UNIVERSITY OF LIVERPOOL

Structural and functional brain alterations in fibromyalgia syndrome patients

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Nicholas Fallon

February 2013

List of abbreviations

___

In order of use:

FMS	Fibromyalgia syndrome
ACR	American College of Rheumatology
MTPS	Manual Tender Point Scale
MRI	Magnetic resonance imaging
fMRI	Functional magnetic resonance imaging
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
DMN	Default mode network
BOLD	Blood oxygen level dependent
VBM	Voxel based morphometry
DTI	Diffusion tensor imaging
FA	Fractional anisotropy
ROI(s)	Region(s) of interest
CIM	Chiari I malformation
EEG	Electroencephalograph
ERD	Event-related desynchronisation
ERS	Event-related synchronisation
PET	Positron emission tomography
EOG	Electrooculagraphic
ECG	Electrocardiographic
ERP	Event-related potential
RF	Radio frequency
TM	Transverse magnetisation
LM	Longitudinal magnetisation
TR	Time to repeat
TE	Time to echo
CBF	Cerebral blood flow
SD	Standard deviation
STAI-S	State anxiety

STAI-T	Trait anxiety
FIQ	Fibromyalgia Impact Questionnaire
BDI	Beck Depression Inventory
PCS	Pain Catastrophising Scale
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
GFP	Global field power
MNI	Montreal Neurological Institute
GLM	General linear model
FDR	False discovery rate
FWE	Family-wise error
TBSS	Tract-Based Spatial Statistics
BA	Brodmann area
PHG	Parahippocampal gyrus
ACC	Anterior cingulate cortex
rACC	Rostral anterior cingulate cortex
PCC	Posterior cingulate cortex
IPL	Inferior parietal lobule
MFG	Medial frontal gyrus
GABA	Gamma-aminobutyric acid

Contents

_____

Appendices 197

List of figures

List of tables

_____

Structural and functional brain alterations in fibromyalgia syndrome patients

Nicholas Fallon

Abstract

Fibromyalgia syndrome (FMS) is a widespread chronic pain disorder affecting 2−5% of the general population and particularly women of middle age (McBeth and Mulvey, 2012). The syndrome is frequently comorbid with a variety of clinical, functional and psychological disorders (Weir et al., 2006) and associated with a large socio-economic burden (Lachaine et al., 2010). In spite of significant previous research, the underlying aetiology and pathophysiology of FMS is not fully understood (Schmidt-Wilcke and Clauw, 2011). However, aberrant structural and functional brain alterations have been proposed as a casual or maintaining factor of the disorder (Schweinhardt et al., 2008). This thesis utilised functional and structural imaging methods and novel experimental paradigms to explore brain alterations in FMS patients. A comprehensive review of previous experimental findings was performed to identify novel research questions. EEG and MRI data for 5 unique studies was collected over two sessions.

In the first study dynamic mechanical stimulation was applied to the forearm of FMS patients and healthy participants, and an ERD analysis of corresponding EEG data was performed. The results revealed that FMS patients exhibited alterations to cortical excitability during brushing stimuli which correlated with clinical measures. These findings indicate that abnormal processing of innocuous somatosensory stimulation may contribute to the pathophysiology and clinical symptom severity of FMS. Secondly, an ERP analysis of EEG data from the

observation of pain and non-pain pictures was performed. FMS patients exhibited differences in ERP components and source activation patterns during observation of pain pictures relative to healthy people. Alterations to processing of observed pain occurred in parahippocampal gyrus and may relate to clinical and psychological aspects of FMS, this finding could be utilised to further understand the heterogeneity of psychological profiles of FMS patients in order to better target therapeutic interventions.

The third study of the thesis describes a novel comparison of functional connectivity with resting-state network structures utilising fMRI recordings. Functional connectivity with default mode network structures was shown to be altered in FMS. This finding may reflect an ongoing time-dependent reorganisation of resting-state networks due to ongoing chronic pain. In the fourth study, a morphological analysis of subcortical structures was performed using high-resolution T1-weighted MR images. FMS patients demonstrated alterations to the morphology of the brainstem, an important structure in descending nociceptive control. Volumetric alterations in this structure correlated with clinical measures of symptom severity suggesting an important role for brainstem alterations in FMS pain symptoms. In the final study the microstructural integrity of white matter was compared between FMS patients and healthy participants. Although no significant differences were identified the findings indicate that FMS is not likely to be related to abnormal development of white matter tracts. Therefore structural alterations associated with FMS are likely to occur only in the grey matter.

Declaration

No part of this work has previously been submitted in support of another application for a degree or qualification at this or any other University or institute of learning.

Acknowledgements

Chapter One

General introduction

1.1 Fibromyalgia syndrome: A brief overview

Fibromyalgia syndrome (FMS) is a chronic pain disorder characterised by widespread pain and tenderness, morning stiffness, sleep disturbance, fatigue, psychological disturbance and cognitive dysfunction (Wolfe et al., 1990; Bennett et al., 2007). The principal characteristic of FMS was first explicitly defined in the 1970s as widespread pain in deep tissues and muscles (Smythe, 1975), and this explanation expanded to include accompanying symptoms such as fatigue and morning stiffness (Smythe and Moldofsky, 1977). Since then the number of recognised symptoms in FMS has steadily increased and a wide range of frequent comorbidities have also been acknowledged (Weir et al., 2006). FMS is now considered to be a heterogeneous and complex disorder and patients are likely to be affected by varying symptoms and related conditions over the course of their lifetime (Clauw, 2009).

Previously investigations of FMS patients have demonstrated genetic and environmental causal factors, as well as various pathophysiological mechanisms which may contribute to the pathogenesis and maintenance of FMS symptoms. However, the exact aetiology and pathophysiology of this complex syndrome is still not adequately understood (Schmidt-Wilcke and Clauw, 2011).

1.1.1. Diagnostic criteria for FMS

Several diagnostic criteria for FMS were suggested in the 1980s (Yunus et al., 1981; Wolfe and Cathey, 1983), although these tended to be based on small study samples and often encompassed arbitrary numbers and locations of tender points (McBeth and Mulvey, 2012). In 1990 the American College of Rheumatology (ACR) criteria (Wolfe et al., 1990) was established using a controlled study with a larger sample population and age-matched control group. The criteria requires pain affecting all 4 quadrants of the body for a period of at least three months, and patients should report pain upon manual palpation examination of no fewer than 11 of 18 designated tender points (Fig.1.1). These criteria were shown to have sensitivity and specificity exceeding 80% (Wolfe et al., 1990). The 1990 ACR criteria were criticised for their use of tender points, which were deemed unsuitable for clinical environments. However, this review also acknowledged their legitimacy for research purposes (Okifuji et al., 1997). The establishment of the ACR criteria proved particularly valuable in allowing standardised study of the disorder; leading to a sharp rise in research of FMS populations (Wolfe and Hauser, 2011; McBeth and Mulvey, 2012). However, clinical diagnosis of FMS is complex and requires comprehension of the wide range of symptoms of the disorder as well the varying comorbidities, rather than sole reliance on the basic ACR premise (Schweinhardt et al., 2008; Clauw, 2009). Recently, new ACR criteria were developed which do not require physical examination and instead classify FMS patients as scoring a widespread pain index score of greater than 7, and a symptom severity score of greater than 5 Points (widespread pain index score of between 3-6 is acceptable if symptom severity score is greater than 9; Wolfe et al., 2010)
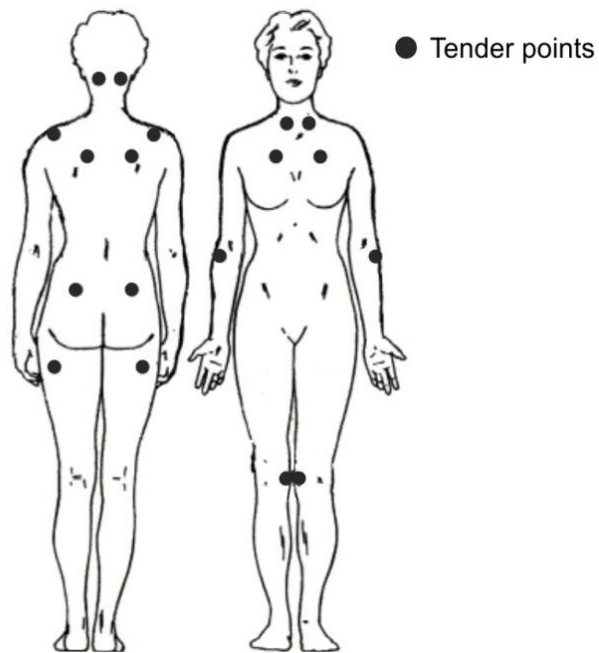
Fig.1.1 Diagram of locations for palpation during the manual tender point scale test

1.2. Epidemiology of FMS

1.2.1. Prevalence of FMS

FMS is one of the most common conditions seen in rheumatology practice (Goldenberg, 1987), exceeded only by osteoarthritis and rheumatoid arthritis (Wolfe and Cathey, 1983). Epidemiological studies have estimated the prevalence of FMS in the general population as high as 11% in Norway (Forseth and Gran, 1992). However, prevalence ranging between 2−5% has been reported in the USA (Wolfe et al., 1995; Burckhardt, 2005; Lawrence et al., 2008), 3.3% in Canada (White et al., 1999), and around 0.7% in Sweden and Finland (Mäkelä and Heliövaara, 1991; Prescott et al., 1993).

Studies consistently indicate a strong female bias in FMS; a meta-analysis of studies revealed that female adult prevalence in western countries ranged between

1−4.9% compared to 0−1.6% in males (Gran, 2003). Similarly, an epidemiological study demonstrated that females are up to 7 times more likely to develop FMS than males (Weir et al., 2006). A pattern of age-related prevalence is also seen in several epidemiological studies of FMS, with increasing likelihood found in the population up until the sixth decade, and decreasing thereafter (Mäkelä and Heliövaara, 1991; Wolfe et al., 1995; White and Harth, 2001). However, some studies indicate that FMS prevalence continues to increase up until the eighth decade (Lawrence et al., 1998; Branco et al., 2010). To summarise, FMS is reported at a prevalence of between 1−11% in general populations although figures of between 2−5% are most common (McBeth and Mulvey, 2012). The syndrome shows a strong female bias (Gran, 2003), and is more common in middle age (McBeth and Mulvey, 2012). Table 1.1 surmises the data from several epidemiological studies of FMS which utilised the 1990 ACR criteria to evaluate prevalence in the general population.

Table 1.1 Epidemiological studies of FMS prevalence in general populations.

Study	Cohort size	Country	Reported prevalence (%)
Forseth & Gran (1992)	2,038	Norway	10.5
Lydell (1992)	1,102	South Africa	3.2
Prescott *et al.* (1993)	1,219	Denmark	0.66
Buskila *et al.* (1993)	338	U.S.A	6.2
Wolfe *et al.* (1995)	3,006	U.S.A	2.0
Clark *et al.* (1998)	548	Mexico	1.2
White *et al.* (1999)	3,395	Canada	3.3
Santos *et al.* (2010)	361	Brazil	5.5

1.2.2. Socio-economic impact

The expense of treatment interventions along with significant indirect costs and reduced patient productivity has led to a recent emphasis on the large socio-economic burden associated with FMS (Lachaine et al., 2010). As a chronic and complex condition, FMS is associated with high healthcare costs (Hughes et al., 2006; White et al., 2008). Retrospective studies of health insurance claims in the United States estimate that FMS patients average between 12−20 doctors visits per year, and directly associated medical costs were evaluated at up to $10,000 USD per annum (Robinson et al., 2003; Berger et al., 2007; White et al., 2008).

However, direct medical costs only account for a proportion of the economic burden associated with FMS (Lachaine et al., 2010). Patients report significant limitations in physical function affecting multiple aspects of daily life (Bennett et al., 2007; Schaefer et al., 2011), which also relate to compromised productivity when in work, increased absenteeism, unemployment and disability (White et al., 2008). Patients may also require additional help and resources such as unpaid assistance from family members for daily living (Boonen et al., 2005; Schaefer et al., 2011). A study found that more than three-quarters of total fibromyalgia-related costs are attributable to lost productivity, disability and indirect costs in Europe (Winkelmann et al., 2011). A similar proportion was also recently reported in the United States (Chandran et al., 2012). The increased use of health services by FMS patients is associated with clinical, psychological and social factors (Bernatsky et al., 2005). Symptom severity and disability have been shown to predict increased medical visits and costs (Walen et al., 2001), as well as psychosocial variables such as substance abuse or psychological distress (Kersh et al., 2001). Studies indicate that additional clinical or psychological comorbidities are amongst the principal determinants of the socio-economic burden of FMS (Walen et al., 2001; Penrod et al., 2004; Bernatsky et al., 2005).

1.2.3. Common comorbidities associated with FMS

FMS is often comorbid with other disorders which can be grouped into clinical, psychological and functional categories. Clinical comorbidities such as osteoarthritis or rheumatoid arthritis are commonplace and may act as confounding or aggravating factors in FMS pain (Atzeni et al., 2011). Although these disorders demonstrate obvious peripheral mechanisms (degeneration or inflammation of the

joints respectively), it was recently conjectured that they may also share central pathophysiological mechanisms with non-inflammatory pain syndromes such as FMS (Lee et al., 2011). Functional regional pain syndromes such as irritable bowel syndrome, interstitial cystitis, temporomandibular disorder, tension-type headache, migraine, and vulvodynia are also commonly associated with FMS (Clauw, 2009), and these disorders may also share pathophysiology with the syndrome (Ablin and Clauw, 2009; Williams and Clauw, 2009).

Psychological disturbance is often associated with FMS, and mood or anxiety disorders may precede or accompany its development (Arnold and Clauw, 2010). Studies estimate the lifetime prevalence of anxiety disorder in FMS between 35−62%, and major depressive disorder between 58− 86% of patients (Thieme et al., 2004; Arnold et al., 2006). In contrast, lifetime prevalence of major depressive disorder in the adult general population was estimated at 8.6% in the United States, and prevalence of any mood disorder at just 11.5% (Jonas et al., 2003). It was proposed that the high prevalence of psychological disturbance in FMS may be due to common pathophysiological mechanisms, rather than arising as a result of FMS pain or vice versa (Hudson and Pope, 1996). Separate interventions are recommended for comorbid psychological disturbances in FMS rather than treating them as part of the same disorder (Thieme et al., 2004). Treatments aimed entirely at relieving psychological disturbance may result in poor therapeutic outcomes for the management of FMS (Williams and Clauw, 2009).

Epidemiological research has indicated that FMS patients are up to 7 times more likely to demonstrate a common comorbid condition than the general population (Weir et al., 2006), and a study showed that up to 90% of FMS patients

report at least 1 associated comorbid condition (Bernatsky et al., 2005). The most frequent clinical comorbidity identified was osteoarthritis which affected 40% of patients; depression was the most common psychological distress, affecting 5% of patients (although lifetime prevalence was not accounted for); the most common functional disorder was irritable bowel syndrome, affecting 36% of patients (Bernatsky et al., 2005).

1.2.4. Risk factors pertaining to FMS

The lack of a peripheral nociceptive cause for FMS pain, coupled with the high prevalence of affective and psychological disturbance led some researchers to construe that FMS may be a psychogenic disorder (Netter and Hennig, 1998). Advances in genetic research, experimental pain testing and neuroimaging have led to advances in our understanding of the aetiology of FMS, although further research is still necessary (Schmidt-Wilcke and Clauw, 2011).

1.2.4.1. Genetic risk factors

Studies have indicated a strong familial component in FMS, it was previously shown that first degree relatives of patients exhibit an 8 times greater risk of developing the disorder than relatives of rheumatoid arthritis patients who were evaluated as a control group (Arnold et al., 2004). There is also evidence of an increased familial risk of common comorbid disorders such as affective disturbance (Hudson et al., 1985; Buskila et al., 1996). Polymorphisms in genes involved in serotonergic, dopaminergic and catecholaminergic systems have been proposed to play a role in the aetiology of FMS (Bondy et al., 1999; Offenbaecher et al., 1999; Buskila, 2009). It is likely that, as a complex and heterogeneous syndrome, a wide

range of genetic factors will be involved in FMS (Bradley, 2009). Due to a small number of studies, relatively small sample sizes and diverse heterogeneity in sample populations, the scientific understanding of the genetic component of FMS aetiology is still at a relatively early stage (Holliday and McBeth, 2011).

1.2.4.2. Social, psychological and environmental risk factors

As well as a probable genetic component, environmental factors are likely to play a role in triggering the onset of FMS. Myofascial pain syndrome (Cakit et al., 2010), physical trauma (particularly to the trunk of the body), acute illness and emotional or psychological distress (Mease et al., 2005) have all been linked with the onset of FMS, however the syndrome will only develop in 5−10% of individuals undergoing such trauma (Bennett et al., 2007). In women, physical abuse has been suggested as a common triggering factor (Ruiz-Perez et al., 2009), and obesity is also often seen in female sufferers (Mork et al., 2010). Psychosocial factors may also play an important role in the development and maintenance of FMS; workplace stress appears to be a frequent cause (Harkness et al., 2004), and life-changing events or family problems have also been proposed as potential triggering factors (Mease et al., 2005). Recent theories have postulated that abnormalities of the human stress response, resulting in exaggerated physiological reactions to stress, could also be a possible cause of FMS (Clauw, 2009).

1.3. Aetiology of FMS

Although the exact aetiology of FMS is still debated, it has been suggested that alterations to processes in the central nervous system may be causal or maintaining factors in the disorder (Yunus, 1992; Clauw and Chrousos, 1997), and such factors have become a primary focus of neuroimaging research.

1.3.1 Central sensitisation hypothesis for FMS

Sensory thresholds are lowered in FMS, and this may be associated with neuroplastic alterations which relate to the development of symptoms in FMS (Staud and Spaeth, 2008). Specifically, a dysfunctional hyperexcitability of pain processing pathways is purported to be responsible for the pain experienced in FMS (Perrot et al., 2008; Petersel et al., 2011). A wealth of psychophysical evidence exists demonstrating reduced pain thresholds in response to pressure, thermal, cold, electrical and laser stimuli in FMS patients (Kosek et al., 1996; Lorenz et al., 1996; Petzke et al., 2003). Cross modal correlations between mechanical and thermal hyperalgesia (Petzke et al., 2003) and enhanced wind-up of repetitive stimuli in FMS patients (Staud et al., 2001; Banic et al., 2004) also suggest that sensory alterations are widespread, indicating central aetiology. An abnormal central pain processing aetiology is also supported by structural and functional neuroimaging studies (for a review see; Jones et al., 2012). EEG studies utilising noxious laser stimuli with FMS patients revealed that reduced pain thresholds correlated with enhanced cortical laser evoked potentials (Gibson et al., 1994; Sorensen et al., 1995; Lorenz et al., 1996; de Tommaso et al., 2011). Functional MRI studies investigating hyperalgesia in FMS also showed augmented cortical activations and subjective pain in patients during noxious stimuli such as mechanical pressure, cold and thermal stimuli (Gracely et al., 2002; Cook et al., 2004; Staud et al., 2008).

