVM and NAc which were both greater in the nociceptive group than in the neuropathic group. The method employed in this study is very similar to the approach taken by myself in the previous chapter, with seeds being created from results from a pain-evoking task paradigm. The areas responding to painful stimuli in the study by Soni et al differ from those found by Vincent (discussed in Chapter 4). This could suggest that in these two conditions, there are different neural mechanisms underpinning the response to neuropathic pain which is evoked by a pinprick. Therefore, it may not be surprising that I have different findings in terms of the connectivity of regions at rest, during background pain.

6.5.3 Between network connectivity

Using seeds defined in Bosma et al[24], I found no significant correlation between painDETECT score and the static functional connectivity between the DMN and salience, DMN and ascending nociceptive or DMN and descending modulatory

networks. This is not greatly different to the findings of Bosma et al, as whilst they found significant differences between the healthy controls and the pain participants, they did not find statistically significant differences between the nociceptive and mixed/neuropathic groups.

When investigating the connectivity between the seeds from Sag et al[31], I found that there was a significant negative correlation between painDETECT and the connectivity of the right ACC and right parahippocampal gyrus. Connectivity between both brain regions was lower the higher patients scored on the painDETECT questionnaire. This is interesting as the ACC is a core brain region within the DPMS[18] and is known to be active during acute painful experiences[17], whereas parahippocampal gyrus has been implicated in the emotional augmentation of pain[32–35]. Potentially, a decrease in the connectivity of these regions with higher painDETECT scores could mean that there is dysfunction in processing of pain, such that the impact of the emotional value of pain is lesser in those with more neuropathic pain. Future studies which can investigate differences specifically between the painDETECT groups should also include a control group with no pain as it would be useful to determine whether this change is only present in those with neuropathic pain, or whether there is a linear correlation between the connectivity and painDETECT, as is suggested by my results.

6.5.4 Differences between my cohort and others discussed

As can be seen in Table 1, there are differences in both the pain condition and the study cohorts between my data and the other studies discussed. As such, I cannot say with certainty why my findings do not match with the findings from the studies I have replicated, as there are several possibilities.

Overall, my results from this chapter (and Chapter 4) lead me to believe that there are differences in the underlying pathology of neuropathic pain in endometriosis compared with other conditions such as osteoarthritis and diabetes. Given the data from this and previous chapters, I believe that chronic neuropathic pelvic pain in women differs in its mechanisms and features to other neuropathic pain conditions.

Sex/gender differences in functional connectivity

Another reason could be due to the fact my cohort is entirely female, whilst the studies focused on in this chapter included female as well as male participants, and do not report sex differences in their respective outcome parameters.

There is ever increasing research interest in sex and gender differences in neuroscience and there have been several studies looking at differences in resting state functional connectivity specifically. Whilst there is not room enough in this thesis to discuss this area in great detail, it is important to acknowledge these when comparing my results in a purely female population to studies carried out in both men and women. As Fauchon et al.[36] summarise, studies of chronic pain which do not examine sexes separately are at risk to miss important findings, as several studies have shown differences between the sexes in resting state connectivity of both healthy controls and chronic pain populations. A systematic review published in 2017[37] investigating sex-based differences in brain alterations across chronic pain conditions, provides strong evidence that future research needs to consider sex differences more carefully. They found that across chronic pain neuroimaging manuscripts, manuscripts looking at sex difference accounts for only 3.6%, mainly in Irritable Bowel Syndrome (IBS) and migraine. Overall, across pain conditions, there are sex differences in the brain. This includes: in IBS, men have increased connectivity of the dorsal anterior insula with the medial prefrontal cortex

(which correlated with visceral sensitivity index)[38]; in migraine women have increased dysfunctional connections between areas including the orbital prefrontal cortex, putamen and caudate[39]. In another study investigating functional connectivity of pain-related brain structures in healthy controls, Dai et al. found significant gender differences[40]. They found specifically that the dorsal dysgranular insula showed increased connectivity with structures located in the right cingulate cortex in males compared to females[40]. Sie et al 2019 found that in early adulthood females had stronger negative connectivity between the medial default mode network and both the posterior mid-cingulate gyrus and right insula, when compared to males[41]. A study investigating the functional connectivity of the subgenual ACC found increased connectivity to both the DMN and sensorimotor networks in women, but not men, with chronic low back pain[42]. Overall, this highlights that there are significant differences in functional connectivity between men and women in chronic pain, therefore studies investigating functional connectivity should consider this in their analysis and potentially report separate analyses for the sexes.

In summary, I believe that one of the reasons for the differences in my work to the other studies discussed in this chapter is that my cohort is entirely female and therefore, I find different functional connectivity than studies in both sexes. Most types of chronic pain are the result of both peripheral and central changes, but the relative significance of the two varies between different syndromes. There are mechanisms by which both central and peripheral changes occur in endometriosis (discussed in more detail in Chapter 1), but the relative contribution of these in women with endometriosis-associated pain is currently unknown and will probably differ on an individual basis.

6.5.5 Strengths & Limitations

As I have discussed, one limitation to my study is that the range of painDETECT scores did not allow for comparisons between painDETECT groups (when painDETECT is used as a grouping variable the cohort n=26 is split into the nociceptive group n=7, mixed n=13 and neuropathic n=6). Other studies described here made comparisons between the nociceptive and mixed/neuropathic group. Whilst in my study the three groups had relatively similar numbers, there were too few in each group to make meaningful comparisons between them and a combination of the mixed and neuropathic group (as seen in Bosma et al[24]) would lead to very unbalanced comparisons. Thus, it could therefore be that while for most analyses I did not find a significant correlation with pain DETECT scores, a group comparison would have reached significance.

Another factor which could explain my non-significant results could be that my study sample is not large enough. However as discussed in the previous chapter, a sample size of n=26 is comparable to those of other studies (n=24 in osteoarthritis[20], n=20 in hereditary and diabetic neuropathies[31], n=30 in diabetic neuropathy[19], n=31 in multiple sclerosis pain[24]) with significant findings.

6.5.6 Future Directions

More work is needed to understand the neural underpinnings of neuropathic pain in endometriosis. Whilst the evidence presented here and in Chapter 4 suggests that there is no difference in the brain's functional connectivity in women with higher painDETECT scores, other aspects of brain function (or structure) which were not investigated as part of this thesis may in fact be altered. Future studies should investigate the dynamic functional connectivity of this cohort with regards to painDETECT score as this method may provide relationships which are not picked out by static functional connectivity[25]. There is growing evidence that brain communication

across regions is intrinsically dynamic[25–30], and findings suggest that flexibility may be related to pain-coping ability[43]. Therefore, the use of dynamic functional connectivity may offer new insights into the understanding of chronic pain.

Further work is also needed to compare the development and maintenance of EAP with and without a neuropathic component. A more detailed investigation of the differences between both subtypes could provide valuable insights which could aid the development of more targeted treatment options.

Future work looking at the neural mechanisms underpinning the therapeutic effects of pain medications may in future allow treatments to be chosen that reverse this change which may then provide relief. Currently, this seems a far-away target but findings such as mine, whilst not ground-breaking, can add to providing a better understanding of what changes occur in the brains of people with neuropathic pain.