Centrally acting pharmacotherapies such as pregabalin, gabapentin, milnacipran and duloxetine are the most commonly prescribed treatments for FMS (Woolf, 2011). The various pharmacological mechanisms of such drugs suggests multiple central pathophysiological mechanisms for FMS symptoms (Petersel et al.,

2011). Pathophysiological explanations for central sensitisation include facilitation of ascending pathways, exaggerated dorsal horn responses to afferent peripheral impulses, dysfunction of descending inhibitory pathways, or neurotransmitter imbalances, all of which may contribute to the clinical symptoms seen in FMS (Graven-Nielsen and Arendt-Nielsen, 2010; Ge et al., 2011; Petersel et al., 2011). The mechanisms underlying central sensitisation are difficult to define and disruption to one (or many) of the complex mechanisms mediating central processing of pain could feasibly cause FMS symptoms. To further complicate matters, it has also been postulated that psychological factors could (at least in part) contribute to central sensitisation (Sarzi-Puttini et al., 2011).

The continuing recognition and support for the central sensitisation hypothesis of FMS has recently led to the suggestion that other, local pain syndromes such as IBS or tension-type headache may also share pathophysiological central sensitisation mechanisms with FMS. It was postulated that such an overlap in pathophysiology may go some way to explain the overlap in symptoms as well as the common comorbidity of these types of disorders (Ablin and Clauw, 2009; Williams and Clauw, 2009; Phillips and Clauw, 2011).

1.3.2. Altered central processing of somatosensory stimuli in FMS

Abnormal processing of peripheral somatosensory input is also likely to contribute to central sensitisation mechanisms in FMS (Petersel et al., 2011). Ongoing peripheral afferent input is known to maintain central sensitisation and has been shown to play an active role in the generation of spontaneous pain, hyperalgesia and allodynia (Staud et al., 2009; Staud, 2010). Persistent peripheral nociceptive activity can lead to chronic pain, particularly if processes such as temporal

summation are facilitated in a state of central sensitisation (Graven-Nielsen and Arendt-Nielsen, 2010). Experimental evidence from neuropathic (Price et al., 1989; Gracely et al., 1992), chronic regional pain syndrome (Price et al., 1998) and irritable bowel syndrome (Verne et al., 2003) patients has shown that peripherally acting analgesics can provide relief in disorders with a strong central sensitisation component. Recent evidence also suggests that peripherally acting analgesics can reduce FMS pain (Staud et al., 2009). Dysfunctional processing of innocuous somatosensory stimuli may result in subjective pain, indicating central amplification of peripheral afferents and such alterations to processing can be observed using objective biomarkers and neuroimaging techniques (Woolf, 2011). It is reasonable to postulate that peripheral input, is required to achieve and maintain central sensitisation, and may be important in the development of disorders such as FMS. The argument for a peripheral contribution to FMS pain was recently bolstered by microneurographic findings suggesting that peripheral small fibre neuropathy may be common in a significant subgroup of FMS patients (Serra, 2012).

1.3.2.1. Allodynia and hyperalgesia

Pain processing can be divided into two categories: nociceptive processing, involving detection of painful stimuli which prevents harm and tissue damage; and pathological pain processing, which serves no advantage and often results in severe distress (Woolf and Mannion, 1999). Allodynia is a pathological pain processing condition whereby innocuous stimuli cause a pain sensation. Hyperalgesia is excessive pain sensation resulting from a painful stimuli. Both are symptoms commonly seen in FMS (Sarzi-Puttini et al., 2011).

Aberrant brain activations have been consistently identified during allodynia using a variety of brain imaging techniques in neuropathic pain populations (for a review see; Jones and Watson, 2007; Moisset and Bouhassira, 2007). Activations in frontal cortices during allodynia pain have been linked to the emotional burden of ongoing pain or an exaggerated cognitive evaluative response (Witting et al., 2006). Activations seen in pain and somatosensory processing structures may represent a more sensory aspect of allodynia pain processing (Maihöfner et al., 2006). However, previous findings would suggest that an 'allodynia matrix' specifically accounting for pathological processing during allodynia pain is unlikely (Moisset and Bouhassira, 2007). Augmented activations and increased subjective pain ratings have been demonstrated in fMRI studies of FMS patients during hyperalgesia (Gracely et al., 2002; Cook et al., 2004; Staud et al., 2008). However, no data exists regarding brain activations in FMS patients during allodynia pain resulting from innocuous stimuli. Further investigation of the central mechanisms underlying these processes could advance treatment and understanding of FMS.

Previously, fMRI studies have investigated hyperalgesia during mechanical pressure in FMS patients, showing augmented cortical activations and subjective pain during stimulation that was non-noxious in healthy people (Gracely et al., 2002). Dynamic mechanical stimulation, such as brushing, was previously utilised to demonstrate augmented activations in cortical regions associated with somatosensory processing such as bilateral primary somatosensory (SI); secondary somatosensory (SII) and thalamus in neuropathic pain patients (Petrovic et al., 1999; Peyron et al., 2004; Witting et al., 2006), and complex regional pain syndrome patients (Mailhöfner et al., 2006). FMS is associated with allodynia to light pressure and severe cases may exhibit allodynia to light touch (Jones and Watson, 2007); patients

also exhibit alterations to central processing of mechanical stimuli (Gracely et al., 2002). Pain resulting from innocuous mechanical stimuli, such as brushing could prove to be an important pathophysiological component of fibromyalgia symptomatology. Despite the previous findings no study has yet investigated somatosensory processing of non-noxious stimuli, such as mechanical brushing, in FMS patients to specifically investigate the mechanisms of allodynia pain in FMS.

1.3.2.2. Dysfunctional endogenous pain modulation and FMS

The duration, quality and intensity of nociceptive input are all highly relevant to pain processing. However, these afferent impulses are also subject to subsequent modulation occurring in the peripheral and central nervous system, which can reduce or augment the intensity of pain sensations (Staud, 2012). Functional imaging studies show that pain modulation within the central nervous system encompasses a network of brain structures including frontal cortices, rostral anterior cingulate cortex (rACC), periaqueductal gray and rostral ventromedial medulla (Mason, 2005; Bingel et al., 2006; Kong et al., 2006; Eippert et al., 2009). It was proposed that pain modulation serves an evolutionary protective function (Dubner and Ren, 1999), for instance by enabling pain reduction in a 'fight or flight' scenario (Ren and Dubner, 2002).

Central sensitisation may result from an imbalance between descending inhibition and ascending facilitation of pain pathways (Graven-Nielsen and Arendt-Nielsen, 2010). This can be assessed experimentally, and dysfunctional endogenous pain modulation has already been demonstrated in various chronic pain conditions, such as temporomandibular disorder (King et al., 2009), osteoarthritis (Kosek and Ordeberg, 2000), chronic tension-type headache (Sandrini et al., 2006) and FMS

itself (Kosek and Hansson, 1997; Lautenbacher and Rollman, 1997; Vierck et al., 2001; Julien et al., 2005). Functional imaging of FMS patients during experimental pain shows reduced activations in regions associated with endogenous pain modulation (Jensen et al., 2009), and reduced connectivity between the brainstem and pain modulatory structures (Jensen et al., 2012). Functional imaging of the brainstem and spinal cord during innocuous and painful touch suggests that dysfunctional descending pain modulation may be important in the experience of allodynia pain (Ghazni et al., 2010). It has also been suggested that dysfunctional descending pain modulation may facilitate the development of chronic pain in FMS and other chronic pain disorders (Arendt-Nielsen and Yarnitsky, 2009; Staud, 2011a; Jones et al., 2012), and it is also likely to be influenced by psychological factors (van Wijk and Veldhuijzen, 2010).

1.4.Pathophysiology of FMS

1.4.1. Tonic ongoing pain in FMS

Chronic pain patients experience an unrelenting pain percept (Foss et al., 2006), and tonic ongoing pain originating from peripheral sources has been proposed as an important component in the development and maintenance of FMS (Staud, 2010, 2011b). It was previously suggested that cortical re-organisation of neural networks may occur as tonic pain persists in FMS (Staud, 2011b), and, due to its ongoing nature, chronic pain may particularly affect resting-state networks (Baliki et al., 2008).

1.4.1.1. Resting-state network abnormalities

The term 'default mode' was initially suggested to describe the increased levels of activation seen in various brain structures during rest (Raichle et al., 2001). Experimental findings have shown that levels of activity in a specific subset of brain regions, referred to as the default mode network (DMN), appear to decrease when switching from a resting-state, such as passive visual fixation or resting with eyes closed, to a goal directed task (Shulman et al., 1997). These findings were consistently repeated and confirmed by meta-analyses (Binder et al., 1999). Regions involved in the DMN include posterior cingulate, precuneus, left and right inferior parietal and medial pre-frontal cortices (Fox et al., 2005; Raichle and Snyder, 2007; Laird et al., 2009).

Studies of clinical populations have previously demonstrated abnormalities in DMN activations in clinical populations. Alzheimer's disease patients show significant reductions, relative to healthy volunteers, in DMN activations (Greicius et al., 2004). In chronic back pain patients the magnitude of deactivation in DMN structures when switching from default mode to a task state was strongly attenuated (Baliki et al., 2008). The extent of dysfunction in the DMN also correlated significantly with the duration of chronic pain, which the authors attributed to re-organisation of DMN as a result of ongoing chronic pain (Baliki et al., 2008). Although DMN activation differences have not been identified in FMS there are alternative ways to analyse resting-state networks in terms of connectivity between structures which have proved to be more successful in FMS research.

1.4.1.2. Functional connectivity alterations

It is possible to investigate functional connectivity between structures using fMRI scans and evaluating blood oxygen level dependent (BOLD) signal changes throughout the brain in order to identify regions with correlated (or anti-correlated) activation patterns (Krienen and Buckner, 2009). These techniques have been employed to further enhance our understanding of resting-state networks such as the DMN. In healthy populations BOLD signal correlations were used to demonstrate significant functional connectivity between the regions commonly identified as the DMN (Greicius et al., 2003). The degree of connectivity between DMN structures may be altered in clinical pathologies (Whitfield-Gabrieli and Ford, 2012). Functional connectivity within the DMN was previously found to be significantly increased in depressed patients relative to healthy people and the length of depressive episode correlated with the degree of augmented connectivity (Greicius et al., 2007).

Altered connectivity between DMN structures has been demonstrated in chronic pain patients (Malinen et al., 2010). A study of DMN connectivity in FMS patients showed augmented connections between DMN structures and insula cortex which was linked to ongoing spontaneous pain (Napadow et al., 2010). Furthermore, this augmented DMN–insula connectivity normalised following successful therapeutic intervention and was associated with a reduction in spontaneous pain (Napadow et al., 2012). Similar abnormal connectivity between DMN and insula was also seen in temporomandibular disorder patients (Ichesco et al., 2012), raising the possibility that this abnormal connectivity may not be specific to FMS, but could be linked to comorbid disorders. Reduced functional connectivity was also previously

identified between the brainstem and rACC in FMS patients during experimental pain, which was attributed to dysfunctional endogenous pain inhibition (Jensen et al., 2012).

Studies have also demonstrated resting-state alterations to functional connectivity between acknowledged pain processing structures in FMS patients (Cifre et al., 2012). Augmented connectivity was seen between anterior cingulate cortex (ACC) and insula cortices, which was attributed to processing of tonic pain, whereas reduced connectivity between thalamus, periaqueductal grey and insula cortices was conjectured to be an indicator of dysfunctional endogenous pain modulation (Cifre et al., 2012). However, the previous studies showing DMN functional connectivity abnormalities in FMS patients exhibit some methodological limitations. Patients aged up to 75 years were utilised in the first study to employ this method in FMS (Napadow et al., 2010). This is problematic as resting-state functional connectivity is known to show age-related alterations in older people (Whitfield-Gabrieli and Ford, 2012). Therefore, a younger sample may be more suitable for analysis. Similarly, the previous investigation of functional connectivity between pain processing structures in FMS used patients of both sexes and a total of only 9 patients (Cifre et al., 2012). No investigations of FMS patients have previously utilised functional connectivity analyses of DMN and pain processing regions identified using recent meta-analyses.

1.4.2. Morphological alterations and FMS

Voxel-based morphometry (VBM) is an analysis technique which employs statistical parametric mapping to analyse anatomical MR images in order to investigate macroscopic alterations in the grey and white matter of the brain

(Ashburner and Friston, 2000). VBM studies in FMS patients have shown a range of regional grey matter differences relative to healthy control subjects, encompassing a wide variety of structures (Kuchinad et al., 2007; Schmidt-Wilcke et al., 2007; Luerding et al., 2008; Lutz et al., 2008; Burgmer et al., 2009; Valet et al., 2009; Robinson et al., 2011). FMS patients have demonstrated local grey matter density reductions in cingulate, insular cortices, medial frontal cortices, parahippocampal gyri (Kuchinad et al., 2007), and in the superior temporal gyrus and left posterior thalamus (Schmidt-Wilcke et al., 2007). Grey matter density increases were found in the left orbitofrontal cortex, cerebellum and bilateral striatum (Schmidt-Wilcke et al., 2007). Analyses excluding age and depression identified reduced grey matter volumes in prefrontal, cingulate and insular cortices of FMS patients (Valet et al., 2009). Conversely an alternative study suggests that grey matter alterations were negated when implementing depression as a covariant (Hsu et al., 2009). VBM analyses of pre-defined regions of interest of FMS patients have shown reduced grey matter volumes in the cingulate, prefrontal and insular cortices, hippocampi and amygdala (Lutz et al., 2008; Burgmer et al., 2009; Robinson et al., 2011).

It was previously suggested that the lack of consistency in the findings may be due to the wide variability in age across studies and/or the relative proportions of different symptoms contributing to FMS diagnosis in a heterogeneous population (May, 2011). VBM has also been used to identify structural differences in irritable bowel syndrome (Seminowicz et al., 2010), tension type headache (Schmidt-Wilcke et al., 2005), chronic fatigue syndrome (de Lange et al., 2005) and post-traumatic stress disorder (Villarreal et al., 2002; Chen et al., 2006) patients. FMS is often comorbid with these disorders, and the overlap of symptoms has led some researchers to propose that similar neurophysiological mechanisms may be involved

(Ablin and Clauw, 2009; Williams and Clauw, 2009; Phillips and Clauw, 2011). It is possible that a particularly high prevalence of a specific comorbidity in a heterogeneous FMS population could drive regional grey matter changes identified using VBM and explain the wide variety in the data.

The cross-sectional nature of structural imaging studies dictates that it is impossible to infer whether structural alterations pre-exist, and increase the probability of spontaneous or environmentally triggered FMS, or occur as a result of prolonged chronic pain symptoms (Schmidt-Wilcke and Clauw, 2011). However, it is postulated that dynamic, reversible neuroplastic alterations are likely in chronic pain disorders (Seifert and Maihöfner, 2011), and a longitudinal study of chronic pain patients with severe osteoarthritis undergoing hip arthroplasty has shown that grey matter alterations normalise post-operatively with pain reduction (Gwilym et al., 2010). Morphological alterations have implications for functional abnormalities, and in the past, grey matter atrophy was purportedly related to dysfunctional endogenous pain inhibition and deficits in cognitive processing in chronic pain populations (Park et al., 2001; Luerding et al., 2008).

Previous morphological findings in FMS patients utilising VBM analyses show a range of discrepancies. The majority of previous studies employed patient cohorts with a mean age of over 50 years (Kuchinad et al., 2007; Schmidt-Wilcke et al., 2007; Luerding et al., 2008; Lutz et al., 2008; Burgmer et al., 2009; Valet et al., 2009). This is problematic as it was previously shown that various brain structures are susceptible to age-related decreases in grey matter volumes (Ziegler et al., 2012). It would therefore be preferable to implement VBM analysis in a younger FMS patient cohort. In order to increase homogeneity within the patient group (and reduce

spurious findings which may be indicative of comorbidities), a strict medication and comorbidity criteria should be employed and patients with past history of major disease, head injury or substance abuse should be excluded.

1.4.2.1. White matter tissue abnormalities and FMS

VBM analyses are only suitable for observing macroscopic changes in morphology, and are predominantly used to investigate regional grey matter alterations. However, diffusion-weighted MR imaging (which is used to assess the directional movement of water through brain tissue), and specifically diffusion tensor imaging (DTI, see chapter two) analysis can be used to assess the complexity of brain microstructure in the white matter of the brain (Sundgren et al., 2004). It has also been hypothesised that DTI analysis may possess improved sensitivity to morphological changes in FMS compared to VBM and other structural analysis methods (Lutz et al., 2008).

Using DTI analysis, white matter alterations have previously been identified in irritable bowel syndrome (Chen et al., 2011), chronic regional pain syndrome (Geha et al., 2008) and neuropathic pain patients (Gustin et al., 2010). The method has also been used to evaluate deterioration of white matter microstructure associated with ageing (Pfefferbaum et al., 2000). In FMS patients, reduced fractional anisotropy (FA) values were observed in the right thalamus, particularly in patients exhibiting greater clinical pain; these changes were attributed to neuronal disorganisation rather than to ongoing axonal degeneration (Sundgren et al., 2007). Similarly, bilateral thalami, insulae and thalamocortical tracts showed significant decreases in FA values relative to healthy people, indicating degeneration or

disorganisation of white matter. Several FA alterations demonstrated correlations with clinical measures of symptom severity (Lutz et al., 2008).

Despite the apparent white matter findings, these studies also raise methodological concerns. No DTI studies of FMS patients report alterations to FA values at a voxelwise corrected level. Also, previous studies have utilised techniques such as VBM to analyse FA data. This method has been criticised due to apparent registration and alignment issues which threaten the validity of interpretations drawn from voxelwise analyses (Ashburner and Friston, 2001). Similarly, the spatial smoothing required by VBM can jeopardise results, and the arbitrary decisions on the degree of smoothing can also influence findings (Jones et al., 2005). Using a recently developed technique, tract-based spatial statistics, it is possible to compare FA throughout the whole brain without the need for smoothing or uncorrected thresholded analyses to identify alterations (Smith et al., 2006). As described earlier, pathophysiological mechanisms including abnormal processing of somatosensory stimuli or alterations to endogenous pain modulation may be involved in the pathogenesis or maintenance of FMS. White matter tract integrity is required for efficient connectivity between brain structures (Stein et al., 2012), and aberrant processing in FMS could arise due to dysfunction of the white matter tracts connecting relevant cortical structures. Despite this no previous study has applied novel techniques such as probabilistic tractography to investigate the degree of white matter connectivity between structures of interest in an FMS population.

1.4.2.2. Pain processing structure abnormalities

Neural processing of pain is complex and encompasses sensory, affective, cognitive, motor and autonomic components (Seifert et al., 2008). Advances in gy

neural imaging techniques have been employed to investigate the brain structures involved in the processing of painful stimuli, including bilateral thalami, SI and SII, insular, cingulate and prefrontal cortices (Iadarola and Coghill, 1999; Peyron et al., 1999; Treede et al., 1999; Apkarian et al., 2005; Maihöfner and Handwerker, 2005; Seifert et al., 2008; Duerden and Albanese, 2011). Regional grey matter reductions have been seen in a variety of pain processing structures in chronic pain disorders including FMS (Jones et al., 2012). Recently, neuroimaging research has investigated whether central nervous system abnormalities in structures responsible for defective pain processing could be the underlying cause of FMS symptoms (Schweinhardt et al., 2008). Regional decreases in grey matter densities in FMS patients relative to healthy people were previously identified in brain areas associated with pain processing such as cingulate, insular, middle frontal cortices, parahippocampal gyri, thalami and superior temporal gyrus (Kuchinad et al., 2007; Schmidt-Wilcke et al., 2007). Similarly, VBM analyses of *a priori* selected regions of interest in pain processing structures show reduced grey matter volumes in the cingulate, prefrontal and insular cortices, hippocampi and amygdala in FMS patients relative to healthy people (Lutz et al., 2008; Burgmer et al., 2009; Robinson et al., 2011).

White matter alterations have also been demonstrated in pain processing structures such as the thalami and insulae of FMS patients (Sundgren et al., 2007; Lutz et al., 2008). Reviewers have hypothesised that white matter alterations in the thalami may be linked to functional changes and increased sensory input to primary somatosensory cortices in FMS patients (Gracely and Ambrose, 2011), which may in turn relate to the hyperalgesia, allodynia or abnormal processing of peripheral afferents which contribute to FMS pain (Staud et al., 2009). Although reduced

functional connectivity has been demonstrated between structures involved in endogenous pain modulation in FMS using fMRI studies (Jensen et al., 2012), no studies have investigated white matter connectivity between such regions in FMS. The wealth of anatomical evidence indicates that structural alterations in pain processing structures of FMS patients are likely, but unfortunately, perhaps as a result of heterogeneity in the patient population, the previous findings demonstrate a lack of consistency and replication.

1.4.2.3. Subcortical alterations and FMS

Morphological alterations in subcortical structures may relate to abnormal processing of peripheral input in FMS (Staud, 2010), and this may relate to dysfunctional endogenous pain modulation (Staud, 2011a). VBM studies have previously reported regional grey matter reductions in subcortical structures such as hippocampi (Lutz et al., 2008), brainstem (May, 2009) and thalami (Schmidt-Wilcke et al., 2007). DTI investigations also indicate morphological alterations in the thalami of FMS patients (Sundgren et al., 2007; Lutz et al., 2008). Reduced resting cerebral blood flow in the thalamus and basal ganglia structures is the most common functional finding using PET with FMS patients (Jones et al., 2012). Recently, a novel method of subcortical shape analysis of structural MRI scans has been established which can identify complex morphological and volumetric alterations to subcortical structures more precisely than VBM (Patenaude et al., 2011).

This method was recently used to elucidate subcortical abnormalities in long term abstinent alcoholics (Sameti et al., 2011), patients with Alzheimer's disease (Zarei et al., 2010) and age-related changes in healthy populations (Goodro et al., 2012). Despite the previous evidence indicative of structural alterations to

subcortical structures in chronic pain disorders including FMS (May, 2009), no previous anatomical studies of FMS patients have applied analysis methods which prioritise the investigation of subcortical structural alterations. Furthermore, in spite of the accepted relevance of subcortical structures in pain processing and particularly endogenous pain modulation, this recently developed, novel technique of subcortical shape analysis is yet to be utilised in any chronic pain population.

1.4.2.4. Arnold Chiari Syndrome

Chiari I malformation (CIM) is a hindbrain malformation where the cerebellar tonsils extend below the foramen magnum, which may develop due to a small posterior fossa (Watson et al., 2011). Studies report that FMS is over 10 times more likely to occur following a neck injury than injury to lower extremities (Buskila and Neumann, 1997; Buskila et al., 1997; Heffez et al., 2004), which suggests an important cervico-spinal aspect of FMS pathophysiology. Furthermore, chronic pain syndromes such as FMS, complex regional pain syndrome and temporomandibular disorder are common among CIM patients (Thimineur et al., 2002), and CIM symptoms show considerable overlap with those seen in FMS (Holman, 2008). These links have led to some FMS patients being surgically treated for presumed CIM pathology, which was shown to be effective in relieving pain and fatigue in suitable FMS patients (Heffez et al., 2004; Heffez et al., 2007). However, the prospect of a surgical treatment for FMS raises economic and efficacy issues, (Wilke, 2001), particularly as these non-randomised studies recruit volunteer FMS patients whose desire for a definitive cure may compromise the validity of the findings (Watson et al., 2011). Previous studies suggesting a link between FMS and CIM are uncontrolled (Heffez et al., 2004; Holman, 2008), and often present a

retrospective analysis of existing MR data (Thimineur et al., 2002). Therefore, it was recently postulated that if CIM was to contribute to FMS it would be likely to only affect a small subset of the heterogeneous population (Watson et al., 2011).

1.5. Psychological aspects of FMS

Fibromyalgia is associated with psychological disturbance such as depression and anxiety (Wolfe et al., 1990; Raphael et al., 2006; Bennett et al., 2007; Fietta and Manganelli, 2007). Depression in FMS patients has been shown to correspond with reduced pain thresholds (Chiu et al., 2005), deficits in endogenous pain inhibition (de Souza et al., 2009), and augmented experimental and clinical pain in patients (Staud et al., 2003; van Middendorp et al., 2010b). FMS patients are also significantly more likely to exhibit general anxiety disorders, post-traumatic stress disorder, obsessive compulsive disorder and social phobias than healthy people (Arnold et al., 2006). FMS may share pathophysiological mechanisms with anxiety disorders (Pae et al., 2009), and familial studies have indicated potential shared genetic loading for FMS and psychological disturbance (Hudson et al., 1985). Similarly to depression, anxiety disorders may be related to increased severity of symptoms and reduced coping capabilities in FMS (Thieme et al., 2004). The prevalence of psychological disturbance coupled with the lack of an apparent physiological explanation led some researchers to categorise FMS as a somatic component of depression (Meyer-Lindenberg and Gallhofer, 1998). However, research has demonstrated that pain in FMS patients appears to be independent of psychological factors (Kurtze et al., 1998; Kurtze and Svebak, 2001).

FMS is associated with emotional disturbances such as increased levels of negative affect, reduced positive affect and altered emotional regulation strategies

relative to healthy people and other chronic pain populations (Zautra et al., 2005; van Middendorp et al., 2008). Increased negative affect and dysfunctional affective processing in FMS patients has been linked to clinical symptom severity (Bartley et al., 2009; van Middendorp et al., 2010a; van Middendorp et al., 2010b). Patients also report cognitive deficits commonly known as 'fibro fog' (Baumstark et al., 1993) which can affect aspects of attention and working memory (Park et al., 2001; Leavitt and Katz, 2006; Dick et al., 2008). It was previously suggested that much of the cognitive impairment seen in FMS can be accounted for by variables such as depression and fatigue (Sephton et al., 2003; Suhr, 2003). However, research has shown that cognitive deficits in FMS are related to, but not dependent on such extraneous factors (Park et al., 2001; Dick et al., 2008).

Researchers have previously utilised the psychological components of fibromyalgia to categorise subgroups within the heterogeneous FMS population. Turk et al. (1996a) categorised patients according to their West-Haven Multidimensional Pain Inventory scores (Kerns et al., 1985), or depending on whether or not their fibromyalgia was judged to be idiopathic or a post-traumatic reaction (Turk et al., 1996b). Subgroups of FMS patients have been shown to display distinct illness behaviours. Specific emotional or affective disorders are more prevalent in specific subgroups, and different subgroups respond to different treatment plans (Thieme et al., 2004). Abnormal levels of stress related adrenocorticotrophic hormone have also been utilised to differentiate between subgroups identified in FMS patients and results indicate that lifestyle stress could be an important factor in psychological pathology associated with FMS (Thieme et al., 2005).

1.5.1. Psychological constructs and FMS

Cognitive-affective factors are also relevant to our understanding of FMS. Patients demonstrate increased vigilance to pain compared with other chronic pain patients (Crombez et al., 2004), which may represent scanning of the body for somatic information due to constant pain (Eccleston and Crombez, 1999), or difficulty in disengaging from pain cues (Van Damme et al., 2002). FMS patients also report increases in helplessness (Palomino et al., 2007) and fear of pain (Turk et al., 2004). Patients also exhibit altered neural processing during anticipation of pain, and this dysfunction is related to psychological and clinical factors (Jones et al., 2012). It is likely that cognitive factors and attentional processes play a significant role in the augmentation of pain in FMS and the subjective experience of the disorder (Crombez et al., 2004). Furthermore, abnormal psychological constructs may interact and influence the development and maintenance of FMS symptoms, as proposed in the fear-avoidance model (Vlaeyen and Linton, 2000), which postulates that chronic pain may develop as a result of avoiding movements or actions due to fear of pain.

Pain catastrophising, an exaggerated negative mindset affecting the anticipation of pain and subjective pain experience (Sullivan et al., 2001), is the psychological construct which has undergone most research in FMS patients. Pain catastrophising is evaluated using the Pain Catastrophising Scale (PCS, Sullivan et al., 1995), and PCS scores have been shown to be directly associated with the degree of disability and functional impairment in FMS patients (Nicassio et al., 1995). Increased PCS scores were previously identified in FMS patients compared with controls (Martin et al., 1996) and other chronic pain patients (Crombez et al., 2004).

Neuroimaging studies using fMRI have shown that increased pain catastrophising in FMS patients correlates with augmented pain activations in brain structures associated with attentional and emotional aspects of pain (Gracely et al., 2004). PCS scores can be used to target specific subgroups with either cognitive behavioural therapy, which demonstrated improved efficacy with high pain catastrophisers, or operant conditioning therapy, which was better suited to low pain catastrophisers (Thieme and Gracely, 2009).

An augmented pain catastrophising trait was also previously linked to exaggerated intensity of pain perceived in others in healthy people (Sullivan et al., 2006). Observing pain in others requires complex affective processing to empathise with the physical and emotional experience of another person (Preston and de Waal, 2002). Functional MRI Studies show that structures commonly activated during pain processing, such as anterior cingulate cortex, bilateral insulae and thalami, are also active when observing pain pictures (Singer et al., 2004; Jackson et al., 2005; Jackson et al., 2006; Singer et al., 2006; Cheng et al., 2007; Gu and Han, 2007; Akitsuki and Decety, 2009; Lamm et al., 2011). This evidence supports the perception-action model of empathy (Preston and de Waal, 2002), which asserts that the perception of another in a particular state leads to the activation of the same state in the observer (Decety and Lamm, 2006; Lang et al., 2011).

In spite of the relevance of psychological constructs such as pain catastrophising to clinical symptoms of FMS, and also to the processing of perceived pain, no studies have yet investigated the neural correlates of observed pain in FMS patients. Alterations to the cortical activations associated with observing pain in

FMS patients could relate to abnormal psychological constructs such as hypervigilance to pain cues, pain catastrophising and empathy.

1.5. *Summary*

FMS is a prevalent chronic pain syndrome, affecting between 2−5% of the general population and it is more common in women and in middle age (McBeth and Mulvey, 2012). FMS shows frequent comorbidity with various clinical, functional and psychological disorders (Weir et al., 2006), and it is associated with a large socio-economic burden incurring substantial direct costs for medical treatment as well as indirect costs due to reduced productivity and disability (Lachaine et al., 2010). Research has employed psychophysical and psychological testing, functional and anatomical neuroimaging techniques, but the underlying aetiology and pathophysiology of the disorder is still not fully understood (Schmidt-Wilcke and Clauw, 2011).

The data from functional and anatomical studies investigating pathophysiological mechanisms of FMS would appear to support a role of central sensitisation in the genesis and maintenance of FMS symptoms, and this is reflected in the (relative) success of centrally acting pharmacotherapies (Woolf, 2011). However, the mechanisms by which such sensitisation may occur in FMS are not yet fully elucidated (Petersel et al., 2011). Experimental evidence exists to support the possibility of reduced endogenous pain modulation in FMS (Julien et al., 2005; Jensen et al., 2009; Jensen et al., 2012), although it is also important not to overlook the peripheral mechanisms which may contribute to central sensitisation and FMS pain (Staud, 2010). Current data suggests a wide range of functional and structural alterations relevant to FMS aetiology and pathophysiology. However, the current

knowledge of alterations to somatosensory processing during innocuous stimuli in FMS is limited, and the diverse findings of morphological alterations in FMS patients raise more questions than they answer. Further structural and functional imaging research is required to successfully integrate the current knowledge into a model of susceptibility and mechanisms underlying the disorder (Clauw, 2009).

Psychological disturbances are commonly associated with FMS (Bennett et al., 2007) and it has been shown that psychological disturbance can influence pain symptom severity (Staud et al., 2003). However, FMS pain is not dependent on psychological comorbidities and therefore should not be considered as a somatic representation of psychological disturbance (Kurtze et al., 1998). Complex psychological constructs such as hypervigilance to pain and pain catastrophising can also affect the symptoms experienced by FMS patients (Crombez et al., 2004), and it was previously proposed that psychological constructs may mediate comorbidity with psychological disturbance in patients (Thieme et al., 2004). However, the exact role of psychological constructs in FMS is not yet fully understood, and new aspects such as the role of specific personality traits (Martinez et al., 2011) are under review. Despite the findings of hypervigilance to pain cues in FMS patients (Crombez et al., 2004), little is known about neural activations associated with observed pain in FMS. Understanding the psychological components of FMS, and particularly those related to pain, may prove vital to furthering our knowledge of the disorder. Specifically, this approach could lead to improved understanding of the heterogeneity seen in the population, and discerning of more suitable therapeutic interventions (Thieme and Gracely, 2009). Further research is needed to elaborate on the interactions between psychological and pathophysiological aspects of the disorder to improve diagnoses and the efficacy of psychological clinical interventions.

Chapter Two

Theoretical basis of methods

---

2. Principles of electroencephalography

2.1. Physiological basis of the EEG signal

Neurons in the brain communicate via action potentials, discrete spikes in voltage generated in the cell body of axons which propagate along the axon fibre to excitatory or inhibitory terminals known as dendrites. Action potentials occur in milliseconds, and are generally not synchronous, therefore any voltage generated is normally cancelled out and undetectable at scalp electrodes (Speckmann and Elger, 2005). Instead, the basic mechanisms underlying the potentials seen using EEG occur in extracellular space, and are referred to as field potentials (Speckmann et al., 1979).When an action potential travels along an axon fibre ending in an excitatory or inhibitory synapse, a post-synaptic potential occurs, neurotransmitters bind with the postsynaptic cell membrane causing ion channels to open and a potential develops between intracellular and extracellular space (Speckmann and Elger, 2005). Unlike action potentials, postsynaptic potentials can last for hundreds of milliseconds. During a coherent response, thousands may occur in a similar location and orientation due to the macroscopic organisation of dendrites (Fisch, 1999). This allows their effects to summate so that they may be detected as a voltage difference on the scalp using EEG (Nunez and Silberstein, 2000; Lopes da Silva and Van Rotterdam, 2005).

2.1.1. EEG rhythms and generators

EEG activity is often described in terms of rhythmic activity. Oscillations of a voltage potential of a regular shape and duration are commonly seen at scalp electrodes and can be described according to their oscillatory frequency (Steriade, 2005). Previously it was proposed that networks of discrete cortical structures generated frequency specific rhythmic activity seen at the scalp (Andersen and Andersson, 1968; Speckmann et al., 1979). However, it has since been postulated that the complex wave sequences seen in EEG are more likely to originate from interactions between multiple source generators and modulating structures (Steriade, 2001). For example, it was proposed that the thalamus and cortex may interact as oscillatory generators under influence of structures such as brainstem (Steriade, 2005).

The oscillatory activity in the EEG signal can be split into bands based upon the frequency of oscillations. The normal amplitude of frequency band oscillations ranges between 10 and 50 µV, with lower frequencies generally exhibiting larger synchronous amplitudes than higher frequencies (Niedermeyer, 2005). Using EEG, it is possible to investigate amplitude increases and decreases in specific frequency bands in response to experimental paradigms. Research studies have previously dedicated particular attention to alpha (8−13 Hz) and beta (16−30 Hz) band oscillations. Increases in amplitude of a specific frequency band associated with a specific event are referred to as event-related synchronisation (ERS), whereas decreases are described as event-related desynchronisation (ERD) (Pfurtscheller and Aranibar, 1977; Pfurtscheller and Lopes da Silva, 1999).

2.1.1.1 Alpha and beta-band oscillatory changes associated with somatosensory stimuli

Alpha rhythm, or alpha-band describes EEG frequencies occurring in the 8−13 Hz range (Niedermeyer, 2005). Alpha-band oscillations exhibit a typical amplitude of up to 50 μV and show their greatest amplitude over occipital, parietal and posterior temporal regions of the scalp, they are most commonly seen during rest with eyes closed (Niedermeyer, 2005). Rolandic-mu rhythm occurs at a similar frequency to alpha-band rhythm, but differs in its principle topography (occurring mainly over central regions) and physiological interpretation (Niedermeyer, 2005). Beta-band oscillations occur within the 16−30 Hz frequency range (Miller, 2007), generally displaying amplitudes not exceeding 30 μV in adults and typically presenting over much of the scalp (Niedermeyer, 2005).

Historical studies identified rhythmic activity in central scalp regions which was attenuated during tactile stimulation (Jasper and Andrews, 1938; Gastaut, 1952). ERD method analysis (Pfurtscheller and Aranibar, 1977; Pfurtscheller and Lopes da Silva, 1999, Chapter 2.3.1) identified short lasting amplitude decreases of 10 and 20 Hz cortical oscillations occurring during somatosensory stimulation (Chatrian et al., 1959; Pfurtscheller, 1981; Salenius et al., 1997; Cheyne et al., 2003; Stancak et al., 2003) including brushing (Cheyne et al., 2003; Gaetz and Cheyne, 2006). Desynchronisation of the 10 Hz oscillations during sensorimotor tasks refers to attenuation of central rolandic-mu rhythm rather than posterior alpha (Salenius et al., 1997). Somatosensory stimuli are also followed by increases in specific cortical oscillations known as ERS (Pfurtscheller, 1981, 1992).