Moreover, future studies should explore neural mechanisms underlying different subtypes of neuropathic pain (e.g., evoked pain, spontaneous pain attacks (like 'electric shocks') and background pain). Whilst work in the field currently has employed techniques to investigate all of these types of pain, I think it is also necessary to determine similarities and differences between them and whether treatments work for specific aspects of the pain phenotype.

6.6 Conclusion

In my analysis I have not been able to confirm previous findings from other types of chronic pain. This highlights the need for neuropathic pain in endometriosis to be further explored so that any changes to central pain processing can be better understood.

6.7 Bibliography

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Chapter 7: Quantitative Sensory Testing (QST)

7.1 Background

Quantitative Sensory Testing (QST) is a psychophysical method of testing the function of the somatosensory nervous system in a given location. It does this by use of a set of calibrated stimuli and participant report of sensory perception. Understanding the patterns of sensory signs and symptoms can aid in understanding the possible neurobiological mechanisms giving rise to the pain. These individual somatosensory profiles, "the mosaic of hyperalgesia, allodynia, and sensory loss" have been shown to vary in different aetiologies and on an individual basis[1,2].

QST has been used both clinically and in research to assess the somatosensory profiles of individuals[3]. The QST paradigm I used was developed by the German Neuropathic Pain Network, DFNS[4]. It comprises a series of standardised tests to assess the detection and pain thresholds for different types of somatosensory stimuli, including hair-like von Frey filaments, needle stimulators (pinpricks) and thermal stimuli. These different stimuli relate to specific neuroanatomical pathways with separate nerve fibre populations. For example, heat and cold stimulation test C and Aδ fibres, whereas von Frey filaments test Aß fibres. The paradigm involves testing two sites, one where the person experiences pain/symptoms and another 'control' site without such symptoms. This can be on the contralateral side if symptoms are for example on the hand or leg. The QST stimuli are physically defined quantities (e.g. 9mN for a pinprick, 32°C for the thermode) but the report from the participant/patient is of course subjective. This allows comparisons between individual responses to physically identical stimuli both between individuals as well as between the test and control site in one person.

QST has been used in a variety of different diseases/conditions (including non-neuropathic pain) as well as in healthy inviduals[3]. There is evidence that different conditions have different sensory profiles, for example, in radiculopathy there is evidence that patients exhibit large loss-of function in vibration detection, whereas this is seen to a lesser degree in carpal tunnel syndrome[5]. There has also been studies showing that across several aetiologies, distinct clusters of sensory profiles can be found[6]. On a test cohort of n=902 (with n=233 validation cohort) they found there were three clusters on the basis of QST measures. These were mainly characterised as 1) mechanical and thermal sensory loss ('sensory loss'), 2) preserved sensory function with cold heat or cold hyperalgesia ('thermal hyperalgesia'), and 3) loss of thermal sensation with mechanical hyperalgesia or allodynia ('mechanical hyperalgesia')[6]. Since this finding an algorithm has been defined to determine on an individual basis which cluster an individual best falls into (three groups described as well as a fourth 'healthy' category in which sensory profile best matches that found in the pain-free population)[7].

QST has been used extensively to investigate neuropathic pain and possible underlying neurobiological mechanisms have been suggested[8]. One such mechanism is deafferentation pain which is defined as pain due to loss of sensory input into the central nervous system[9]. This can occur when there is an injury to the dorsal root which means second order neurons cannot receive anatomical/physiological input from the afferent neurons[5]. Another mechanism is peripheral sensitization, which occurs when the threshold for activation in the nociceptor terminal in decreased, one way this can arise is by inflammatory mediators triggering an increase in the insertion of ion channels into the membrane[10]. Lastly, central sensitization can be hypothesised from QST responses, which as the name suggests is due to changes in synaptic transmission and plasticity in the spinal cord and/or cortex[10]. QST measures have shown that some people with

neuropathic pain show gain of function, such that they report greater sensation (lower thresholds/larger pain scores) suggesting allodynia (pain due to a stimulus that does not normally provoke pain) and hyperalgesia (increased pain from a stimulus that normally provokes pain), whereas others show loss of function, such that they have relative 'numbness' of the area and do not feel sensations that a healthy person might. Both of these can also be seen in the same person, such that they may have gain of function in certain pathways (in response to certain stimuli) and loss of function in others.

In this chapter I carry out some pilot analysis of QST data and I aim to:

- Make comparisons between our data in women with endometriosis-associated pain and DFNS reference data from healthy controls.
- Compare data from the test site to the control site in the group
- Investigate how QST measures relate to painDETECT measures in this cohort.
- Use algorithm to stratify individual participants into previously reported subgroups based on sensory profile

7.2 Methods

7.2.1 Data collection

Participants were recruited as part of the Translational Research in Pelvic Pain (TRiPP) study[11]. The TRiPP study aims to investigate chronic pelvic pain, specifically bladder pain syndrome and endometriosis-associated pain. One of the aims of the TRiPP study is to better phenotype pelvic pain in women so that better animal models can be identified. Whilst this is a multicentre study, all data discussed in this chapter was collected in Oxford at the Women's Centre of the John Radcliffe Hospital. Recruitment took place from January 2020 until March 2020 when the study was suspended due to COVID-19. The number of participants is therefore lower than originally planned. All

participants were recruited from previous endometriosis study participants who had pelvic pain symptoms with surgically confirmed endometriosis and who had consented to be contacted again for future work. Inclusion criteria were: female, aged 18 – 50 years, willing and able to give informed consent, at least one pelvic pain (i.e. dysmenorrhea or dyspareunia) scored as more than 3 out of 10; women who were pregnant, lactating or planning pregnancy or women who had any (other) serious disease or disorder which may have put them at risk or influenced the results were excluded. Participants were asked to take part in three study sessions (one of which was QST) which could be completed at separate times or in one combined session (as convenient for participant). All QST sessions were carried out in an air-conditioned room at approximately 20°C. Participants were also asked to complete a battery of questionnaires, which included painDETECT as well as other validated questionnaires and pain measures. Before the session participants were also asked to complete a "How are you today?" questionnaire which asked them specific questions about their pain/mood at the time of the session so these could be controlled for in further analysis, as well as questions about medications taken that day.

The DFNS protocol which is used in this chapter is highly standardised, more details of this protocol can be found in Chapter 2.

7.2.2 Data analysis

Please see Chapter 2 for analysis methods.

To relate QST measures to painDETECT scores, correlation analyses were carried out. Normality tests were carried out on each painDETECT sensation variable (i.e. those scored out of 5, excluding question on time course and spatial properties of the pain).

For normally distributed variables Pearson's correlation was used; non-normal measures non-parametric Spearman's correlation was used. Each QST variable was correlated against each sensory painDETECT variable.

Multiple comparison correction was not used for these tests as it is pilot data.

I used this pilot data in a power calculation to determine the total sample size needed to identify significantly different QST profiles to the healthy control reference cohort. For this I used a power value of 0.8 (80%) and a significance threshold of $p<0.05$. Participant z-transformed QST scores for all variables were averaged to give an overall effect size.