Generally, ERD associated with somatosensory stimulation is localised to foci overlying somatosensory cortices (Pfurtscheller, 1992; Neuper and Pfurtscheller, 2001; Cheyne et al., 2003; Stancak et al., 2003; Gaetz and Cheyne, 2006), and ERD overlying somatosensory cortices contralateral to the stimuli is generally stronger than that located over ipsilateral hemisphere (Stancak, 2006). It was previously demonstrated that patterns of somatosensory ERD/ERS resemble the somatotopic organisation of the sensorimotor cortex (Gaetz and Cheyne, 2006) and source localisation methods were used to locate the source generators of 10 and 20 Hz oscillatory changes, associated with sensorimotor stimulation, to primary somatosensory and motor cortices (Salmelin and Hari, 1994).

The amplitude changes of different frequency bands are independent. Therefore, ERD accompanying sensorimotor tasks can be complemented by concurrent ERS in the same cortical region in an alternative frequency band (Stancak, 2006). To further complicate matters ERD foci may be surrounded by ERS, a phenomenon referred to focal ERD/surround ERS (Suffczynski et al., 2001) which may reflect activity of specific cortical region whilst surrounding areas are inactive (Lopes da Silva, 2006). Amplitude reductions of cortical oscillations in the 8−13 Hz and 16−30 Hz bands, localised to specific cortical regions, can be physiologically interpreted as a correlate of cortical activation (Pfurtscheller and Lopes da Silva, 1999). This is supported by multi-modal findings which show that the amplitude of oscillatory field potentials correlate with hemodynamic measures of brain activation (Logothetis et al., 2001), and ERD was shown to co-occur with increases in BOLD-fMRI signal (Singh et al., 2002; Babiloni et al., 2005; Mantini et al., 2007; Formaggio et al., 2008). The physiological relevance of ERD and ERS accompanying tasks such as experimental somatosensory stimuli suggest that it is

particularly suited to investigations of somatosensory processing. It has not yet been utilised profoundly in clinical populations although previous studies demonstrated reduced amplitudes of beta-band ERS following somatosensory stimuli in chronic region pain syndrome patients (Juottonen et al., 2002) and altered ERD patterns in chronic lower back pain patients during somatomotor stimulation (Jacobs et al., 2010).

2.2. EEG signal acquisition and processing

In the most basic terms, an EEG recording encompasses the measurement and amplification of fluctuating electrical field potentials across time (Kamp et al., 2005). EEG recordings are well established in terms of clinical use, and EEG is currently utilised as a diagnostic and monitoring tool for epilepsy (Thompson and Ebersole, 1999) and sleep disorders (Freedman, 1986). Recent developments suggest that clinical use could eventually extend to include diagnosis of autism (Murias et al., 2007).

During a conventional EEG, electrodes are positioned on the scalp and a suitable conducting gel, paste or liquid is used to apply electrodes. The location of electrodes commonly corresponds to (a contemporary derivative of) the Standardised International 10–20 system, which is based upon relative distance measurements using internationally recognised anatomical landmarks on the skull (Jasper, 1958; Klem et al., 1999). This standardisation means that the names and corresponding locations of electrodes are consistent across various countries and laboratories. A typical adult scalp EEG signal ranges between approximately 10 and 100 µV in amplitude (Aurlien et al., 2004), and needs to be considerably amplified before it can be accurately measured (Luck, 2005). The resulting amplified voltage fluctuations

are digitised, and the digital recording is used for display and analysis purposes. Each individual electrode signal represents the voltage difference between a specific electrode and a reference electrode signal (Luck, 2005). There are numerous methods used to provide the reference value; such as the mean recordings from electrodes positioned over bilateral mastoid processes, a common average reference which represents the mean signal of all EEG channels or Laplacian data which is a comparison between each electrode and the weighted average of the immediately surrounding electrodes (Nunez et al., 1997).

2.2.1. Volume conduction problem

The voltage difference detected by scalp EEG electrodes relates to the location, size and orientation of the dipole caused by cortical activity. However, it also depends on the conductivity and resistance of the brain, fluid and skull tissues, referred to as volume conductivity (Lopes da Silva and Van Rotterdam, 2005). Mathematical rules can be applied to understand how conductivity influences the measurements from the scalp. For example, as voltage fields reduce by the square of distance, deeper sources are more difficult to detect than those near the scalp (Klein and Thorne, 2007). Algorithms designed to explain, eliminate or reduce the attenuating effects of volume conductivity are continually being developed and improved. A classical model using 3 concentric spheres with various conductivities was developed in the 1960's (Geddes and Baker, 1967), and iterative improvements, such as more realistic head models with more accurate conductivities (Goncalves et al., 2000) are continually being developed.

2.2.1.1. Advantages and limitations of EEG recordings

EEG has superb temporal resolution and can detect electrical changes over the course of milliseconds (Schneider and Strüder, 2012). This allows for a more direct understanding of the processing of stimuli, and experimental manipulations can be used to investigate specific aspects of sensory or cognitive processing which can be more accurate and revealing than behavioural measures such as reaction times (Luck et al., 2000). EEG also provides a comparatively direct measurement of neuronal activity as opposed to indirect hemodynamic responses measured in fMRI or positron emission tomography (PET) (Hari et al., 2010). There are also considerable practical advantages to EEG recordings. Some EEG systems, such as the Biosemi Ag-AgCl active-two electrode system (Biosemi B.V, Amsterdam, Netherlands), are mobile and can be transported to appropriate locations for the study of clinical populations. Furthermore, this particular system does not require electrical field shielding and minimises electrode impedance outputs by integrating the first stage of amplification in the electrode itself (Metting van Rijn et al., 1990). Another important practical consideration relates to the fact that EEG research is comparably inexpensive compared to fMRI, magnetoencephalography or PET (Schneider and Strüder, 2012).

The major limitation of EEG investigation, in comparison to functional methods such as fMRI for instance, is reduced spatial resolution. As mentioned earlier, because EEG is recorded via electrodes located on the scalp the electrical signal is attenuated by the tissues it has to pass through, such as the meninges, cerebrospinal fluid and skull (Nunez et al., 1997). Definitive identification of the source of the electrical activity is impossible, commonly known as the inverse

problem. Complex analysis methods allow for the implementation of mathematical algorithms which may be used to reconstruct intracranial sources for a given EEG signal, but these are limited by the accuracy of conductivity models and brain templates utilised to subjective data (Schneider and Strüder, 2012).

2.2.1.2. Artifact rejection in EEG analysis

EEG electrodes are also sensitive to artifacts, electrical signals which do not originate from within the brain. There are physiological causes of artifacts, such as electrical activity in muscles associated with eye blinks (electrooculagraphic activity, EOG), heart beats (electrocardiographic activity, ECG), muscle movements and respiration. Other artifacts can originate from electrode problems, or electrical noise from alternating current electrical appliances (causing a 50 Hz wavelength artifact in the recording). Such issues can be corrected manually by disregarding trials containing artifacts following a visual inspection. Alternatively, independent component analysis techniques (Jung et al., 2000) can be employed. These utilise mathematical algorithms to isolate the average EEG signal component responsible for a specific artifact, e.g. EOG or ECG artifacts. This component is then subtracted from the EEG signal to leave behind 'clean' data (Luck, 2005).

2.3. Quantitative Analyses

2.3.1. Event-related potential analysis

The term 'event-related potential' (ERP) refers to the time-locked EEG changes seen at electrodes in relation to the onset of a stimulus such as a visual, somatosensory or auditory presentation (Lopes da Silva, 2005). ERPs are utilised for quantitative analyses of EEG to compare neurophysiological responses to specific

stimuli or events between groups or conditions (Lopes da Silva, 2005). In the past, ERP analysis has been utilised to assess clinical disorders and to infer the nature of neurophysiological dysfunction (Duncan et al., 2009).

A relatively large number of ERP responses are required for successful analysis (Lopes da Silva, 2005). However, as ERPs represent time-locked responses, sufficient ERP waveforms can be time-averaged to generate a robust mean waveform with positive and negative voltage deflections which are referred to as components (Luck, 2005). ERP components can be quantitatively investigated in terms of latency or amplitude and this is done extensively in cognitive neuroscience and psychophysiological research (Luck, 2005). Deviations in component amplitudes and/or latencies can be used to make neurophysiological inferences about a process or population (Duncan et al., 2009). Using source localisation methods, it is also possible to infer the cortical regions responsible for deviations in ERP components (Lopes da Silva, 2005). The advantages of ERP analysis overlap with those of EEG itself. ERP method benefits from excellent temporal resolution; it is a direct measure neuronal activity and is relative low in cost compared to fMRI. The obvious disadvantage of ERP method is the large number of trials required for quantitative assessment. It is also important that trials do not coincide with ongoing spontaneous activity which could be misinterpreted as event-related data (Lopes da Silva, 2005).

2.3.2. Event-related synchronisation/desynchronisation analysis

Even during periods of relative inactivity, spontaneous, ongoing brain activity occurs and features as rhythmic activity in EEG recordings (Niedermeyer, 2005). Short lasting amplitude increases or decreases of these spontaneous cortical rhythms, known as event-related synchronisation/desynchronisation (ERS/ERD)

(Pfurtscheller and Aranibar, 1977; Pfurtscheller and Lopes da Silva, 1999) may accompany events such as sensory stimuli or cognitive processes. These changes in oscillatory patterns can be quantified by comparing oscillatory power during an event or task related period to a reference interval using the following equation:

$$\text{ERD}j \; = \; \frac{Pj - R}{R} \times 100$$

where j is the band power time series sample, P_j is the power at the jth sample and R is the average power in the reference interval (Pfurtscheller and Aranibar, 1977).

Increases and decreases in amplitude of frequency band oscillations accompany events such as somatosensory stimulation (Chatrian et al., 1959; Pfurtscheller, 1981; Stancak et al., 2003) or motor movements (Pfurtscheller et al., 1993; Stancak and Pfurtscheller, 1996, 1997; Neuper and Pfurtscheller, 2001). ERD/ERS analysis method is used to quantitatively compare event-related amplitude increases or decreases between groups or across various experimental conditions. Amplitude reductions of alpha and beta-bands oscillations can be physiologically interpreted as a correlate of underlying cortical activation (Pfurtscheller and Lopes da Silva, 1999), which accords with hemodynamic findings which demonstrate that BOLD-fMRI signal increases correlate with oscillatory field potential amplitude changes (Singh et al., 2002; Babiloni et al., 2005; Mantini et al., 2007; Formaggio et al., 2008). ERD method was previously utilised for clinical research, aberrant responses were seen in Parkinson's disease patients during motor movements (Labyt et al., 2003) and the technique was also used to predict recovery in patients following a cerebrovascular accident (Platz et al., 2002). This methodology has also been used to study oscillatory amplitude decreases associated with painful stimuli (Ploner et al., 2006; Stancak et al., 2012b).

2.4 Principles of magnetic resonance imaging

2.4.1 Introduction and physics of MRI

Magnetic resonance imaging (MRI) is a safe and non-invasive technique used to generate images of internal body structures for clinical and research purposes (Mandeville and Rosen, 2002). The principles underlying this process depend primarily on the measurement of the activity of protons, most commonly found in the form of H^+ hydrogen ions, because they have a large magnetic moment (they spin at an angle which causes a magnetic field). H^+ hydrogen ions are abundant in living tissues, with two occurring in every water molecule (Narashiman and Jacobs, 2002). When a participant is placed in a strong, static magnetic field the protons align with the direction of the magnetic field (longitudinal magnetisation, LM) (Hendee and Morgan, 1984). During an MRI scan a radio frequency (RF) pulse is delivered at a specific frequency, known as the larmor frequency, selected to yield energy only to the appropriate nuclei (H^+ ions), a phenomenon known as resonance. This energy causes the protons to fall out of the state of alignment and their spin axis to tilt (or precess, a state known as transverse magnetisation, TM). Following the offset of the RF pulse the precessional protons immediately begin to re-align with the static magnetic field (longitudinal relaxation). This relaxation generates a radio frequency which causes a small current in a receiver coil located inside the scanner (Hendee and Morgan, 1984). The time taken for the precessing nuclei to return to a longitudinal magnetic state is known as T1 (Hendee and Morgan, 1984).

Conversely, the RF pulse will also cause the TM nuclei to precess in phase, but as soon as the pulse is switched off this state relaxes and the time taken for all of the ions to move out of phase is known as T2 (transverse relaxation) (Narashiman

and Jacobs, 2002). Longitudinal relaxation and transverse relaxation are independent processes but T2 can never exceed T1 (Hendee and Morgan, 1984). Different tissues possess differing T1 and T2 latencies; water has a long T1 and T2 whereas lipids have short T1 and T2 (Hendee and Morgan, 1984; Mandeville and Rosen, 2002). Depending on the parameters used the image can be manipulated to enhance the contrast between specific tissues or tissue properties. By altering time to repeat (the time between RF pulses, TR), or time to echo (time between RF pulse and reception of the signal, TE) it is possible to enhance the contrast of the image to focus on particular tissues. Short TR and TE will cause a T1-weighted image, substances with a short T1, such as lipids will exude a stronger signal intensity, appearing brighter. Alternatively, long TR and TE produces a T2 weighted scan, where long T2 substances (water) will appear brighter (Mandeville and Rosen, 2002).

Spatial information to identify the origin of the MR signal is acquired by applying further magnetic fields, known as gradients (Narashiman and Jacobs, 2002). This causes a distribution of resonance frequencies throughout the scan image with spatial information mapped onto the frequency scale, known as frequency encoding. The gradient field also causes spins to dephase and further spatial information is acquired by considering the duration and magnitude of gradient fields and measuring phasing/dephasing of spins (phase encoding). By tailoring the RF frequency used to target nuclei, the gradient duration and magnitude, and combining frequency and phase encoding, pulse sequences are designed to focus on specific aspects for the image (Narashiman and Jacobs, 2002). The process of image reconstruction is similar to that used in computerised tomography. Complex algorithms such as fast Fourier transforms are applied to phase and frequency encoded data, although the exact nature of mathematical transformation depends on

the pulse sequence and selected acquisition method (Hendee and Morgan, 1984; Narashiman and Jacobs, 2002). Eventually, by considering the intensity and location of signal, an image can be computed.

2.4.2. Functional MRI

MRI is unable to directly measure neuronal activity (Mandeville and Rosen, 2002), but with manipulation of parameters it can be used to image brain function indirectly. Long TR and TE parameters may be used to generate a T2 weighted image, which weights the scan in favour of imaging water. As water molecules behave differently in the vicinity of paramagnetic fields, contrast agents with paramagnetic properties in the blood can be used to evaluate blood flow or volume changes (Narashiman and Jacobs, 2002). The first functional recording of brain activation in humans using MRI utilised a contrast agent to identify regional blood volume increases during a visual stimulus (Belliveau et al., 1991), and water diffusion was also used to image for blood flow abnormalities as an early detection tool for ischemia (Moseley et al., 1990a). In order to suitably link blood flow to neuronal activation, an appropriate contrast agent is necessary. Exogenous contrasts agents were initially utilised but they are associated with the same drawbacks as contrasts agents in PET and suffered from poor temporal resolution (Mandeville and Rosen, 2002). Instead, a safe endogenous contrast agent would be preferable, and this is what is commonly used in fMRI today.

2.4.2.1. The BOLD Signal

Deoxyhemoglobin in deoxygenated blood possesses paramagnetic properties and attenuates MR signal more than oxyhemoglobin which is diamagnetic, this difference causes T2 signal intensity changes (Thulborn et al., 1982). During

localised cortical activation of brain structures increases in regional CBF exceed the cerebral metabolic oxygen utilisation rate in the region resulting in a surplus of oxygenated blood (Fox and Raichle, 1986). T2 signal loss around veins was shown to vary depending on blood oxygenation levels (Ogawa et al., 1990b) and the blood oxygen level dependent (BOLD) signal was proposed as a naturally occurring contrast agent to infer hemodynamic responses (Ogawa et al., 1990a; Ogawa et al., 1990b). BOLD signal intensity reflects the surplus of oxygenated blood in a region and thus regional activation (Kim and Ogawa, 2012). A study utilised simultaneous fMRI and EEG recordings to show that the BOLD signal correlates more with local field potentials than with individual neuronal activity (Logothetis et al., 2001). However, it should be noted that the exact nature of the relationship between BOLD signal and neuronal activation is complex and still debated (Ekstrom, 2010).

BOLD was previously purported to be superior to exogenous contrast agents in terms of safety and temporal resolution (Ogawa et al., 1990a). BOLD signal acquisition has since been refined in terms of hardware, analysis methods and experimental design parameters and is considered one of the most important functional measures of neuroimaging (Kim and Ogawa, 2012). In a typical fMRI scan the brain is scanned at a low spatial resolution to allow for a rapid rate of image acquisition, and the scans are T2 weighted to detect the alteration caused by deoxygenation. As deoxyhemoglobin attenuates the MR signal, a regional hemodynamic response associated with a surplus of oxygenated blood results in a greater signal intensity (Mandeville and Rosen, 2002).

2.4.3. High-resolution T1-weighted structural images

Anatomical T1-weighted MRI scans utilise superior spatial resolution than functional data and are routinely acquired in neuroimaging studies (Howarth et al., 2006). Employing short TR and TE times results in a T1-weighted scan, and substances with a short T1 (such as lipids) generate a greater signal intensity than those with a long T1 (water). As white matter axons in the brain are surrounded by a fatty myelin sheath, T1-weighted images result in an excellent contrast between grey and white matter (Narashiman and Jacobs, 2002). These images can be used to accurately co-register findings from functional scans, or for clinical evaluation. Researchers can employ voxel-based morphometry analysis of high-resolution T1-weighted images to assess regional variations in macroscopic grey and white matter (Ashburner and Friston, 2000), or to assess subcortical alterations to geometric shape (Patenaude et al., 2011). Scanning parameters can be specifically tailored to prioritise resolution and contrast for the research of specific structures or tissues (Thomas et al., 2005), and images can be pre-processed prior to analyses to further improve tissue classification for research purposes (Ashburner and Friston, 2005).

2.4.4. Diffusion tensor imaging (DTI)

As detection of the MR signal relies on H^+ nuclei (protons) which are primarily present in water molecules, movement of water can affect MR spins-relaxation times and therefore signal (Mori and Zhang, 2006). Diffusion-weighted imaging employs complex pulse sequences with multiple stimulating echo pulses to allow for sensitivity to water diffusion effects. Multiple gradient fields are also utilised to infer the location, direction and degree of diffusion of water molecules (Narashiman and Jacobs, 2002). The direction of water diffusion is sensitive to the

direction or axis of the gradient from which it is measured; therefore, to effectively assign the direction of diffusion in each voxel it would be necessary to measure diffusion along an infinite number of axes. To combat this problem, the concept of the diffusion-tensor was introduced in the early 1990's (Basser et al., 1994).

Diffusion-tensor imaging (DTI) is an MRI method which measures the directional diffusion of water molecules throughout each voxel in the brain. DTI scans employ multidimensional vector algorithms and utilise at least six diffusion gradients. This is the minimum number of gradients required to elucidate the characteristics of a diffusion ellipsoid which is used to represent the directional water diffusion in each voxel and to generate a directional tensor for each voxel (Mori and Zhang, 2006). Water in the white matter of the brain diffuses more rapidly in the direction analogous with the structure of the axon than in any other directions (Stejskal, 1965). Therefore, when anisotropic (directional) diffusion is mapped throughout the brain it can be used to provide an alternative form of image contrast which is indicative of aligned anisotropic structures (Chenevert et al., 1990; Moseley et al., 1990b; Turner et al., 1990). The resolution of a DTI scan means that, although the movement of water molecules is identified at the molecular level, the actual data represents the average of anisotropic movement evident within the resolution of a single voxel. Therefore, diffusion data is used to infer bundles of fibers or tracts in white matter rather than individual axons (Beaulieu, 2002).

After the diffusion ellipsoid and tensor is determined, the vector of the longest axis (eigenvector v1) can be calculated to elucidate the fiber orientation (Mori and Zhang, 2006). The data from the diffusion-tensor of each voxel can be used to calculate fractional anisotropy (Pierpaoli and Basser, 1996), the relative

degree of directional diffusion, which can be utilised in quantitative analyses of white-matter tracts throughout the brain. The principle fibre direction for each voxel can also be utilised to trace white matter pathways using methods such as probabilistic tractography (Mori and Zhang, 2006). For visualisation purposes, DTI data is usually displayed using a colour-coded orientation map to indicate the direction of movement, as well as the degree of diffusion (Makris et al., 1997; Pajevic and Pierpaoli, 1999).

2.4.4.1. Fractional anisotropy

Water molecules in the brain are constantly in motion. Anisotropy is the term used to describe the directional properties of a process such as the diffusion of water molecules. Fractional anisotropy (FA, Pierpaoli and Basser, 1996) refers to a value, between zero and one, which is used to quantify relative directional properties of the diffusion process to describe the relative degree of anisotropic to isotropic diffusion. A higher fractional anisotropic value would indicate an ellipsoid that was 'cigar shaped', and indicative of a strong, linear directional diffusion (Westin et al., 2002). The actual FA value is calculated by using mathematical operators to divide the difference in diffusivity between the longest axis and the average of the two shorter axes of the voxel ellipsoid, by the mean diffusivity of all axes. The resulting value is then normalised to achieve a relative FA value of between $0-1$ (0 = purely random diffusion, 1 = purely anisotropic diffusion = 1) (Westin et al., 2002).

2.5. Summary

To summarise, EEG and MRI may both provide rich datasets which can be analysed using a wide variety of procedures. Each method brings its own particular strengths and weaknesses, with the excellent temporal resolution of EEG comparable

to the superior spatial resolution of MRI. The present thesis employs multi-modal imaging of FMS patients and a healthy control group to investigate a variety of research questions. EEG and MRI data are analysed to evaluate structural and functional abnormalities evident in FMS patients relative to healthy people. EEG analysis methods range from classical ERP analysis of a novel paradigm, to ERD analysis, which has scarcely been utilised with chronic pain patients. MR analysis methods include novel approaches to functional and structural imaging analysis including recently developed techniques such as probabilistic tractography of DTI data and subcortical shape analysis of T1-weighted anatomical images.

Chapter Three

Problems and hypotheses

---

3. FMS: The problem

Fibromyalgia may be caused by structural and functional alterations to central processing (Woolf, 2011). However, the specific mechanisms by which such abnormal processing could arise and be maintained in FMS are not yet fully understood (Schmidt-Wilcke and Clauw, 2011). Evidence suggests that a complex array of morphological, neurophysiological and psychological alterations could be responsible for FMS symptoms (Petersel et al., 2011). However, the present understanding of complex pathophysiology of FMS has resulted in a wide-range of treatment plans which usually exhibit relatively low efficacy, and often leave patients unsatisfied (Schmidt-Wilcke and Clauw, 2011).

The study of a complex clinical syndrome such as FMS for eventual dissemination as a thesis brings about a unique set of challenges. The study of National Health Service (NHS) patients in the United Kingdom requires that experimenters undergo appropriate clinical training and successfully apply for either a research passport or honorary contract with the NHS. A further requirement asserts that research sessions can only be performed on suitable NHS premises. However, the primary concern regarding research of this nature relates to required ethical approval application procedures. In order to commence the study of NHS patients, ethical approval is required from the National Research Ethics Committee (see appendix). This encompasses a thorough, time-consuming and demanding process,

and approval is required for each specific study design. Within the time-constraints of a standard research degree, it is not plausible to collect and analyse data in order to design subsequent studies based on the findings. This eliminates the option to submit sequential approval applications for the study of an NHS patient population. To circumvent this problem it was decided to design all studies simultaneously. The current literature pertaining to structural and functional brain imaging of FMS patients was evaluated in detail, and research questions that have not been adequately addressed in previous studies were identified.

All data was acquired in a minimal number of sessions to reduce participant drop-out rate. Two data collection sessions were utilised, the first using EEG recordings and the second encompassing multi-modal MRI scanning. This resulted in the collection of a novel data set in an FMS patient population. Innovative EEG paradigms and various MRI acquisition methods were selected, which require multiple analysis techniques. This method of data collection has unique advantages compared to sequential studies. Each individual study was driven by a relevant research question that was not satisfactorily covered in the existing FMS literature, rather than all studies being driven by a single initial research question. It is an approach which fundamentally requires that the thesis encompasses several recording and analysis methods. The result is a comprehensive account of novel studies utilising multi-modal brain imaging techniques.

In this thesis, functional brain imaging techniques have been employed to evaluate central processing during rest and in the presence of somatosensory and psychological challenges. Structural neuroimaging methods were also used to assess alterations to cortical grey and white matter and subcortical structures. By utilising

newly developed imaging analysis methods and novel experimental paradigms it is hoped that it will be possible to elaborate on the functional and anatomical alterations seen in the central nervous system of FMS patients in previous studies. Further understanding of the central mechanisms involved in FMS is necessary if we are to improve treatment plans in future.

3.1. Brain responses during somatosensory stimulation in FMS patients

FMS patients frequently demonstrate allodynia (Bennett et al., 2007, Chapter 1.3.2.1) and alterations to processing of somatosensory stimuli (Gracely et al., 2002, Chapter 1.3.2), and pain resulting from innocuous stimuli may be an important pathophysiological component of FMS. Dynamic mechanical stimulation, such as brushing, can be utilised to investigate brain activation changes during allodynia. Using this method, abnormal brush-evoked activations were previously seen in neuropathic pain patients (Petrovic et al., 1999; Peyron et al., 2004; Witting et al., 2006) and complex regional pain syndrome patients (Mailhöfner et al., 2006).

As described in Chapter 2, ERD method is suitable for investigations of brain responses to somatosensory stimulation (Stancak, 2006). Alpha and beta-band ERD are linked to underlying cortical excitability (Pfurtscheller and Lopes da Silva, 1999), and this method was previously utilised to investigate brain activations during brushing stimulation in healthy people (Cheyne et al., 2003; Gaetz and Cheyne, 2006). Altered patterns of ERD during somatosensory stimulation infer alterations in cortical excitability, and could result from structural and functional brain alterations. ERD analysis was previously used to investigate cortical activation differences in chronic lower back pain patients, relative to a group of healthy control subjects, during somatomotor stimulation (Jacobs et al., 2010).

No study has yet employed ERD technique during innocuous somatosensory stimulation of FMS patients. Little is known about the mechanisms underlying processing of innocuous somatosensory stimuli in FMS, and improved understanding of this process may reveal more about the central mechanisms underlying the syndrome such as central sensitisation.

- Research question 1: Is the central processing of somatosensory afferents affected in FMS?

Abnormal processing of somatosensory input, such as allodynia, has been linked to the development and maintenance of central sensitisation in FMS (Staud, 2010, 2011b). Despite this, little is known about the central processing alterations that may accompany allodynia in FMS, and further understanding of this aspect of the disorder could reveal more of its underlying pathophysiology. This thesis utilised EEG recordings and ERD methodology to investigate cortical oscillatory changes and corresponding pain during innocuous dynamic mechanical somatosensory stimulation (brushing) in FMS patients to investigate the following hypothesis:

- FMS patients will report subjective pain during innocuous somatosensory stimulation and exhibit alterations to cortical oscillatory amplitude changes, relative to healthy controls.

3.2. Cortical activations in FMS patients during observation of pain pictures

Chapter 1.5.2 examines the evidence of alterations to psychological constructs and their relationship to pain processing in FMS patients. Hypervigilance to pain cues and increased pain catastrophising trait were previously identified in FMS patients (Crombez et al., 2004). It was also shown that psychological factors can affect the symptoms experienced in FMS (Zautra et al., 2005; van Middendorp et

al., 2008; Bartley et al., 2009). ERP technique has been shown to be effective for evaluating neurophysiological responses to emotional stimuli (Lopes da Silva, 2005), and ERP findings can infer neurophysiological dysfunction in clinical disorders (Duncan et al., 2009). ERP technique was previously employed to examine responses to visual pain stimuli in healthy people (Fan and Han, 2008; Han et al., 2008; Proverbio et al., 2009; Decety et al., 2010; Li and Han, 2010; Ibáñez et al., 2011). Early ERP components (110−180 ms post-stimuli) were shown to be modulated when observing pain pictures, and the degree of modulation was associated with the subjective intensity and unpleasantness of perceived pain in healthy subjects (Fan and Han, 2008; Han et al., 2008; Proverbio et al., 2009; Li and Han, 2010). Later components around 300 ms post-stimuli were also shown to be stronger during viewing of pain pictures, and also in females, relative to males. This finding was attributed to a stronger empathic response in females (Proverbio et al., 2009).

Compared to healthy people, FMS patients demonstrate increased subjective displeasure (Bartley et al., 2009), and alterations to somatosensory-evoked potentials (Montoya et al., 2005) when viewing negative affective images. However, it is not known whether the cortical processes associated with observing pain could be affected in FMS. ERP alterations when observing pain pictures may relate to structural or functional brain alterations which could impact upon the symptoms of FMS. Improved understanding of the cortical processes associated with alterations to psychological factors of FMS would enhance the awareness of psychological disturbance in FMS and could lead to more targeted therapies.

- Research question 2: Are the cortical processes associated with observation of pain affected in FMS?

In this thesis an ERP analyses of EEG data was performed to investigate whether cortical activations during observation of pain pictures were altered in FMS patients relative to healthy volunteers. The following hypothesis was investigated:

- FMS patients, relative to healthy control subjects, will attribute stronger pain ratings to pain pictures and manifest alterations to ERP components.

3.3. Resting-state functional connectivity alterations in FMS patients

Ongoing tonic pain is an important symptom of FMS and may contribute to the pathogenesis and maintenance of central processing alterations in the syndrome (Staud, 2010, 2011b). It was previously shown that chronic pain can cause a time-dependent re-organisation of the DMN in chronic back pain patients (Baliki et al., 2008). Resting-state fMRI can be analysed using seed-correlation analysis to evaluate functional connectivity between specific cortical structures (Biswal et al., 1995; Fox et al., 2005; Whitfield-Gabrieli and Nieto-Castanon, 2012). Functional connectivity between structures can be altered as a result of clinical and psychological pathology, and changes may indicate the pathological mechanisms of disorders (Whitfield-Gabrieli and Ford, 2012). Few studies have previously demonstrated alterations to functional connectivity with DMN or pain processing cortical structures in FMS patients (Napadow et al., 2010; Cifre et al., 2012). However, as pointed out in Chapter 1.4.1.2, these few existing studies exhibit some methodological weaknesses which could be improved by incorporating more

stringent exclusion criteria during patient selection, and by utilising novel and recently developed analysis techniques.

In order to expand and elaborate upon previous findings, a younger, homogenous FMS patient population should be utilised as well as the most recently available functional connectivity analysis techniques and appropriate seed regions of interest such as those from large scale meta-analyses.

- Research question 3: Does ongoing pain in FMS affect resting-state functional networks?

Using a novel functional connectivity analysis technique (Whitfield-Gabrieli and Nieto-Castanon, 2012), this thesis aims to investigate whether FMS patients demonstrate alterations to functional connectivity between DMN and pain processing structures at rest to investigate the following hypothesis:

- Functional connectivity with default mode network and pain processing structures will be altered in FMS patients relative to healthy people.

3.4. Morphological alterations to cortical and subcortical structures in FMS patients

Chapter 1.4.2 describes the wide-range of morphological findings previously identified in FMS patients. Macroscopic methods such as VBM have failed to achieve consensus or consistency in results and the wide range of variability could be due to the age of the patients studied in different investigations, or heterogeneity of comorbidities and symptom profiles in experimental populations (May, 2011). Methodological issues surrounding tissue classification or arbitrary smoothing could also affect the validity of VBM findings (Jones et al., 2005; Smith et al., 2006; Patenaude et al., 2011), and it is not suited for the study of deeper regions such as basal ganglia structures. Alternative methods, such as geometric shape analysis of

subcortical structures may be preferable to VBM for identification of subtle morphological alterations (Patenaude et al., 2011). This method can identify the location and direction of complex morphological alterations through direct measurement of geometric shape more precisely than VBM (Patenaude et al., 2011).

Due to the wide range of variability in previous VBM findings, a study is needed to utilise alternative techniques which may be more sensitive to alterations in FMS. Similarly, care should be taken to maximise the heterogeneity within the patient population and reduce the effects of external factors which may affect brain morphology such as old age. This thesis attempts to investigate whether FMS patients exhibit central morphological alterations compared to healthy people, particularly in subcortical structures.

- Research question 4: Is the morphology of subcortical structures of the brain affected in FMS?

The study described in this thesis utilised a novel technique of geometric shape analysis of subcortical structures (which was never previously employed in a chronic pain patient sample) using high-resolution T1-weighted anatomical MR scans. The following hypothesis was considered:

- FMS patients will demonstrate abnormalities to the shape and volume of subcortical structures relative to healthy people.

3.5. Microstructural alterations to white matter structures in FMS patients

As described in Chapter 1.4.2.1, studies of various chronic pain populations have revealed white matter alterations in various pain processing structures (Geha et al., 2008; Gustin et al., 2010; Chen et al., 2011). However, few studies have investigated white matter anatomical alterations in FMS patients. The previous

findings suggest that pain processing structures may demonstrate alterations in the microstructural integrity of white matter in FMS patients (Sundgren et al., 2007; Lutz et al., 2008). White matter alterations in FMS patients could relate to functional abnormalities such as altered functional connectivity between structures, or aberrant processing of somatosensory afferents. Such dysfunction could feasibly cause the symptoms seen in FMS. A previous study reported links between specific white matter abnormalities and symptoms such as fatgue, stress and ongoing pain in FMS patients (Lutz et al., 2008). However, it was recently acknowledged that further understanding is needed to elucidate the underlying pathophysiological relationship between white matter alterations and FMS (Gracely and Ambrose, 2011).

Existing DTI investigations of FMS patients employed techniques such as VBM comparisons of FA values elucidated from DTI images. This method of voxelwise analyses was previously criticised due to methodological issues which threaten the interpretation of results (Ashburner and Friston, 2001). Tract-based spatial statistics (TBSS, Chapter 4.2.6.1) represents a recent advance in statistical comparison of DTI scans which can be used to examine FA values across the whole brain whilst accounting for anatomical variation in white matter tracts without the need for data smoothing (Smith et al., 2006). However, TBSS analysis has never been employed to investigate white matter alterations in FMS patients.

Previously, functional alterations to processing of mechanical stimuli (Gracely et al., 2002), and endogenous pain modulation (Jensen et al., 2009; Jensen et al., 2012) were shown in FMS patients. However, it is not known whether white matter connectivity between the structures involved in processing of somatosensory afferents or endogenous pain modulation could be responsible for such alterations. Novel techniques such as probabilistic tractography, which can be utilised to

investigate white matter connectivity alterations between relevant regions of interest, have not yet been utilised in FMS patients.

- Research question 5: Is the integrity of white matter microstructure altered in FMS patients?

In the present study, DTI scans were analysed to investigate the microstructural integrity of white matter anatomy throughout the entire brain of FMS patients using TBSS analysis in order to evaluate white matter alterations that may relate to FMS symptoms. To further expand upon previous findings probabilistic tractography was performed to investigate white matter connectivity between structures of interest located in regions associated with endogenous pain modulation and somatosensory processing, these specific systems were seleted due to their relevance for FMS based upon the central sensitisation hypothesis. The following hypothesis was investigated:

- FMS patients will show reduced white matter integrity in structures of the brain associated with pain processing, as well as alterations to the connectivity of tracts between structures involved in endogenous pain modulation and somatosensory processing.

3.6. Summary

In order to expand upon the existing knowledge of structural and functional brain alterations associated with FMS, this thesis utilised 5 studies encompassing a variety of EEG and MR analysis techniques. For the EEG data, novel paradigms were employed to investigate phenomenon that were never previously examined in FMS patients. ERD analysis was also utilised for the first time in an FMS patient

group. MR data was analysed using new and novel techniques to enhance the understanding of structural alterations in FMS. Morphological alterations in subcortical structures were investigated for the first time in FMS patients, and contemporary methods were utilised to investigate white matter alterations in specific tracts of high relevance to FMS symptoms. An improved method of functional connectivity analysis was also employed to analyse resting-state fMRI as well as seed locations from recent meta-analyses to build upon the findings of previous investigations.

Chapter 4

Methods, Procedures & Materials

4.1 EEG studies

4.1.1. Patients

Nineteen female patients (age 40.01 ± 7.95 years, mean ± SD) diagnosed with fibromyalgia syndrome took part in the study. Patients were recruited from outpatient fibromyalgia clinics at two regional NHS Foundation Trust hospitals; the Walton Centre, Liverpool, United Kingdom, and Wirral University Teaching Hospital, Wirral, United Kingdom. Informed consent was obtained from all participants in accordance with the Declaration of Helsinki. The study was approved by the National Research Ethics Committee of the United Kingdom and the Research Governance Committees of both NHS Foundation Trust hospitals.

All patients fulfilled ACR criteria for diagnosis with fibromyalgia on the day of recording (Wolfe et al., 1990). Patients with additional disease or disorders such as hypertension, diabetes, previous brain trauma or neurological disorders were excluded. Patients with disorders commonly comorbid with FMS such as arthritis, or functional syndromes such as irritable bowel syndrome, interstitial cystitis, temporomandibular disorder or migraine were not excluded provided that the patient categorised FMS as their primary diagnosis, and declared no transient symptoms from other disorders on the day of testing. Patients aged between 22–52 years were considered for participation. This age criterion is stringent by comparison to previous

studies, and was selected to minimise age-related structural and functional brain alterations in the sample. Mean duration of symptoms was 9.62 ± 6.97 years (mean $\pm$ SD), and mean time since diagnosis was 2.62 ± 1.45 years (mean $\pm$ SD).