7.3 Results

7.3.1 General results

Due to the suspension of the study (COVID-19), I was only able to test N=9 patients in the study. This small sample showed large variability in their sensory profiles.

Individual QST profiles

Figure 1 shows the sensory profile of each participant across all tests. Any score outside of the grey area on the graph is considered pathological. The graphs highlight the large variability seen in my sample. In some participants the results for the test site and control site are very similar (see A, G and I) whereas others show marked differences between sites. For example, one participant (Figure 1C) exhibited a significant loss of function for WDT at the test site, but a slight gain of function at the control site.

Similarly, another participant (Figure 1E) showed a large gain of function in MPS at the test site, indicating hyperalgesia, but a normal result at the control site.

QST measures

Figure 2 shows results for each participant by test. For WDT, CPT, HPT, MPT, MPS, WUR, PHS, results were largely within the normal (with at least 7 of the 9 participants). In contrast, for TSL results for both sites, for all participants are below the norm, showing that these women have a loss of function and are less sensitive to temperature change than the healthy controls. Equally, the pressure pain thresholds for both sites, our participants were above the norm, suggesting that they had gain of function and lower pain thresholds. As you can see from Figures 2H & 2M, some of our participants experience hyperalgesia/allodynia; for MPS (2H) one participant shows gain of function at the test site, but not the control site; DMA (2M) shows that there are two participants have scores above the norm.

7.3.2 QST comparison between the test and control sites

Using Student t-tests I compared the z-transformed scores for the test and control sites. I found a significant difference in CDT ($p=0.014$) and PPT ($p=0.002$). The CDT showed greater loss of function at the test site, compared to the control site (as can be seen in Figure 2A). For PPT the test site exhibited lower thresholds (allodynia), showing a greater gain of function than the control site (as seen in Figure 2K).

7.3.3 QST comparison to norm data of healthy participants

Using the adapted t-test described by Magerl and collegues[12], I found significant differences in our data compared with the healthy controls reference data. Participants' TSL were significantly different from the norm data at the test and control both sites (for control site $t(16)=-9.46$ $p<0.05$, for test site $t(16)=-8.77$ $p<0.05$) (Figure 2C), for both sites all participants showed a clear pathological loss of function. CPT was significantly different at the control site only ($t(16)=-2.45$) (as can be seen in Figure 2D), with our participants having generally a loss of function, although all data stay within the 95% confidence interval from healthy controls (grey area on graph). CDT was significantly different at the test site ($t(16)=-3.17$) (Figure 2A), with our participants having a loss of function with some individuals outside the grey area. Similarly, for PPT (Figure 2K) for both the test and control sites, all participants show a significant gain of function (for control site $t(16)=16.12$, for test site $t(16)=13.39$). For MDT (Figure 2F) and VDT (Figure 2J) there is also significant differences at both sites (MDT at control site $t(16)=-3.2$, at test site $t(16)=-2.17$; for VDT at control site $t(16)=-2.63$, at test site $t(16)=-2.15$), generally there is a loss of function in our participants. For other measures there was no significant difference in comparison to reference data at either site.

7.3.4 QST correlation with painDETECT measures

As not all variables were normally distributed, I report non-parametric Spearman rank correlation coefficients to characterise the relation between (z-transformed) QST measures and painDETECT scores. As two of the participants have not yet completed painDETECT, my sample size for this analysis is n=7.

I carried out correlations between painDETECT measures and QST measures at the test site, as the painDETECT questions in this context will be in relation to their pelvic pain.

There was a positive correlation between WDT and mechanical allodynia (rho=0.757, p=0.049). I found significant negative correlations between CDT and prickling/tingling sensation (rho=-0.926, p=0.003); between MPS and prickling/tingling sensation (rho=-0.810, p=0.027); and finally, between VDT and numbness (rho=-0.820, p=0.024).

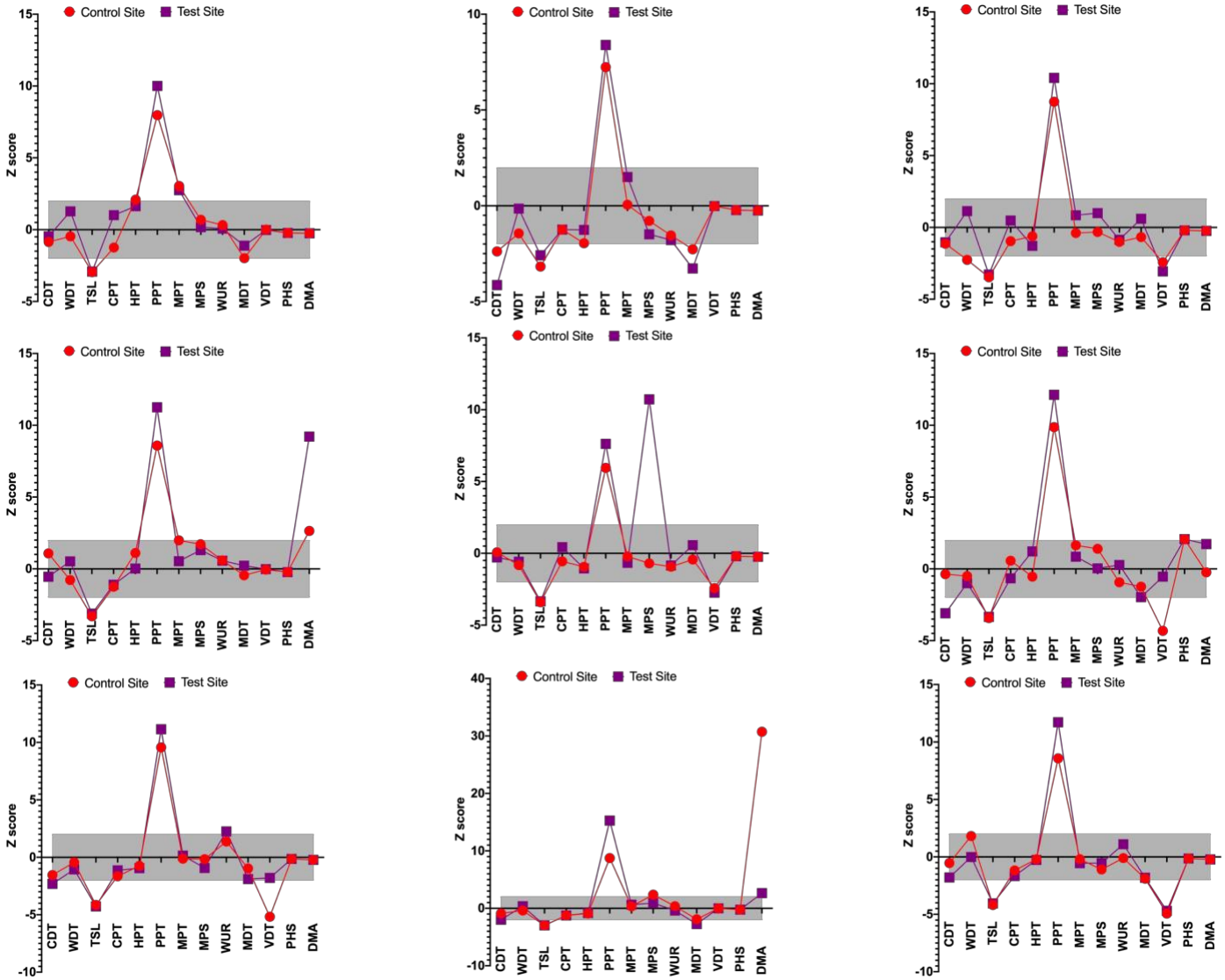

Figure 1: Individual QST profiles. The grey area shows the 95% confidence intervals from the reference data (-1.96 to 1.96). CDT = cold detection threshold; WDT = warm detection threshold; TSL = thermal sensory limen; CPT = cold pain threshold; HPT = hot pain threshold; PPT = pressure pain threshold; MPT = mechanical pain threshold; MPS = mechanical pain sensitivity; WUR = wind up ratio; MDT = mechanical detection threshold; VDT = vibration detection threshold; PHS = paradoxical heat sensation; DMA = dynamic mechanical allodynia

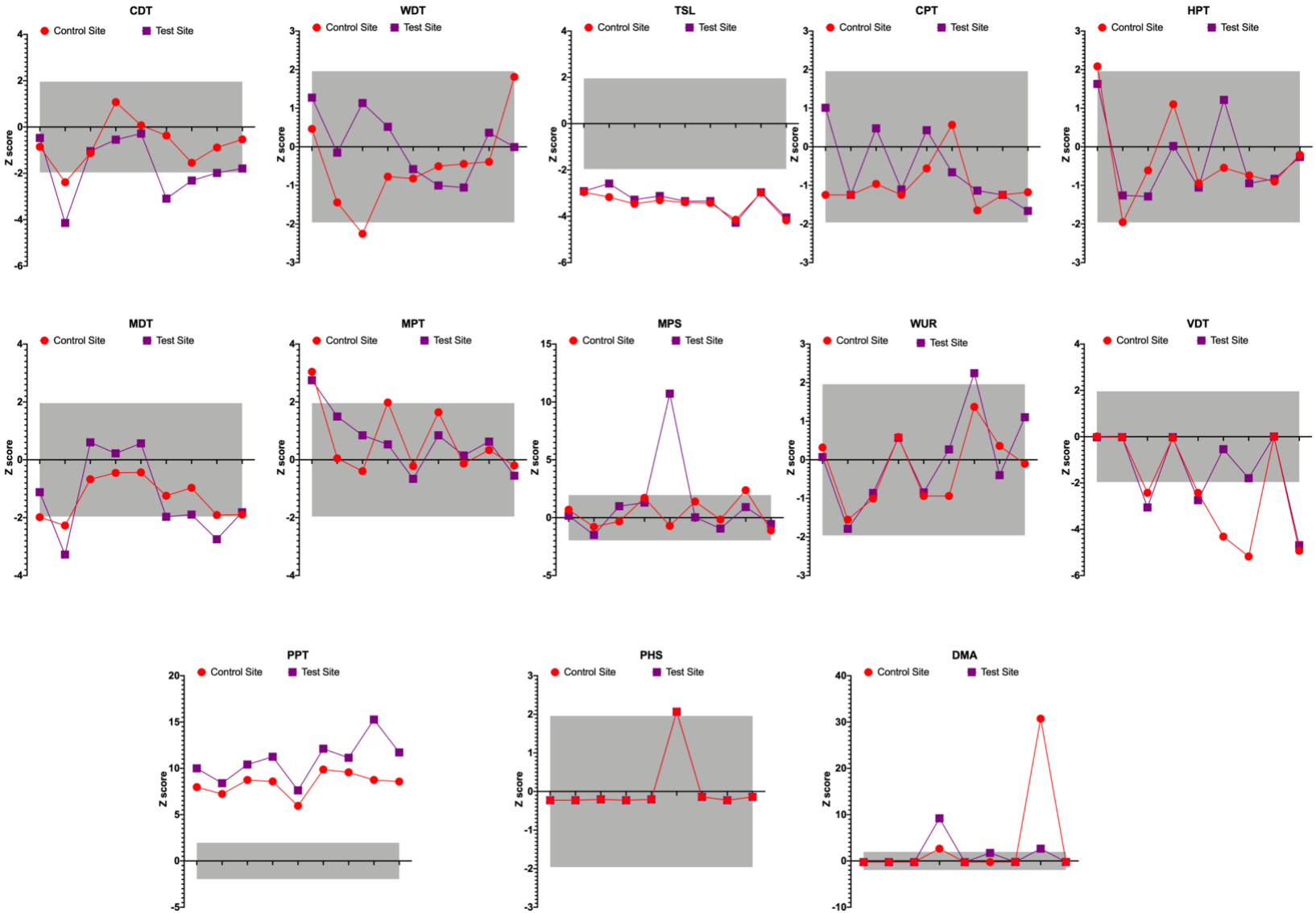

Figure 2: Profiles for each QST measure. Individual participants are represented on the x axis. The grey area shows the 95% confidence intervals from the reference data (-1.96 to 1.96). CDT = cold detection threshold; WDT = warm detection threshold; TSL = thermal sensory limen; CPT = cold pain threshold; HPT = hot pain threshold; PPT = pressure pain threshold; MPT = mechanical pain threshold; MPS = mechanical pain sensitivity; WUR = wind up ratio; MDT = mechanical detection threshold; VDT = vibration detection threshold; PHS = paradoxical heat sensation; DMA = dynamic mechanical allodynia

7.3.5 Algorithm-based stratifying on sensory profiles

I found that of the n=9 participants; n=2 were classified as having a 'healthy' sensory profile, n=2 were classified as 'thermal hyperalgesia', n=3 were 'sensory loss' and n=2 had 'mechanical hyperalgesia'.

7.3.6 Power calculation based on pilot data

Using the data available at this stage I carried out a power calculation to determine the sample size required to identify a significant difference in subjects compared to reference healthy controls. I found a sample size of 130 is needed for power of 0.803 (>80%) and significance level of $p<0.05$ using an effect size of 0.35, which is the mean z-transformed QST variable score for my cohort.

7.4 Discussion

7.4.1 Overview

This data, whilst preliminary, gives some interesting results. There is large variation between participants, which highlights the heterogeneity of this cohort, as is seen in other conditions.

7.4.2 QST measures: comparison with norm data

For CDT (Figure 2A) there is a significant difference between the test and control sites, with the test site having more loss of function with some participants falling outside the 95% confidence interval from healthy controls, suggesting that the symptom is

pathological. Decreased sensitivity for non-painful stimuli is hypoesthesia. One possible underlying neurobiological mechanism for hypoesthesia is deafferentation.