As some of drugs used to alleviate pain can modulate the brain activity, withdrawal of some of pain medication was required for 3 to 5 days before sessions. Patients using medications with central nervous system effects, who were not deemed suitable for withdrawal by the clinical team, were excluded. Common medications at stable low doses such as pregabalin (up to 75 mg twice a day), gabapentin (up to 300 mg twice a day) and amitriptyline (up to 10 mg at night), were considered acceptable for the criteria of minimal central nervous effects as designated by the clinical team. Analgesics (such as co-codamol) were withdrawn prior to the recording sessions. For example, a patient taking 6 to 8 tablets of mild co-codamol (8/500mg) a day was asked to discontinue use for 3 days prior to testing, a patient using up to 8 tablets of strong cocodamol (30/500mg), or high doses of dihydrocodeine, was asked to taper the dose over a period of 2 days before discontinuing use for 3 days prior to testing; withdrawal was managed by the clinical team during consultation. Analgesic medications with minimal central nervous system effects such as paracetomol were permitted.

At their request, 4 patients on low dose anti-depressant medication (e.g. 10 mg citalopram per day) were permitted to take part after undergoing withdrawal for at least 5 days prior to recordings, 6 patients were using no medications for management of their FMS. The remaining patients were either using permissible doses of common medications with minimal central nervous efficacy and/or withdrew from non-permitted medications, such as co-codamol, for at least 3 days prior to recordings. For further information regarding the precise medication

withdrawal procedures for all common FMS medications, and exclusion criteria concerning specific comorbidities see the relevant sections in the original ethics application (Appendix.1).

Table 4.1 shows the epidemiological data for all FMS patients participating in the EEG session as well as various clinical and psychological scale scores. All participants completed a series of questionnaires incorporating the Beck Depression Inventory (BDI, Beck et al., 1961) and State and Trait Anxiety Index (STAI, Spielberger et al., 1970) to evaluate mood disorder, Pain Catastrophising Scale (PCS, Sullivan et al., 1995) to measure psychological contructs specific to pain, and the Fibromyalgia Impact Questionnaire (FIQ, Burckhardt et al., 1991) to evaluate the impact of the disorder on quality of life.

4.1.3. Controls

Eighteen, age matched, female controls (age 39.23 ± 7.99 years, mean ± SD) were recruited through internet and campus advertisement. Volunteers aged between 22–52 years were considered and age matched to recruited FMS patients. Volunteers taking regular medication and/or currently diagnosed with any disease or disorder were excluded. All patients and volunteers were compensated for time and travel expenses. Table 4.2 shows the epidemiological data for all healthy control subjects participating in the EEG session as well as various clinical and psychological scale scores.

4.1.4. EEG recordings

Participants were accompanied to the Sensory-Motor Laboratory of Liverpool Pain Research Institute, a dedicated pain measurement laboratory facility, where they underwent electrode preparation. EEG data was recorded using the 64 channel

Biosemi Ag-ACl active-two electrode system (Biosemi B.V, Amsterdam, Netherlands). Electrodes positions were allocated according to the extended 10–20 system with respect to three anatomical landmarks; two pre-auricular points and the nasion. Two bipolar, flat Ag-ACl external reference electrodes were attached to the mastoid process behind each ear. Vertical electro-oculograms were recorded using bipolar electrodes positioned above and below the right eye. The recording bandpass filter was 0.16−100 Hz, and the sampling rate was 512 Hz.

Table 4.1 FMS patient characteristics and clinical data.

Ppt Code	Age	Years Symptoms	Years diagnosis	Hand	Brush	MTPS EEG	MTPS MRI	STAI-S	STAI-T	FIQ	BDI	PCS
FM01	40	12.00	1.50	R	1	6.00	5.50	35.00	52.00	64.70	16.00	16.00
FM02	42	5.00	1.50	R	3	4.61		37.00	41.00	59.02	20.00	21.00
FM03	39	3.00	2.50	R	1	5.83	5.83	41.00	51.00	71.99	21.00	20.00
FM04	22	6.00	0.50	R	1	3.44	3.44	46.00	54.00	68.32	22.00	10.00
FM05	43	4.50	3.00	R	0	6.33	5.83	63.00	62.00	77.75	31.00	14.00
FM06	44	15.00	5.00	R	2	2.28	1.93	41.00	37.00	61.92	8.00	1.00
FM07	33	7.00	3.00	R	1	4.89	4.89	33.00	41.00	55.55	17.00	16.00
FM08	43	4.00	3.00	R	1	4.39	3.72	38.00	54.00	72.15	21.00	12.00
FM09	41	18.00	1.00	R	2	3.56		25.00	44.00	19.11	5.00	14.00
FM10	48	6.00	6.00	R	0	1.61	1.94	28.00	36.00	39.22	14.00	3.00
FM11	37	3.50	3.50	L	2	7.67	7.67	41.00	32.00	56.40	6.00	0.00
FM12	49	3.00	2.00	L	0	2.22	2.44	24.00	22.00	25.36	5.00	2.00
FM13	52	10.00	2.00	R	0	4.94	3.50	35.00	42.00	48.96	14.00	19.00
FM14	46	2.00	1.00	R	2	7.78		57.00	53.00	75.37	20.00	25.00
FM15	45	18.00	3.00	R	0	4.78	3.28	21.00	24.00	48.42	6.00	5.00
FM16	30	4.00	2.00	R	2	8.17	7.89	54.00	71.00	82.56	41.00	28.00
FM17	26	6.00	3.00	L	1	6.44	6.33	39.00	60.00	74.74	20.00	5.00
FM18	32	16.00	4.00	L	0	6.56	6.67	38.00	55.00	69.67	33.00	33.00
FM19	37	4.50	2.00	R	1	4.61	3.89	53.00	70.00	80.23	37.00	31.00

R=Right; L=Left; MTPS = manual tender point scale; STAI-S =state anxiety; STAI-T = trait anxiety; FIQ = Firomyalgia Impact Questionairre; BDI = BeckDepression Inventory; PCS = Pain Catastrophising Scale; Shading = excluded from MRI session

Table 4.2 Healthy control group characteristics and clinical data.

PPT Code	Group	Age	Hand	Brush	MTPS EEG	STAI-S	STAI-T	FIQ	BDI	PCS
FM21	HC	32	R	0	0.78	39.00	45.00	14.36	2.00	4.00
FM22	HC	40	R	0	0.00	20.00	20.00	1.91	0.00	0.00
FM23	HC	30	R	0	0.00	24.00	21.00	0.00	2.00	0.00
FM24	HC	41	R	0	1.17	26.00	34.00	7.00	6.00	9.00
FM25	HC	38	R	0	0.22	32.00	51.00	10.50	6.00	14.00
FM26	HC	51	R	0	0.22	20.00	24.00	0.00	0.00	5.00
FM27	HC	46	R	0	0.00	32.00	38.00	1.00	0.00	5.00
FM28	HC	47	R	0	0.50	23.00	25.00	0.00	2.00	2.00
FM29	HC	37	R	0	0.28	20.00	38.00	4.00	4.00	0.00
FM30	HC	37	R	0	0.44	31.00	31.00	1.43	2.00	11.00
FM31	HC	37	R	0	0.00	20.00	25.00	0.00	2.00	0.00
FM32	HC	48	R	0	0.17	23.00	43.00	11.00	8.00	1.00
FM33	HC	40	R	0	0.00	26.00	40.00	19.36	15.00	16.00
FM34	HC	22	R	0	0.00	28.00	36.00	14.50	0.00	1.00
FM35	HC	27	R	0	0.22	37.00	36.00	8.86	4.00	4.00
FM36	HC	48	R	0	0.17	27.00	34.00	1.43	11.00	4.00
FM37	HC	31	R	0	0.00	25.00	43.00	11.00	2.00	19.00
FM38	HC	45	R	0	0.00	21.00	28.00	0.00	0.00	0.00

R=Right; L=Left; MTPS = manual tender point scale; STAI-S =state anxiety; STAI-T = trait anxiety; FIQ = Firomyalgia Impact Questionairre; BDI = BeckDepression Inventory; PCS = Pain Catastrophising Scale; Shading = excluded from MRI session

4.1.5 Brain responses in FMS patients during somatosensory stimulation: procedure

During the experiment participants were seated in a comfortable chair with eyes closed. Their right forearm rested on a supporting plinth, angled parallel to the floor and with the height adjusted to the participant's comfort preference. The experiment consisted of 2 blocks, each consisting of 20 cycles of 4 s of rest followed by 4 s of mechanical brush stimulation. The experimenter was positioned to the right of the participant and a monitor located in front of the experimenter displayed a visual indicator of brushing and resting periods matched to EEG triggers. During brush periods the experimenter commenced brushing of the participant's right forearm. Brush strokes consisted of one continuous motion from the tip of the right elbow anterior in direction for a distance of approximately 4–5 cm at a rate of 2–3 cm/s for 2 s. Without removing the brush the experimenter reversed direction before returning the brush at a similar speed and pressure to the tip of the elbow. This region of the forearm was selected to encompass an FMS tender point (lateral epicondyle), which also refers to a succinct region of the somatosensory cortex homunculus (Nakamura et al., 1998). The brush was removed for the duration of the rest period. The brush used was a standard soft bristled paintbrush; bristles were 6 cm in length, 4 cm wide and 2 cm deep.

Prior to the experiment participants were informed of the procedure and the brushing action was demonstrated by the experimenter. Participants were instructed that the experiment was only investigating pain evoked by the brushing action and not 'ongoing aches and pains'. After each block participants were asked "Did you feel any pain specifically during brushing?" If they answered affirmatively, participants were asked "Would you describe the pain as slight, moderate or severe?"

Responses were scored on a 4-point Likert scale as follows; 0 for 'no pain reported', 1 for 'slight pain', 2 for 'moderate pain' and 3 for 'severe pain'.

4.1.6 Cortical activations in FMS patients during observation of pain pictures: procedure

To investigate cortical activations during observation of pain pictures, participants remained seated in the same comfortable armchair and viewed a 19 inch computer monitor positioned 1.0 m in front of them. Their right forearm rested on a table, with the height adjusted to the participant's comfort preference, a computer mouse was place in their right hand with the right forefinger positioned over the left mouse button. The experiment consisted of a single recording encompassing viewing of 100 trials and lasting 20 minutes. Each trial began with 3 s resting interval during which subjects viewed a black fixation cross on a grey background, a colour photographic image was then presented on the grey background for 3 s followed by a second resting interval of 2 s and a 4 s response epoch (Fig. 4.1). During the response epoch a seven-point rating scale with anchors 'no pain at all' (1) and 'worst possible pain' (7) was presented in the form of seven horizontally aligned dark grey rectangles appearing on a light grey background. Participants were required to repeatedly click the left mouse button with their right forefinger to increment the scale, highlighting subsequent rectangles in yellow, to attribute the amount of pain they considered to be evident in the scene.
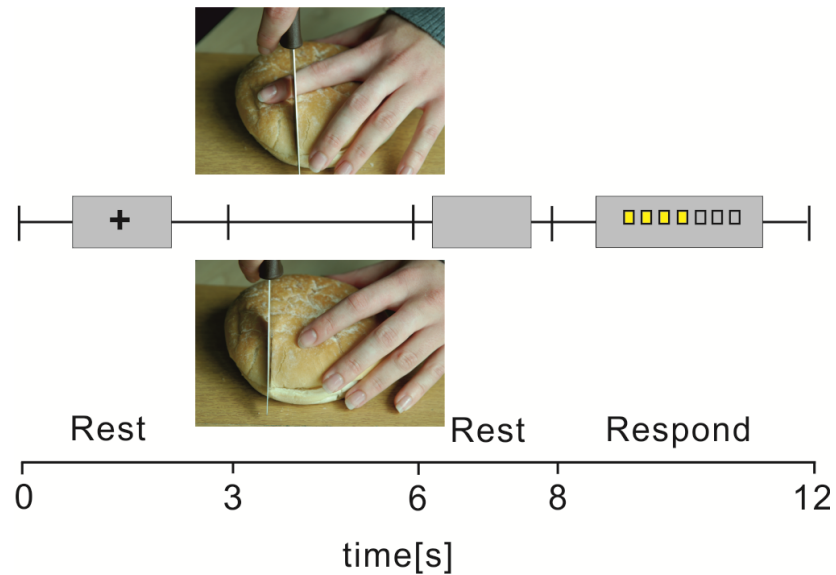
Fig. 4.1 Flowchart of the observation of pain pictures experiment. The figure illustrates one trial of the experiment, beginning with a rest interval (3 s), visual presentation of a pain or non-pain image (3 s), followed by a second rest interval (2 s), and a response period (4 s). During the response period subjects used repeated mouse clicks to increment a scale and attribute the amount of pain they considered to be evident in the image.

The images employed were similar to those used in previous studies (Jackson et al., 2005; Jackson et al., 2006; Gu and Han, 2007; Lamm et al., 2007; Akitsuki and Decety, 2009), 50 images displayed hands or feet in situations containing pain, such as a knife cutting bread in a manner which would endanger the hand, or a foot standing on a shard of glass (Fig.4.1). A further 50 non-pain images which were graphically matched to pain scenes, such as a knife safely cutting bread, were also displayed. Following the experiment, participants rated each photograph in terms of emotional valence and arousal using 9-point Self Assessment Manikin scales (Bradley and Lang, 1994).

At the end of the EEG recording session participants underwent a clinical MTPS examination (Wolfe et al., 1990). Eighteen anatomically standard FMS tender

points were palpated for 4 s using the thumb pad of the dominant hand. Pressure began at 1 kg force and was incremented by 1 kg per second until a maximum pressure of 4 kg is achieved. Following examination of each point patients reported whether they felt any pain during palpation and rated the pain verbally on a scale of 0 for 'no pain', to 10 for 'worst pain ever experienced'. Participants also completed a series of questionnaires (described earlier in section 4.11) incorporating the BDI, STAI PCS and FIQ.

4.1.5 Brain responses in FMS patients during somatosensory stimulation: analyses

EEG data from the brushing investigation was analysed using FieldTrip (Oostenveld et al., 2011) and EEGlab (Delorme and Makeig, 2004) toolboxes in Matlab v.7.8 (The Mathworks Inc, USA). For each participant 40 paired rest-brush EEG epochs of 8 s duration (4 s rest and 4 s brushing) were visually inspected for artifacts. Epochs containing motion, electrode or muscle artifacts were rejected. Monopolar EEG data was spatially transformed using the Laplacian operator method (Thickbroom et al., 1984) to transform EEG data into reference-free data. This method improves the spatial resolution of EEG amplitude distributions by both reducing volume conduction effects and removing reference electrode effects. Laplacian filtered data was previously shown to be suitable for source distribution analysis of different frequency bands (Srinivasan et al., 2006). A further visual inspection of the spatially filtered data was performed and epochs containing artifacts were rejected. The average number of epochs remaining after both rounds of artifact correction was 28.1 ± 8.6 (mean $\pm$ SD) and 28.5 ± 6.7 in patient and healthy control groups respectively.

4.1.7.1 ERD analysis

Power spectral densities were computed by averaging Fast Fourier Transform power spectra in a 512 sample (1s) data window identically aligned relative to the onset of brushing. Blackman window data smoothing was applied prior to Fast Fourier Transform. This spectral window was shifted in 0.0625 s intervals (32 samples) to yield a power time series of 112 points representing the interval from -3.5 to 3.5 s relative to onset of brushing. Frequency components showing the largest amplitude decreases during brushing were identified in both alpha (8–13 Hz) and beta (16–30 Hz) bands for each participant by analysing amplitude changes in 6 electrodes overlying the contralateral somatomotor cortices. The frequency component showing the largest amplitude decrease was used as an anchor to define a 3 Hz (alpha) and 5 Hz (beta) window centred on the select frequency for each participant. Absolute band power for optimal alpha and beta frequencies was computed in resting and brushing intervals by squaring EEG signal amplitudes in the pre-identified participant select frequency bands, and averaging over all trials for each participant (Pfurtscheller and Lopes da Silva, 1999). As brushing stimuli were manually applied by the experimenter, and to prevent overlapping of brush/rest oscillatory changes between epochs, a 2 s window for rest (-3 s to -1 s relative to onset of brushing) and brush (1 s to 3 s relative to onset of brushing) epochs were exported for ERD analysis. ERD related to brushing was calculated for each participant according to the formula established by Pfurtscheller and Aranibar (1977). Finally, individual frequency specific brush ERD data were averaged for both patient and healthy control groups in optimal alpha and optimal beta-bands. In order to ascertain the quality of EEG recordings, power spectral densities were computed for each subject in two electrodes located over contralateral and ipsilateral

central-parietal sites (CP3 and CP4 respectively) across all frequency components in rest and brush epochs.

4.1.7.2 Statistical analysis

To evaluate differences in mean ERD during brushing between FMS patients and healthy controls a Student's independent *t*-test was computed for each electrode in each optimal frequency band using Matlab v.7.8. Electrodes showing statistically significant differences between groups were combined into clusters and averaged. A 95% confidence level was employed and permutation analysis technique (Maris and Oostenveld, 2007) was used to correct for false positive results due to the performance of multiple tests over 64 electrodes and multiple frequency bands. Clusters of electrodes demonstrating differences in mean ERD between groups were checked for outliers (outside 1.5*interquartile range) and mean differences re-computed to reveal the most robust differences. Mean individual ERD in each cluster was calculated for each participant. In order to investigate potential correlations between oscillatory changes during brushing and clinical or psychological data Pearson's correlation analysis was performed within the patient group for clinical and psychological variables and ERD in each significant cluster of electrodes. Spearman's correlation analysis was performed for brush pain ratings and the various measures to investigate potential psychological causes of subjective pain during brushing.

To control for the possibility of resting-state spectral power affecting subsequent brush-related ERD values, mean resting-state power spectral densities were analysed in alpha and beta-bands from 2 electrodes located over contralateral and ipsilateral central-parietal sites (CP3 and CP4 respectively). A 2×2 mixed

ANOVA was performed to compare resting spectral power between groups and hemispheres in alpha and beta-bands. All statistical analyses were carried out in SPSS v.17 (SPSS Inc, Chicago, USA).