For WDT (Figure 2B) some participants have a marked difference between the test and control sites, for example participant 3 has gain of function at the test site and loss of function at the control site, whereas participant 9 had gain of function at the control site with normal function at the test site. Nearly all the data for WDT falls within the 95% confidence interval from healthy participants, which suggests that warm detection is not greatly different in our participants compared to healthy controls.

TSL is significantly different in our participants, compared to healthy control reference data (Figure 2C), in that our participants all exhibit loss of function at both the test and control site. This is interesting as their warm and cool detection thresholds are not different between participants and controls. TSL assesses how well participants detect changes in temperature (both warming and cooling), so this suggests that our participants are less able to distinguish these changes in temperature than healthy controls. As this is also a form of hypoesthesia, deafferentation could be the neurobiological mechanism.

CPT is, for all-bar-one participants, showing loss of function at the control site (see Figure 2D). At the test site there are much more variations between gain and loss of function. Participant 6 and participant 9 have lower Z scores at the test site than the control site; all other participants have lower scores at the control site. All participant responses fall within the 95% confidence interval from healthy control reference data, but the results from the control site are statistically significantly different from the 'normal' reference data as they are predominantly below the 'norm' Z=0.

HPT (Figure 2E) has nearly all data within the 95% confidence intervals from healthy control reference data. Some participants have differences between the test and control sites but these differences are not consistent across the group. For example, participant 4 shows gain of function at the control site, with 'normal' function (Z=0) at the test site, whereas participant 6 shows loss of function at the control site and gain of function (lower thresholds) at the test site.

Our participant results for MDT (Figure 2F) are significantly different to the reference data for both the test and control sites despite a lot of the results sitting within the 95% confidence interval. Generally, our participants show a loss of function, showing they have mechanical hypoesthesia, which could be the result of deafferentation. Interestingly some participants have greater loss of function at the test site (participants 2, 6, 7 and 8), whereas others have greater loss of function at the control site (participants 1, 3, 4 and 5).

MPT is again varied between participants (Figure 2G). Participant 1 has allodynic responses at both the control and test sites to a similar degree, both of which are considered to be pathological and could be the result of central sensitization or peripheral sensitization. In contrast, participant 2 shows an allodynic response at the test site and a 'normal' response at the control site. Participant 4 shows an allodynic response at the test site, with greater allodynia at the control site.

MPS (Figure 2H) for most participants falls within the 95% confidence interval from the reference data. However, participant 5 shows a very large hyperalgesic (gain of function) response at the test site, with a slight loss of function at the control site. Whilst increased sensitivity to pinprick stimuli could be the result of either peripheral or central

sensitization, the fact that this is only seen at the test site would suggest that this is potentially due to peripheral sensitization.

WUR (Figure 2I) shows very similar patterns for the test and control sites, especially for participants 1, 2, 3, 4 and 5. Again, most of the data falls within the 95% confidence intervals from the reference data. Participant 7 shows gain of function at the test site, outside the confidence intervals, with a lower gain of function at the control site. The WUR is calculated as a ratio of the pain score from a series of stimuli to the pain score from a single stimulus. This repeated noxious stimulus is associated with a progressive increase in perceived pain intensity. A gain of function in this measure shows that this normal increase is exaggerated. This enhanced wind-up is seen in post-herpetic neuralgia and central pain[1].

For VDT (Figure 2K) there is no gain of function, at either the test or control site. Some participants had significant loss of function (outside grey region of 95% confidence interval from reference data) at both the test and control site (participants 3, 5 and 9), whereas others had loss of function outside the grey region for only the control site (participants 6 and 7). One potential underlying neurobiological mechanism of this hypoesthesia is deafferentation.

For PPT (Figure 2L) all data were considerably above the maximum of the 95% confidence interval. This shows that these women had hyperalgesia at both their control and test sites, although for all participants greater hyperalgesia was seen at the test site. It is very interesting that this finding is so far out of the normal range. Increased sensitivity to blunt pressure suggests central sensitization[6]. Whilst there is currently no adequate evidence for the role central sensitization may play in PPT, the fact that findings across both sites are pathological may indicate alteration to central processing.

For PHS (Figure 2M) no log transformations are carried out. Whilst participant 6 has gain of function, it is important to put this score into the correct context. As this data is raw when it is Z transformed, results outside the grey region, on an individual level, need to be considered cautiously. When looking at the raw data, out of the 3 possible occurrences of PHS in the study, participant 6 experienced one (at both sites), whereas the other participants experienced none (at either site). As the reference data is collected from a large sample, the reference values are between 0.06 and 0.1. Since one would not expect any occurrence of PHS in a healthy participant, any number greater than 0 is considered pathological. Whilst it is interesting that one participant experienced PHS, more participants are needed to draw conclusions from the parameter especially.

DMA is an interesting measure as it assesses pain scores to stimuli that are non-noxious. Three participants exhibited gain of function at the test site (participants 4, 6 and 8). Interestingly, participant 8 showed even greater gain of function at the control site, showing very high levels of allodynia. For this variable there appears to be a relatively clear split between those with close to normal (Z=0) responses for both the test and control site (participants 1, 2, 5, 7 and 9) and those who display allodynia (at one or both sites). Generally, the presence of allodynia is indicative of central sensitization.

7.4.3 Comparison between test and control sites

Looking for differences between the test and control sites is an important in terms of QST findings. This is because if the test site is outside the norm, but the control site isn't, the pathology is very local, not widespread. However, if both sites have pathological scores, this could be a sign that the problem is more widespread, and potentially due to central alterations.

I found a significant difference in PPT and CDT between the control and test site. One explanation for this could be that there have been localised changes to nerve fibres at the test site which has altered the processing of pain. Interestingly, the CDT showed a loss of function at the test site, whereas the PPT showed gain of function. The PPT results suggest that they have a lower threshold for pressure pain at the test site compared with the control site at the group level, indicating they have hyperalgesia at this site. The CDT shows that they were, at a group level, less able to detect a change in temperature to 'cool', suggesting some loss of temperature sensation.

7.4.4 Group level statistical comparison to reference data

I found some measures were significantly different between both the test and control sites compared with the healthy control reference data: TSL, MDT, VDT, PPT. PPT is the only one of these which is a pain threshold measure, and our participants demonstrated a significant gain of function (hyperalgesia) at both the test and control site. The other measures are detection thresholds, and our participants generally showed loss of function, which is most prominent in TSL where for both the control and test site all our participants are below the lower 95% confidence interval limit.

For both TSL and PPT our data falls clearly outside the 95% confidence interval, which suggests that these measures are significantly different in our cohort in comparison to healthy controls, and it would be interesting to see if this pattern continues with the complete cohort.

7.4.5 QST correlation with painDETECT

It is useful to investigate the correlation between QST measures and self-reported measures, such as painDETECT as self-reported measures are, naturally, easier to administer both for research (enabling larger sample sizes) and clinically (especially given that specialised training is required for QST). One would expect there to be correlations between: painDETECT assessment of painful light touch and QST DMA; painDETECT painful cold or heat and increased sensitivity to thermal tests in QST; painDETECT variable light pressure triggering pain and PPT; and painDETECT measure of 'numbness' and a loss of detection (MDT, VDT, CDT, WDT) in QST. However, previously published work has investigated this and found in n=96 patients with chronic neuropathic pain that there was only a small to moderate concordance between expected pairings, with the greatest (but only moderate) associated between painDETECT painful light touch and DMA in QST[13].

I found a negative correlation between VDT and numbness, which would be expected given the relative scores (i.e., negative scores in VDT show loss of sensation/numbness, whereas greater painDETECT scores show numbness/loss of sensation).

Given the small number of participants (n=7) for this analysis it is not possible to draw strong conclusions. However, it is very intriguing that although QST measures correlated strongly with painDETECT measures, as expected, several of them are not as expected. QST of course measures responses to fixed stimuli, whereas painDETECT questions are about both spontaneous (un-stimulated) pain (do you suffer from a burning sensation?) and pain triggers (is cold or heat in this area occasionally painful?). It is interesting to see a correlation between spontaneous and provoked pain measures in these two types of assessment and it will be important to see whether these relationships are present in the full cohort.

7.4.6 Stratification based on sensory profiles

Cluster analysis carried out by Baron and colleagues showed that QST assessed sensory profiles can be used to classify patients into one of three groups[6,7], has since been used in other populations[5]. Such stratification of patients may reflect different neurobiological mechanisms causing pain and hence, different subgroups may respond differently to treatments. I have found that of my n=9 participants, n=2 are classified as healthy whilst n=3 fall into each of 'sensory loss', and n=2 into both 'thermal hyperalgesia' and 'mechanical hyperalgesia'. Whilst in a cohort this size it is not meaningful to calculate percentages in each classification, other conditions also see patients falling into each of these categories[5].

7.4.7 Strengths & Limitations

QST, unlike other methods of the somatosensory system (such as nerve conduction assessment), can detect both negative and positive sensory signs (i.e., both gain- and loss-of function) in a non-invasive way. It enables the assessment of the nervous system and can be used to hypothesise mechanisms, which is a key aspect of understanding chronic pain. Whilst QST has been utilised in many pain conditions[3], this is the first report of DFNS QST protocol being carried out in an endometriosis-associated pain cohort.

The clear main limitation of this analysis is the fact it is a pilot analysis only, as the full sample size has not yet been reached. My findings so far, whilst intriguing, therefore need to be interpreted with caution.

Another limitation is that due to the location of pain in this population it is not possible to use the contralateral side, whilst collaborators at the DFNS agreed that the foot was the best control site there may still be differences due to site. Significant differences between face, hand and foot have been described across QST parameters, for example in healthy controls for PPT the face has the lowest threshold, followed by the hand and finally the foot (which also showed the greatest between subject variation)[8]. This is not helped by the fact that there is no reference data for the abdomen to ensure there is comparability. Since the TRiPP study includes a pain-free control group, once the full dataset is acquired, the difference between the abdomen and foot can be better determined.

7.4.8 Future Directions

Once the full dataset is available for analysis it will be possible to repeat the analysis I have carried out, in several symptom groups. One of these groups will be healthy controls, which will enable a comparison between the test and control site to determine whether differences seen in this pilot analysis (and any found in the full analysis) are truly due to the presence of pain at the test site. We will also have bladder pain (with no endometriosis) participants and participants with pelvic pain with no bladder symptoms and no endometriosis. This will enable us to investigate whether there are differences in the QST profiles depending on pathology of pain or type of symptom (i.e. presence of bladder pain).

Whilst current pain ratings and current medications were available for these participants, due to this being a pilot study, these were not controlled for in analysis. It will be interesting to assess how these affect QST measures in women with pelvic pain,

although there is evidence that medication is not associated with relevant differences in sensory phenotypes[6,14].

It will also be interesting, with the full cohort, to determine the proportion of women falling into each of the cluster classifications, and to determine whether this differs between the patient groups we have as has been shown in different neuropathic pain conditions[5]. Further, a comparison of these clusters and those found in painDETECT (discussed in Chapter 3 and 4) would be of great interest to determine whether these are measuring the same phenomenon.

7.5 Conclusion

Whilst this data is preliminary analysis only, there are some very interesting findings which will be investigated further when the full dataset is available. I have found that women with endometriosis-associated pain have significantly lower pressure pain thresholds compared to age-matched healthy control women and also have reduced sensitivity to cooling of the skin. I have found some measures show similar patterns at both the control and test site and others differ significantly in this cohort.

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Chapter 8: Discussion

8.1 Thesis summary

In this thesis I have investigated the presence and characteristics of neuropathic pain in endometriosis.

In Chapter 3 I explored the prevalence of neuropathic pain in women with endometriosis in a large cohort using painDETECT and found that 40% of respondents were categorised as having neuropathic pain, with a further 35% having mixed neuropathic/nociceptive pain. Those with neuropathic pain had greater psychological and cognitive burden and had greater pain intensity scores. An increased number of surgeries to the abdomen was associated with neuropathic pain. When investigating the sensory symptoms profiles, I found that the most common symptom was sudden pain attacks (like electric shocks), with 79% of those with neuropathic or mixed pain experiencing them at a clinically significant level. I found that those with a neuropathic component could be subdivided into two groups on the basis of their symptom profiles.

In Chapter 4 I replicated these findings in a cohort of women with chronic pelvic pain of no known pathology. I found very similar clusters to be present in these women, validating my previous work and opening the door for future research into sex and gender differences in symptom profiles in neuropathic pain. In this chapter I also investigated whether those with neuropathic pain responded differently to gabapentin/placebo and whilst there was insufficient power, there appears to be a trend towards a relationship.

In Chapters 5 and 6 I studied the central nervous system, specifically looking at the resting state connectivity of the brain, with relation to painDETECT score. My results suggest that there are key differences in the processing of provoked and spontaneous/background pain when investigating neuropathic pain in this population. I was unable to replicate findings from other neuropathic pain conditions in my cohort, which again may be due to my population being entirely female.

In Chapter 7 I presented pilot data from QST assessment of women with endometriosis-associated pain. I found that the participants in our study had greatly reduced pain thresholds for pressure pain at both the test and control site but showed loss of sensation with regards to temperature change detection at both sites. The participants presented with a range of gain-of-function and loss-of-function responses highlighting the heterogeneity of chronic pain.

When taken together, the data presented in this thesis show that there is a neuropathic component in a proportion of women with endometriosis-associated pain. Whilst the mechanisms giving rise to and maintaining this pain need to be further investigated, I have discussed some key possibilities. My thesis has a large focus on better phenotyping endometriosis-associated pain, which has been done by a variety of methods.