4.1.7.3 Beamformer analysis

In order to localise the cortical sources accounting for surface ERD changes during brushing, EEG data were analysed using dynamic imaging of coherent sources (DICS) method (Gross et al., 2001) in BESA v5.2 (MEGIS, Germany). This technique applies a spatial filtering technique known as beamformer analysis to localise coherent sources of brain oscillatory changes in a desired frequency band (Gross et al., 2001). Beamformer analysis (VanVeen et al., 1997) is a distributed source modelling technique which utilises spatial filtering to extract components of a signal with specific spatial characteristic, such as those originating at a predetermined voxel. Sequential implementations of this analysis can be used to create a statistical volumetric map of source frequency amplitude changes throughout the entire brain volume (Brookes et al., 2008). The time course of activity is then computed and the resulting time series can be considered as an estimate of the oscillatory source activity in the desired frequency range throughout the entire brain volume (Gross et al., 2001).

In the present study, beamformer analysis was employed to evaluate source activity using a grid of approximately 4600 voxels sized $7 \times 7 \times 7$ mm^3. Resulting volumetric maps were exported to SPM8 (Welcome Trust Centre for Neuroimaging, University College London, United Kingdom), spatially normalised to Montreal Neurological Institute (MNI) space and resampled to $1 \times 1 \times 1$ mm^3 voxels. Univariate Student's *t*-test analyses were performed using the resulting statistical

volumetric maps for patient and healthy control groups to evaluate sources of frequency specific amplitude changes during brushing. Regions of interest (ROIs) were selected for each group by identifying clusters of voxels showing the most significant source amplitude changes (corrected $P < 0.001$) during brushing stimulation. MNI co-ordinates for the exact locations of t-maxima within each cluster were identified and clusters of frequency specific source amplitude decreases, significant in the patient group- but not control subjects during brushing, were selected for ROI analysis. A spherical ROI, 8 mm in diameter and centred on the patient group t-maxima for each source, was assigned using MarsBaR toolbox (MRC Cognition and Brain Sciences Unit, Cambridge, United Kingdom) in SPM8 and mean frequency amplitude changes extracted for each participant in each ROI. A two-way ANOVA showed a significant group effect on source activation values ($F(1,35) = 4.82$, $P = 0.035$), and a significant effect or source ($F(4,35) = 14.94$, $P < 0.001$). A two-way ANOVA for repeated measures (group $\times$ source) analysis was performed in SPSS v.17, to compare variance between groups in source amplitude changes associated with brushing for each ROI. Exploratory one-way ANOVA analysis was utilised to investigate the specific sources driving any main effects or interactions.

4.1.8 Cortical activations in FMS patients during observation of pain pictures: analyses

EEG data from observing pictures was processed using BESA v. 5.2 (MEGIS, Germany). Data was first spatially transformed into reference-free data using common average reference method (Lehmann, 1987). The oculographic and, when necessary, electrocardiographic artifacts were removed by principal

component analysis (Berg and Scherg, 1994). Data was visually inspected for presence of any movement or muscle artifacts, and epochs contaminated with artifacts were excluded. The mean number of trials remaining after artifact correction was 42.6 ± 5.3 (mean ± SD) and 42.6 ± 5.0 in patient and healthy control groups for pain pictures and 43.5 ± 4.5 and 42.3 ± 6.1 for non-pain pictures. A Student's independent t-test indicated no difference between groups in the mean number of trials for either picture type ($P > 0.05$).

ERPs associated with the onset of viewing photographs were computed separately for FMS patients and healthy control subject responses to pain- and non-pain pictures by averaging the respective epochs in the intervals ranging from 200 ms before stimulus onset to 1200 ms after stimulus onset (717 time points) in each surface electrode. The baseline period ranged from −200 ms to 0 ms relative to the onset of visual stimulus. ERP signals were bandpass-filtered from 0.5 to 40 Hz and the data for each subject, condition and electrode was extracted using Matlab v.7.13 (The Mathworks Inc, USA).

Global field power (GFP) can be used to identify temporal "events" which show consistent topography across trials (Koenig and Melie-Garcia, 2010; Tzovara et al., 2012). Grand averaged GFP encompassing all groups and conditions as well as individual GFP for each group and condition and butterfly plots of all electrode responses were computed using Matlab. Peaks in grand averaged GFP and butterfly plots were used to define the centre of 15 ms epochs of interest in which to perform statistical comparisons between groups and conditions. In each time epoch of interest up to four electrodes overlying topographic peaks of positive or negative voltage deflections were selected for statistical analysis on the basis of observed effects from

surface isopotential maps (Ibáñez et al., 2011). Mean amplitudes for each electrode of interest in each time epoch were exported to SPSS 19.0 (SPSS Inc., New York, USA).

Three-way analysis of variance (ANOVA) for repeated measures with one between subjects factor (group) and two within subject factors (picture type and electrode) was performed in each time epoch of interest to investigate main effects of group, picture type, electrode and interactions. In the event of a significant main effect or interaction, an exploratory two-way ANOVA for repeated measures (group × picture type) was computed for each electrode to identify specific electrodes showing significant main effects or interactions in each time epoch. Post-hoc Student's independent samples *t*-tests were performed to investigate significant interaction effects from ANOVA analyses. All P values from ANOVA analyses were adjusted with Greenhouse-Geisser ε correction to account for violation of the assumption of sphericity. This sequential use of ANOVA is common practice in EEG studies analysing multiple electrode sites and is referred to as 'the standard approach' (Luck, 2005).

4.1.8.1 Source analysis

Source analysis was carried out in BESA v. 5.2 using local autoregressive average (LAURA) distributed source localisation method (Grave de Peralta Menendez et al., 2001; Grave de Peralta Menendez and Gonzalez Andino, 2002; Grave de Peralta Menendez et al., 2004). LAURA source localisation is an inverse solution which incorporates the minimum norm algorithm (Hämäläinen and Ilmoniemi, 1994) with additional biophysical constraints (Menendez et al., 2001). The minimum norm algorithm selects the inverse solution with lowest overall

intensity to elucidate a unique explanation for surface data, then biophysical constraints are applied to account for interfering factors such as the volume conduction of the head. This method uses a local auto-regressive average operator, with coefficients dependent on the distances between solution points and relating to electromagnetic and biophysical laws that state that the strength of the source will decline with distance and also that the source activity at one point is related to the activity at neighbouring points (Grave de Peralta Menendez and Gonzalez Andino, 2002). Simulation studies show that LAURA can accurately resolve multiple, simultaneous sources (Grave de Peralta Menendez et al., 2001) and performs better than alternative methods such as LORETA (Menendez et al., 2001). In this study, LAURA maps were exported to ANALYZE format with $7\times7\times7$ mm^3 voxels for every subject and condition across all 717 time points and mean LAURA source data for the 15 ms time epochs of interest were exported using Matlab v.7.13.

For each time epoch, a univariate Student's *t*-test was performed using the average of all subjects and conditions to identify the strongest sources of activation. Peak_nii (http://www.martinos.org/~mclaren /ftp/Utilities_DGM) was used to identify up to 10 peak voxels in each univariate *t*-map, images were thresholded at *t* > 10 and peaks that were less than 2.5cm apart were collapsed whilst maintaining the location of *t*-maxima. Finally, spherical ROIs, 10 mm in diameter and centred on the *t*-maxima of the sources identified by Peak_nii program, were generated using MarsBaR toolbox in SPM8. Mean source activations were extracted from ROIs in the LAURA volumes for each time epoch of interest in each participant and condition.

A three-way ANOVA for repeated measures with one between subject factor (group) and two within subject factors (picture type, source activation) was computed in each time epoch of interest to investigate main effects of group, picture type, source location and interactions. Exploratory two-way ANOVA for repeated measures (group × picture type) were performed to identify specific sources showing significant main effects or interactions in each time epoch. Post-hoc Student's independent samples *t*-tests were performed to investigate the specific effects driving significant interactions. Two way (group × picture type) analysis of covariance (ANCOVA) for repeated measures was computed for LAURA source activations demonstrating significant main effects or interactions. PCS, BDI and FIQ scores were employed as covariates to investigate whether these measures were responsible for the main effects or interactions seen in source activation patterns. All P values from ANOVA analyses were adjusted with Greenhouse-Geisser ε correction to account for violation of the assumption of sphericity.

4.1.8.2 Behavioural statistical analysis

Subjective ratings of pain, valence, and arousal attributed to images were analysed using a two-way (group × picture type) ANOVA for repeated measures in SPSS v19.0. To investigate the role of subjective measures of pain, valence and arousal for each type of image on source activations a two-way (group × picture type) ANCOVA for repeated measures was computed in BMDP2V program (Statistical Solutions Ltd, Cork, Ireland). This program allows assessment of whether changes in a dependent measure, e.g., source activation, are associated with a specific change of a covariate across the different levels of a within subject factor, such as the picture type. A 95% confidence level was employed throughout.

4.2 MRI studies

4.2.1 Patients

Sixteen female patients (age 38.5 ± 8.45 years, mean ± SD) who had attended the EEG session were selected for MRI recordings; three of the original patient group were excluded due to internal metal or claustrophobia. Mean duration of symptoms in the MRI patient group was 9.13 ± 6.80 years, and mean time since diagnosis was 2.88 ± 1.34 years (mean ± SD). All patients fulfilled ACR criteria for diagnosis with fibromyalgia on the day of scanning (Wolfe et al., 1990). Patients with additional disease or disorders (not commonly comorbid with FMS such as diabetes or hypertension) were excluded, as were patients with a past history of major disease, alcohol/drug abuse or serious head or brain injury. Table 4.1 shows the epidemiological data for all FMS patients participating in the MRI recording session as well as various clinical and psychological scale scores.

4.2.2 Controls

Fifteen age-matched female controls (age 39.40 ± 8.65 years, mean ± SD) took part in the MRI recording session, three of the original healthy group were excluded due to internal metal or claustrophobia. Volunteers taking regular medication, currently diagnosed with any disease or disorder or demonstrating a history of major disease, alcohol/drug abuse or serious head or brain injury were excluded. Table 4.2 shows the epidemiological data for all healthy control participants participating in the MRI recording session as well as various clinical and psychological scale scores.

4.2.3 MRI data acquisition

Participants attended the Magnetic Resonance and Image Analysis Research Centre (MARIARC) at the University of Liverpool. All participants underwent safety screening, performed by a senior radiologist to confirm their suitability for the session. Magnetic resonance images were acquired using a whole-body 3 tesla Siemens Trio MRI imaging system (Siemens, Magnetom, Erlangen, Germany) and an 8-channel head coil. As required by MARIARC safety protocol, a clinical T2-weighted anatomical scan was acquired. This scan was not used for research purposes, but was evaluated by a qualified clinician for medical anomalies or incidental findings that would require further investigation. Following the clinical scan, a high-resolution 3-dimensional T1-weighted image was acquired using a modified driven equilibrium Fourier transform (MDEFT) sequence (TR = 7.92 ms, TE = 2.48 ms, flip angle = 16°, 176 sagittal slices, slice thickness 1mm, matrix 256 × 256, in-plane voxel size 1 mm × 1 mm, total acquisition time 12:51 mins). Diffusion-weighted images were then recorded using a spin echo echo-planar imaging sequence (SE-EPI, TE = 93 ms, TR = 6800 ms, 54 axial slices, isotropic resolution 2.5 mm^3) comprising 7 images with no diffusion weighting (b = 0) and 60 images with isotropically distributed diffusion-sensising gradients (b = 1000 s/mm^2).

At this stage participants were briefly removed from the scanner so that MR compatible headphones could be fitted appropriately. The subjects were then re-localised before the final functional scan. Resting-state fMRI data was acquired using a T2-weighted sequence (32 axial slices, 0.7mm spacing, TR = 2.0 s, TE = 30 ms, flip angle = 90°, field of view = 192mm, voxel size = 3× 3 ×3.5 mm). During the 20 minute resting-state fMRI acquisition period (600 scans), subjects were asked to remain awake with their eyes closed. Fifteen auditory stimuli (a one second beep

tone) were delivered via headphones at pseudorandom intervals (every 60−90 s, mean onset 75 s). Participants were instructed to respond to the auditory stimulus by pressing a button on a button box placed in their right hand. Stimulus-response epochs were later excluded as confounds. This method was previously shown to provide appropriate data for resting-state network analysis (Fair et al., 2007).

Following completion of all MRI recordings all patients were again required to undergo a clinical MTPS examination (Wolfe et al., 1990). They also completed the FIQ (Burckhardt et al., 1991) to evaluate the impact of FMS symptoms on their quality of life in the week preceding the scanning session.

4.2.4 Resting-state functional alterations in FMS patients

Spatial pre-processing of functional resting-state data was performed in SPM8 running in Matlab v.7.13. Functional volumes underwent realignment, slice-timing correction, normalisation to MNI space using the normalised EPI template image in SPM and spatial smoothing (8mm full width half maximum Gaussian kernel filter). Noise correction was performed using the anatomical component-based noise correction (aCompCor) method (Behzadi et al., 2007) implemented in the Functional Connectivity Toolbox (CONN, Whitfield-Gabrieli and Nieto-Castanon, 2012) in SPM8. During pre-processing in CONN, the high-resolution T1-weighted anatomical volumes were automatically segmented into grey matter, white matter and cerebrospinal fluid and normalised to MNI space. The BOLD time series from subject-specific white matter and cerebro-spinal fluid, and the temporal time series associated with the motion correction parameters applied during functional pre-processing steps, were employed as confounds and removed from BOLD data using linear regression. The residual BOLD data was previously shown to benefit

from improved specificity, sensitivity and validity for subsequent functional connectivity analyses (Shehzad et al., 2009; Whitfield-Gabrieli and Nieto-Castanon, 2012). Sound conditions, representing the 15 second period beginning 1 second before onset of a sound stimuli and 14 seconds post stimuli, were defined and included as confounds so as to only investigate the remaining resting-state data (Fair et al., 2007). Finally, BOLD data was bandpass filtered (0.008-0.09 Hz) to reduce low-frequency drift and noise effects.

4.2.4.1 Seed regions of interest

ROI seeds consisting of 10 mm diameter spheres centred on co-ordinates for DMN structures from a recent meta-analysis of DMN studies (Laird et al., 2009), were defined using MarsBaR software in SPM8. Table 4.3 shows the hemisphere, anatomical location, Brodmann area (if applicable) and MNI co-ordinates of the DMN seeds. Similarly, 21 identical ROIs were centred on co-ordinates of regions associated with activations during noxious muscle pain according to a recent meta-analysis of functional neuroimaging studies of experimental pain (Duerden and Albanese, 2011). Table 4.4 shows the hemisphere, anatomical location, Brodmann area and MNI co-ordinates of the pain processing ROIs.

TABLE 4.3 Seed regions of interest selected for the default mode network

Hemisphere	Region	BA	x	y	z
Left	Precuneus (pC)	7	−4	−58	44
Left	Posterior Cingulate (PCC)	31	−4	−52	22
Right	Ventral Anterior Cingulate (vACC)	32	2	32	−8
Right	Inferior Parietal Lobule (RIPL)	40	52	−28	24
Left	Medial Prefrontal Cortex (MPFC)	9	−2	50	18
Right	Middle Temporal Gyrus (RMTG)	39	46	−66	16
Left	Middle Frontal Gyrus (LMFG)	8	−26	16	44
Left	Inferior Parietal Lobule (LIPL)	40	−56	−36	28
Left	Middle Temporal Gyrus (LMTG)	39	−42	−66	18

x, y, z = MNI co-ordinates (mm), BA = Brodmann area

TABLE 4.4 Seed ROIs selected associated with activation during noxious muscular pain.

Hemisphere	Region	BA	x	y	z
Right	Anterior insula		36	14	10
Left	Thalamus		−12	−16	8
Left	Cingulate gyrus	24	0	16	24
Left	Mid-insula		−34	2	20
Left	Posterior insula		−36	−22	12
Right	Posterior parietal cortex	40	64	−22	22
Right	Middle frontal gyrus	10	32	42	16
Left	Precentral	6	−56	−2	8
Left	Inferior parietal lobule		−58	−38	28
Left	Secondary somatosensory cortex	41	−58	−18	14
Left	Posterior cingulate gyrus	23	−4	−26	28
Left	Cingulate gyrus	32	−8	30	24
Left	Posterior insula		−38	−18	−6
Right	Cingulate gyrus	32	8	10	38
Left	Superior temporal gyrus	22	−50	6	−6
Left	Precuneus	7	−8	−70	36
Left	Cerebellum		−2	−26	−14
Right	Cerebellum		24	−62	−18
Left	Cerebellum		−38	−54	−36
Left	Middle frontal gyrus	9	−30	40	28
Left	Anterior insula		−28	16	2

x, y, z = MNI co-ordinates (mm), BA = Brodmann area

4.2.4.2 *Seed-to-voxel analysis*

Individual correlation maps were generated by extracting the residual BOLD time course from each seed ROI and calculating Pearson's correlation coefficients with the BOLD time course of each voxel throughout the whole brain. The resulting coefficients were converted to normally distributed scores using Fisher's transformation and entered into general linear model (GLM) analysis to generate statistical parametric maps of voxelwise functional connectivity for each seed ROI. The value of each voxel represents the relative degree of functional connectivity with each seed (Whitfield-Gabrieli et al., 2011). Second-level GLM analysis of relative functional connectivity values was performed using a two-sided independent *t*-test, implemented in the CONN toolbox, to investigate differences in seed-to-voxel connectivity between groups. As in previous studies (Woodward et al., 2011; Ichesco et al., 2012) voxel level statistics throughout the whole brain were performed at uncorrected level (P < 0.001) before false discovery rate (FDR) (Benjamini and Hochberg, 1995) correction was applied at the cluster level (P < 0.05).

4.2.4.3 *ROI-ROI analysis*

To further examine whether functional connectivity between DMN and pain processing structures differs in FMS, the BOLD time series extracted from each seed ROI was correlated with the extracted time-series from all other seeds in the corresponding network. These values were entered into second-level GLM analysis, implemented in the CONN toolbox, and a two-sided independent samples *t*-test was performed to evaluate between-group differences in ROI-ROI connectivity for each seed region. Results were thresholded at P < 0.05 and FDR correction was applied to correct for multiple tests required.

4.2.4.4 Correlations between relative functional connectivity and clinical measures

For each cluster of voxels showing a significant group difference in connectivity with DMN or pain processing structures in seed-to-voxel analyses, the Fischer transformed correlation coefficients were extracted for each subject. These values indicate the relative connectivity strength with network seed ROIs for each individual subject. Pearson's correlation analysis was performed within the patient group to investigate the relationship between relative functional connectivity with seed ROIs and clinical measures including duration of symptoms and MTPS scores (Wolfe et al., 1990).

4.2.5 Morphological alterations to subcortical and cortical structures in FMS

In order to analyse T1-weighted scans, subcortical geometric shape analysis and voxel based morphometry analysis was utilised.