8.2 Clinical relevance

Whilst endometriosis-associated pain syndrome is defined in the IASP taxonomy of pain[1], clinically endometriosis is still predominantly managed by gynaecologists and the pain symptoms considered to arise either from the ectopic tissue implants themselves or the inflammatory environment of the pelvis. Thus, current guidelines[2–4] all recommend

simple analgesics (non-steroidal anti-inflammatories[5]) and either hormonal suppression or surgical ablation/excision of the lesions. Pain management programmes are usually recommended only when treatment has failed and to date, no medication specifically targeting a neuropathic component (e.g. amitriptyline, gabapentin, pregabalin, duloxetine etc) has been trialled in endometriosis-associated pain. Many women with endometriosis-associated pain undergo repeated surgeries for either fertility indications or in the belief that pain symptoms may improve, at least temporarily. For instance, a recent retrospective study found that over half of the sample (n= 486) had undergone more than one surgical procedure related to their endometriosis in a 10 year period[6]. All these interventions are offered based on the understanding that endometriosis-associated pain is nociceptive in nature. However, my data indicate, in line with other conditions associated with chronic pain (e.g. rheumatoid arthritis[7–9], lower back pain[10–12] and bladder pain syndrome[13,14]), that there is a neuropathic-like component for a large proportion of women.

It is important to emphasise that neuropathic pain is not simply more intense or more widespread than nociceptive pain. There is ample evidence from studies in animals and humans showing that neuropathic and nociceptive pain differ considerably with respect to their underlying pathology[15–18] (although both types of pain often co-occur[19,20]), including differences in cognitive-affective processing[21–24]. Endometriosis-associated pain is well known to be associated with psychological distress, and reasons proposed for this finding include the impact on fertility, intimate relationships and role functioning, in addition to the difficult journey women undergo on the path to diagnosis. My finding that women with a neuropathic component to their pain not only experience greater psychological distress, but also more fatigue and cognitive impairment (even after controlling for pain intensity; Chapter 3) adds to this knowledge.

My findings have several direct implications for the diagnosis and treatment of endometriosis-associated pain. First, the high prevalence of neuropathic pain in a support group cohort (as discussed in Chapter 3) clearly argues for a routine assessment of a potential neuropathic component. The painDETECT questionnaire is a relatively short and easy to complete screening tool, which could be integrated into any standard gynaecology consultation.

Secondly, neuropathic adjunctive analgesics could be considered in the treatment of endometriosis-associated pain if a neuropathic component is confirmed, although at present efficacy in this patient group has not been demonstrated. In the future, treatment decisions may be guided by the symptom profile assessed by painDETECT or QST measures (adapted for bedside use), if these are shown to respond differently to treatments. My findings from GaPP2, the gabapentin trial in CPP (Chapter 4) highlight the need to carefully select the population for such clinical trials. Neuropathic adjuncts need to be trialled in women with endometriosis-associated pain but the sample should be selected to include only women with neuropathic-like characteristics (for example those in the neuropathic painDETECT group). This will take us from a 'one size fits all' approach and lead the way to more personalised treatments.

The relationship between the number of surgeries and a neuropathic component seen in Chapter 3 cannot determine causality; surgery could be causing damage to nerves which leads to neuropathic pain, or individuals with neuropathic pain may not respond to surgery and therefore may be offered repeated surgery with the aim to alleviate symptoms. This does however suggest that any decision about further surgical interventions should consider the role of neuropathic pain on a patient-by-patient basis and perhaps consider alternative treatment strategies (Chapter 3).

My findings, in Chapters 5 and 6 especially, indicate the need to investigate the differences between provoked and spontaneous pain in more detail. Clinically, it is important to know these differences and whether they respond differently to treatment options as well as which is most bothersome for the individual (i.e. which one should treatment target, if possible).

A better understanding of the full symptom profile of individuals is vital to move treatment options forward and to be able to offer personalised treatments. With a condition as heterogeneous as endometriosis, it is especially important to assess all aspects of pain symptoms including descriptors, triggers and time-courses.

8.3 Neuropathic or nociplastic pain

Nociplastic pain is a relatively new and somewhat controversial term, defined as "pain that arises from altered nociception despite no clear evidence of actual or threatened tissue damage causing the activation of peripheral nociceptors or evidence for disease or lesion to the somatosensory system causing pain"[31]. Whilst this definition requires the pain to not be either nociceptive or neuropathic in nature, the authors of this newly proposed term do suggest that nociplastic pain could occur alongside nociceptive pain[31]. This term has received mixed responses, with some people believing that there is no need for an additional pain term[32] and others agreeing with the need for a term but preferring 'centralized pain'[33] as a concept already used in pain research and understood by clinicians.

The definition of nociplastic pain requires no clear evidence of disease or lesion to the somatosensory nervous system, contrasting with neuropathic pain for which disease/lesion of the somatosensory nervous system is a requirement. For a diagnosis of

'definite' neuropathic pain a diagnostic test confirming such disease/lesion is necessary, similarly without carrying out such tests one could not say that pain is completely nociplastic as the absence of disease/lesion of the somatosensory nervous hasn't been shown. The symptoms that patients with nociplastic and neuropathic pain present with are overlapping, for example primary hyperalgesia to pressure[34]. Thus, currently it is not possible to definitively distinguish between neuropathic pain and nociplastic pain using questionnaires[34], and there has been suggestion that some questionnaires validated as screening tools for neuropathic pain actually may rather reflect nociplastic pain[35]. Currently, the suggested questionnaires to assess nociplastic pain are the Central Sensitization Index or the Fibromyalgia Scale[35], both of which assess aspects such as how widespread the pain is. In Chapter 3 I use the Fibromyalgia Scale alongside painDETECT, and find that there is not a strong correlation between the two, suggesting that in fact they are assessing different concepts as they were designed and validated to do. Also in this Chapter and later in Chapter 7 I find sensory alterations that are consistent with neuropathic pain and not nociplastic pain, such as numbness and loss of function responses as well as paraesthesia.

Therefore, whilst future studies may show some people with endometriosis have a nociplastic component to their pain, this does not take-away from my findings of neuropathic pain in this thesis. As has been apparent throughout my thesis, there are large heterogeneities in the pain experienced by these women. By using validated screening tools I have found that a proportion women have neuropathic pain, however it would not be surprising to me if nociplastic pain was present both in women with nociceptive and neuropathic pain in this population. Whilst currently contentious, I am yet to see justification as to why nociplastic and neuropathic pain could not be present in the same person, given knowledge about the chronification of pain and the central

changes that have been shown in peripheral neuropathic pain. Ultimately, as given the nature of pain, it remains difficult to determine what the origin and amplification source of pain is and whether continued pain would be the result of peripheral injury (to non-neuronal tissue or nerve) or alterations to the nociceptive pathway.

8.4 Challenges in endometriosis research

As with all investigations into pain perception, all data analysed here is subjective. Whilst there is a push for objective measures of pain, patient reports are arguably the most useful. Limitations to specific studies are discussed in the relevant chapters.

Generally, one challenge to research into endometriosis-associated pain is the lack of an non-invasive diagnostic test[25]. Given that surgery is required to clinically diagnose endometriosis, it is especially difficult to detangle what is due to the endometriosis and what is the result of surgery. Whilst this can be overcome by prospectively collecting data before surgery, the data in this thesis has been collected post-surgery.

Internationally, there is a significant diagnostic delay in endometriosis[26], this often means that women have been experiencing pain for many years before any endometriosis treatments are offered to them. During this time women experience stigma and often have to fight to be believed. This will no doubt impact their psychological health, which can in turn influence their pain perception[27–29]. It is currently not clear what impact this diagnostic delay has on ultimate treatment success but there is little doubt that the delay is damaging to women[30]. The fact that many women whose data I have used in this thesis have been suffering pain for a decade(s) should not be dismissed, especially when comparing to other chronic pain conditions which do not necessarily have such long symptom duration.

Naturally, whenever research is carried out on volunteer participants there is an aspect of bias. For my study discussed in Chapter 3, data was collected from support group websites; one would expect women on such groups to be potentially the most severely impacted by their endometriosis. For the studies discussed in Chapters 5, 6 and 7, visits were carried out at the John Radcliffe hospital, so women had to be able to travel to sessions, as well as donate their time to the study. I am very grateful to all the women who have taken part in this research; their eagerness to take part in research (from which they do not benefit) has highlighted to me the need for better understanding and treatment of endometriosis and associated pain.

8.5 Future directions

Whilst the evidence in this thesis aims to look at neuropathic pain from a broad perspective, there are naturally many more avenues which need investigating.

Firstly, the mechanism giving rise to neuropathic pain in endometriosis needs to be better understood. In this thesis I have given examples of mechanisms which could cause damage to peripheral nerves but currently there is no causal evidence of these. More work on the molecular changes in the pelvis of women with endometriosis-associated pain is therefore needed, to see if there is evidence for a molecular underpinning of neuropathic pain, for example with more evidence of inflammatory mediators present in the pelvis, relating those to pain measures (for example painDETECT). Also, the role of surgery in neuropathic pain needs further investigation, to determine whether surgery is causing neuropathic pain in some women, why there may be differences in the risk of neuropathic pain post-surgery and whether potentially different surgical techniques carry greater risk.

The central mechanisms of neuropathic pain, which I have investigated in Chapters 5 and 6, also need to be further explored to determine differences in mechanisms of spontaneous and provoked pain. This also needs to be translated into treatment targets, which may become possible with current work investigating the cerebral signature of analgesia. A longitudinal study, whilst difficult, would be useful to determine whether alterations to the processing of pain in women with endometriosis changes before and after surgery, especially with regards to the presence of a neuropathic component. Generally, for investigating the effect of treatments, using fMRI before and after the duration of treatment is incredibly useful as it helps to understand both the mechanism of the pain and the treatment action.

As I have mentioned, there is a desperate need to trial treatments for endometriosis-associated pain and neuropathic adjuncts (such as gabapentin, amitriptyline, duloxetine and pregabalin) are good candidates. Given the evidence from the GaPP2 study in Chapter 4, I would suggest such a trial selects women that had a neuropathic component to their pain. Given these drugs are effective in neuropathic pain conditions, they are the most likely to get a significant therapeutic response.

Fundamental to all this research is the proper phenotyping of the pain experienced by women with endometriosis-associated pain. Work needs to be carried out to understand whether patients feel that their pain has been completely assessed by measures widely used, so that these can be adopted into clinical practice. As I have discussed, more work is needed to explore the differences in spontaneous and provoked pain and how best to assess these symptoms needs to be determined.

8.6 Concluding remarks

The data presented in this thesis suggest that a neuropathic pain component is present in a substantial proportion of women with endometriosis-associated pain. Those with a neuropathic-like component can be subdivided into two groups with different sensory symptom profiles, a finding which I have replicated in a cohort of women with chronic pelvic pain with no known pathology. Whilst more research is needed, there is an interesting interaction between painDETECT group and treatment with gabapentin and placebo in women with chronic pelvic pain, which indicates the need to carry out more studies with subgroups of patients to get closer to individualised treatment.

Ultimately, I hope that my work will create new avenues to explore in endometriosis-associated pain which can be translated into meaningful improvement on the impact endometriosis has on the lives of millions of women worldwide. Only once we have a better understanding of the symptoms of endometriosis-associated pain and the mechanisms giving rise to them can we successfully offer (personalised) treatments.

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Name:_____ Control site:_____

Date of birth:_____ Date:_____ Test site:_____

Localisation of pain:_____ Pain intensity prior to QST:_____(0-100)

Room temperature:_____℃ Skin temperature Control site:_____℃ Test site:_____℃

QST - Documentation Form

Control site

CDT	WDT	TSL	CPT	HPT
°C	°C	°C	°C	°C
°C	°C	°C	°C	°C
°C	°C	°C	°C	°C
		°C		
		°C	PHS	
		°C	Number of paradoxical heat sensations	/3

Test site

CDT	WDT	TSL	CPT	HPT
°C	°C	°C	°C	°C
°C	°C	°C	°C	°C
°C	°C	°C	°C	°C
		°C		
		°C	PHS	
		°C	Number of paradoxical heat sensations	/3

MDT; Mechanical detection threshold (von Frey hairs)

Control site

felt	not felt
mN	mN
	mN
mN	
	mN
mN	
	mN
mN	
	mN
mN	

Test site

felt	not felt
mN	mN
	mN
mN	
	mN
mN	
	mN
mN	
	mN
mN	

MPT; Mechanical pain threshold (Pinprick)

Control site

blunt	pricking
mN	mN
	mN
mN	
	mN
mN	
	mN
mN	
	mN
mN	

Test site

blunt	pricking
mN	mN
	mN
mN	
	mN
mN	
	mN
mN	
	mN
mN	

SR-Function; MPS: Pinprick; DMA: Brush (BR), Q-tip (QT), Cotton wisp (CW) [a]

Control site

128		CW		32		256		BR	
CW		256		128		8		32	
32		128		BR		CW		16	
256		8		CW		QT		128	
BR		32		16		128		512	
8		QT		256		64		CW	
16		BR		512		32		64	
QT		64		8		512		256	
512		16		64		BR		QT	
64		512		QT		16		8	

Test site

8		16		QT		512		64	
QT		BR		64		16		512	
256		512		8		64		QT	
64		32		512		BR		16	
CW		64		256		QT		8	
512		128		16		32		BR	
128		QT		CW		8		256	
16		CW		BR		128		32	
32		8		128		256		CW	
BR		256		32		CW		128	

WUR; Wind-up ratio (Series of 10 stimuli/single stimulus) [b]

Control site

Single stimulus	Stimulus series
Stimulus intensity	mN

Test site

Single stimulus	Stimulus series
Stimulus intensity	mN

VDT; Vibration detektion threshold

Control site Test site

Bony prominence:_____

/8	/8
/8	/8
/8	/8

PPT; Pressure pain threshold

Control site Test site

Muscle:_____

kg or kPa	kg or kPa

The patient has understood the instructions and complied with them.

☐ yes ☐ no ☐ unsure

a In case a stimulus is rated "100", this and any more severe stimuli should no longer be applied to the affected skin area during the test and a rating of "100" should be noted for all the stimuli.
b Should the pinprick stimulator with the intensity of 256 mN (128 mN in the face) not be tolerable for the patient, the next lower intensity may be used. In this case this should be documented on the documentation sheet and during data entry. If necessary, repeat the testing in the control site with the lower stimulus intensity to ensure a consistent measurement.

Appendix 1: QST form