4.2.5.1 Subcortical shape and volumetric analysis

Fifteen subcortical structures (brainstem, bilateral thalami, hippocampi, amygdalae, putamen, caudate nucleus, accumbens nucleus and pallidum) were segmented from each subject's high-resolution T1-weighted structural scan using the Oxford Centre for Functional MRI of the Brain's (FMRIB) integrated registration and segmentation tool (FIRST) toolbox (Patenaude et al., 2011) in FMRIBs software library (FSL, http://www.fmrib.ox.ac.uk/fsl, Smith et al., 2004). This method implements a probabilistic adaptation of the active appearance model using a large, manually labelled data set as a training template wherein the subcortical structures are parameterised as surface meshes with established vertices. In an automated process, the images are linearly registered (12 degrees of freedom) to the training

template and subsequently to a standard MNI space subcortical mask template in FSL. Finally, shape and intensity variations in the images are used to automatically segment subcortical structures. This method of shape analysis was recently validated and shown to be consistent over a variety of magnetic field strengths and acquisition systems (Goodro et al., 2012).

To evaluate group differences in the shape of subcortical structures vertex analysis was performed. A multivariate Gaussian model of vertex location and intensity variation was used to generate a surface mesh for each structure in each subject. The number and correspondence of mesh vertices is constant across subjects to allow for point-to-point comparisons such as group differences in mean vertex positions. Corresponding vertices of surface meshes for each individual subject were compared in standard MNI space using the multivariate GLM with Pillai's trace as the test statistic. This generates a multivariate F-statistic for each vertex which is sensitive to between group differences in geometric vertex co-ordinates in any direction (Patenaude et al., 2011). Individual vertex results were corrected for multiple comparisons (across vertices) using FDR method (Benjamini and Hochberg, 1995), $P < 0.05$ was considered significant. Bonferroni-Šidák correction was also employed to account for the multiple comparisons required across 15 structures. For any structures demonstrating a significant shape alteration in FMS patients relative to healthy participants, correlation analysis was implemented in FIRST in the FMS patient group only. MTPS, FIQ and BDI scores were independently implemented as correlates in the design and vertex analysis was performed to evaluate whether these measures correlated with vertex positions. FDR correction was applied to correct for multiple tests across vertices.

As recent studies point to an association between Chiari I malformation and FMS (Thimineur et al., 2002; Heffez, 2011; Watson et al., 2011) each brain was assessed for Chiari I malformation by measuring the distance from the inferior tip of the cerebellar tonsils to the line connecting basion and opisthion (Watson et al., 2011). This measure was of importance for evaluation of potential shape or volume differences in brainstem because increased pressure of cerebrovascular fluid below foramen magnum in Chiari I malformation may affect this structure.

The surface meshes generated for each subject were then transformed back into native space using the inverse transformation of the earlier registration, and boundary corrected. The volume (mm^3) of each subcortical structure was calculated. For each subcortical structure demonstrating a significant difference in shape analysis, volumetric data was exported for each individual participant. Mean values for FMS patient group and healthy control groups were compared using Student's independent sample *t*-tests. Individual values in the FMS patient group were also utilised for Pearson's correlation analyses with clinical measures (MTPS scores) in SPSS v.19.

4.2.5.2 Voxel based morphometry analysis

VBM pre-processing and analysis was performed using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm) in SPM8 running in Matlab v.7.8. VBM consists of several steps including spatial normalisation of images, segmentation of grey matter and other tissues before analysis of global and local differences in voxel intensities. Images were registered to MNI space and an optimized method of VBM using the VBM8 toolbox was implemented for segmentation of grey and white matter, default settings were used unless otherwise indicated. A hidden Markov

random field model is applied as part of this procedure to reduce noise effects. As spatial normalisation causes expansion or contraction of some brain regions, segmented images were scaled by the degree of contraction (modulated), so that the total volume of gray or white matter in the images remains the same as in the original images. Images were smoothed using an isotropic Gaussian kernel of 10 mm full width at half maximum. A data quality check based on inhomogeneity measures of the sample, as implemented in VBM8 toolbox, was used to check for anomalous data (outside 2 standard deviations) which was visually inspected and subsequently excluded if necessary. Using these criteria no data required exclusion from the sample.

A voxel-wise comparison was performed between the FMS patient and healthy control groups using the GLM implemented in VBM8. As in previous clinical studies (Schmidt-Wilcke et al., 2006; Buckalew et al., 2008), pain-related grey matter alterations may be small, and so whole brain differences in voxel intensities were initially evaluated using threshold of $P < 0.001$ (uncorrected). A threshold was also applied to only consider spatially extended clusters encompassing at least 30 voxels. Subsequently, clusters of voxels demonstrating grey matter volume differences at the uncorrected level were compared using a statistical threshold corrected for FWE at cluster level ($P < 0.05$ corrected).

4.2.5.3 Total intracranial volumes and correlations with clinical measures

Total volumes of grey matter, white matter and total intracranial volumes for each participant were exported and mean values for FMS patient group and healthy control groups were compared using Student's independent sample *t*-tests. Pearson's correlation analysis was performed to evaluate possible correlations between total

grey matter volumes in the patient group and clinical measures of symptom severity (MTPS and FIQ score). All statistical comparisons were performed in SPSS v.19.

4.2.6 Microstructural alterations to white matter structures in FMS patients

Diffusion-weighted MR images were analysed to investigate white matter integrity throughout the entire brain in FMS and healthy control groups. FA values, indicating the relative degree of directional water diffusion in the brain, can be used to quantify the local integrity of white matter microstructure throughout each voxel of the brain in each subject (Smith et al., 2006). Also, the probabilistic anatomical connectivity between ROIs involved in somatosensory processing and endogenous pain modulation was compared using probabilistic tractography.

4.2.6.1 Tract-based spatial statistics

In this study, diffusion-weighted data was analysed using the FMRIB diffusion toolbox (FDT, Behrens et al., 2003; Behrens et al., 2007; Jbabdi et al., 2012) in FSL (Smith et al., 2004; Jenkinson et al., 2012). In an automated process, DTI images were first skull-stripped using the FSL brain extraction tool (Smith, 2002) and volumes were corrected for motion and eddy-current artifacts by affine-nonlinear registration of each diffusion-weighted image to the baseline ($b = 0$) image. Using the FDT toolbox, a diffusion tensor model was applied to each voxel in the brain in order to generate a voxelwise map of FA values for each individual subject. Individual FA maps were then projected onto a 1×1×1 mm standard MNI FA template using linear affine registration in FMRIBs linear integration registration toolbox (Jenkinson and Smith, 2001) in FSL. The transfer of individual FA values to the template is configured to account for individual residual variation in the locations of tracts. Individual MNI-registered FA maps were averaged to generate a study

specific template, which is 'thinned' using non-maximum-suppression perpendicular to the anatomical tract structure in order to generate a white matter skeleton representing the white mater tracts that are most common across all participants. This template was then thresholded to include only voxels with FA values of greater than 0.2, so as to only incorporate voxels indicative of white matter (Smith et al., 2006). Individual FA images were projected onto the mean FA skeleton so that each voxel takes the FA value from the nearest locally relevant tract. This process solves the alignment problems evident in previous analysis methods.

Voxelwise statistics were performed to test for group comparisons using tract-based spatial statistics (TBSS). TBSS is a novel, fully automated method which investigates data throughout the entire brain volume. As all subjects FA images are aligned to a common registration target template this method does not necessitate smoothing of the data (Smith et al., 2006). To account for multiple tests across voxels, permutation analysis with 5000 permutations was employed using the randomise tool in FSL. A statistical threshold of $P < 0.05$ was required for significance. Threshold-free cluster enhancement (Smith and Nichols, 2009) was utilised to enhance clusters showing differences. This technique eliminates the need for an initial uncorrected cluster-forming threshold or data smoothing (Smith and Nichols, 2009). Finally, mean FA values throughout the entire study-specific white matter template were extracted for each individual subject and mean FA across the whole of the white matter was compared between groups using a Student's independent samples t-test in SPSS v.19.

4.2.6.2 Regions of interest utilised for probabilistic tractography

In order to investigate the degree of white matter connectivity between structures associated with endogenous pain modulation and somatosensory processing, ROIs were defined in structures based on previous research and DTI investigations. ROIs were selected in the rACC and the brainstem for their relevance to endogenous pain modulation based on previous functional imaging studies (Bingel et al., 2006; Eippert et al., 2009). Seed (rACC), target (brainstem), and waypoint masks (left/right anterior thalami) were defined using the Harvard-Oxford atlas in FSL. Suitable anatomical waypoint masks are used to restrict analyses to the specific direct connectivity between seed and target structures (Stein et al., 2012). For each ROI a binary mask image was first generated in MNI space and affine registered to subject's native image space using the inverse transform of the registration employed in TBSS analyses. The rACC was defined by combining the ACC and subcallosal cortex masks from the Harvard-Oxford atlas before manually removing voxels with MNI co-ordinates above z= 7 mm (Hadjipavlou et al., 2006; Stein et al., 2012). The brainstem mask and anterior thalami waypoint masks were defined using the Harvard-Oxford subcortical atlas in FSL. Similar ROIs were previously employed to investigate connectivity between endogenous pain modulation structures during experimental pain in healthy people (Hadjipavlou et al., 2006; Stein et al., 2012). Fig.4.2A shows the locations of ROIs utilised for investigation of endogenous pain modulation in transverse, axial and sagittal views, as well as a 3-dimensional representation of the ROI masks in a normalised MNI template brain.

To investigate anatomical connectivity between somatosensory processing structures, seed ROIs in the thalamus and target ROIs in the primary somatosensory

cortices were defined in each hemisphere using Harvard-Oxford subcortical and cortical atlases in FSL. A waypoint mask encompassing the spino-thalamic tract in each hemisphere was defined using the Johns-Hopkins University white mater atlas (Wakana et al., 2007; Hua et al., 2008). These ROIs were selected based on previous DTI research of thalamocortical connectivity with somatosensory cortices (Sudhyadhom et al., 2012). Fig.4.2B shows the locations of ROIs utilised for investigation of somatosensory processing.
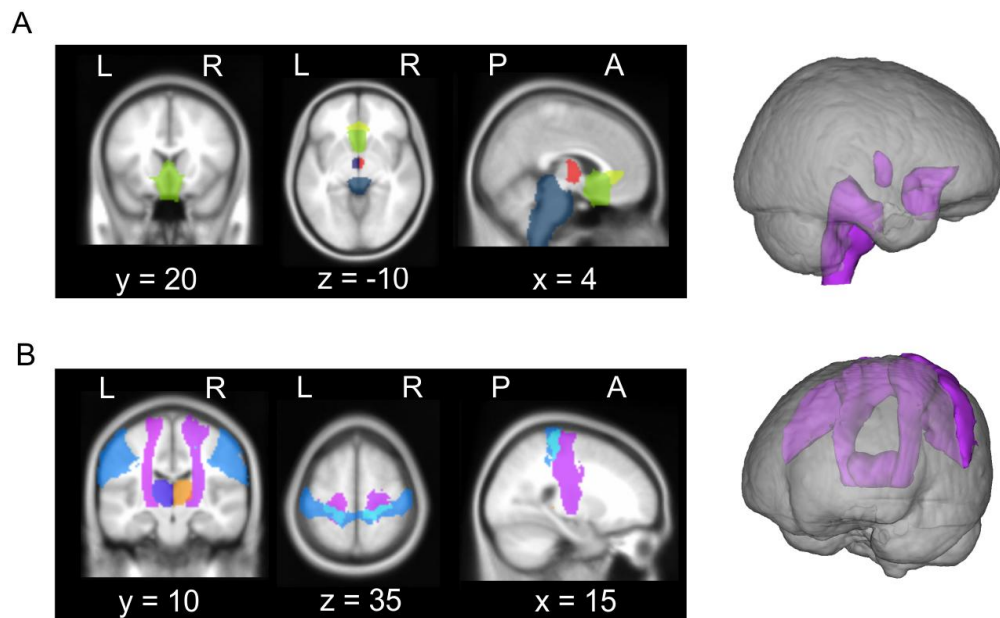
Fig.4.2 Locations of ROIs utilised for probabilistic tractography. **A** ROIs employed for analysis of endogenous pain modulation in transverse, axial and sagittal views. A 3-dimensional representation of the ROI masks is also displayed in a normalised MNI template brain. **B** ROIs utilised to investigate somatosensory processing structures.

4.2.6.3, Probabilistic tractography

In order to analyse relative connectivity in specific tracts of interest in FMS, fiber tracking was performed using a probabilistic tractography method in the FDT toolbox in FSL. Default parameters (5000 streamline samples, 0.5 mm step lengths, curvature thresholds = 0.2) were utilised as in previous seminal studies (Behrens et al., 2003; Behrens et al., 2007). Probabilistic tractography uses a probabilistic diffusion model to estimate the fibre distribution in each voxel. This model allows for multiple fibre orientations in each voxel and calculates the probability of each orientation. In the present thesis, probabilistic tractography was employed to perform seed-target classification analysis in the left and right hemisphere to investigate the white matter connectivity between seed and target ROIs. 5000 streamlines were traced from each seed ROI voxel. Each streamline follows a random path depending on the probabilities of the local fibre distributions at each voxel. Iterative analysis with a large number of streamlines yields the most common pathways which accord to local fibre orientations demonstrating the highest probability. Connectivity scores between seed and target ROIs were calculated by evaluating the proportion of streamlines which reach the target ROI after accounting for exclusions due to waypoint masking.

To control for the effect of spurious connections whilst maintaining sensitivity to weaker pathways, results were thresholded to exclude pathways with less that 10 streamlines (0.2 %) passing through them (Moayedi et al., 2012). The number of samples reaching the target ROIs was exported for each subject and the values were normalised to account for individual differences by dividing by the total number of samples (following exclusions due to waypoint masking) in each subject.

The normalised values range between 0−1, and are an indicator of relative connectivity strength between the structures of interest in each individual participant (Forstmann et al., 2010; Coxon et al., 2012).

To visualise connectivity between the structures, the pathways from individual participants (thresholded at 0.2% streamlines) were binarised and combined for each group. Images which indicate the relative frequency of pathways in each voxel in FMS patient and healthy control groups were generated. These binary images were thresholded to display only pathways that were evident in at least 50% of subjects in each group (Hadjipavlou et al., 2006).

4.2.6.4 Statistical analysis

Normalised connectivity values for rACC seed to brainstem tracts via the anterior thalamic waypoints, and from thalamus to SI via the spino-thalamic tract waypoints in each hemisphere were extracted and entered into 2×2 mixed ANOVA analysis (between groups factor; group, within groups factor; hemisphere). All statistical analyses were carried out in SPSS v.19 and results of $P < 0.05$ were considered significant.

Chapter Five

Results

5.1 Brain responses in FMS patients during somatosensory stimulation

FMS patients demonstrate hyperalgesia and allodynia (Bennett et al., 2007; Clauw, 2009) and pain resulting from innocuous stimuli is likely to be an important pathophysiological component of FMS symptoms. Currently no data is available regarding the spatio-temporal patterns of ERD during mechanical stimulation in FMS patients, nor is it clear whether such changes would be associated with clinical measures or the psychological profile of patients with FMS. This study investigated the cortical oscillatory changes associated with mechanical brushing stimulation in FMS patients. It was hypothesised that FMS patients would report subjective pain during brushing and show alterations in alpha and beta-band ERD amplitudes indicative of altered cortical excitability.

5.1.1. Clinical and psychological characteristics and analysis of subjective pain during brushing

Thirteen out of 19 patients reported pain during brushing, the mean pain rating in patients (1.05 ± 0.91, mean $\pm$ SD) indicates a pain sensation approximate to 'slight pain' on the Likert scale ranging from 'no pain' (0) to 'severe pain' (3). As all healthy subjects rated brushing pain as '0', a one-sample *t*-test was performed. The results revealed a significant difference between the mean pain ratings for patients (1.05 ± 0.91) and controls (0.00) ; $t(35) = 3.91$, $P < 0.001$.

A discriminant analysis was performed with brushing pain ratings, MTPS, STAI, BDI, FIQ and PCS scores as predictor variables to investigate differences in variance between groups for each variable, as well as the relative strength of each variable in predicting group membership. Univariate ANOVA revealed that the variance in patient and healthy control groups differed significantly for all variables (Table 5.1). A single discriminant function was calculated to demonstrate the effectiveness of all predictors, this function was significant for distinguishing patient and healthy groups ($\chi^2 = 68.9$, df = 8, P < 0.001). The correlation coefficients between various predictor variables and the discriminant function (Table 5.1) signify the relative strength of each variable in successfully identifying FMS and healthy group membership. These values suggest that FIQ and MTPS scores were the strongest predictors of fibromyalgia group membership in the population. BDI score was the next best predictor followed by brush pain ratings, STAI and PCS scores (Table 5.1).

5.1.2 Event-related desynchronisation of *alpha and beta-band rhythms*

Fig. 5.1A shows mean amplitude changes for one patient in 8–13 Hz and 16–24 Hz bands and time-frequency plots during rest and brushing in electrodes CP3 (left panel) and CP4 (right panel). Strong beta-band ERD was seen in both illustrated electrodes during brush periods. Topographic maps in Fig. 5.1A illustrate the patient's mean amplitude changes across the whole head surface in 8–13 Hz and 16–24 Hz bands from rest (-1.0 to -3.0 s) to brush periods (1.0 to 3.0 s relative to onset of brushing) as well as locations of the electrodes illustrated in time-frequency plots (white circles). Amplitudes of beta-band frequency components decreased bilaterally during brushing, whereas amplitude decreases of alpha-band power were only found in contralateral electrodes. Fig.5.1B shows the amplitude changes in one healthy

control participant matched for scale, electrodes, frequency bands and time course. Note the absence of ipsilateral beta desynchronisation in the band power, time-frequency and topographic representations.

Table 5.1 Clinical and psychological characteristics of the research sample. The mean scores and standard deviations for each psychological or clinical test in patient and control groups. F and P values from ANOVA illustrate significant differences in variance between groups for each variable. The correlation coefficient refers to each variable's correlation with the discriminant function; values indicate the relative contribution of each variable to the discrimination of FMS patient or healthy control group membership.

Variable	Patients Mean ± SD		Controls Mean ± SD		F	P	Correlation coefficient
Fibromyalgia Impact	60.60	17.88	5.91	6.27	150.72	<0.001	0.72
Manual Tender Point	4.95	1.91	0.23	0.32	107.37	<0.001	0.61
Beck Depression	18.79	10.75	3.67	4.17	31.12	<0.001	0.33
Pain Rating	1.05	0.91	0.00	0.00	22.97	<0.001	0.28
State Anxiety	39.42	11.35	26.33	5.85	19.10	<0.001	0.26
Pain Catastrophising	14.47	10.25	4.67	5.97	12.45	0.001	0.21
Trait Anxiety	47.42	13.83	34.00	8.81	12.24	0.001	0.21
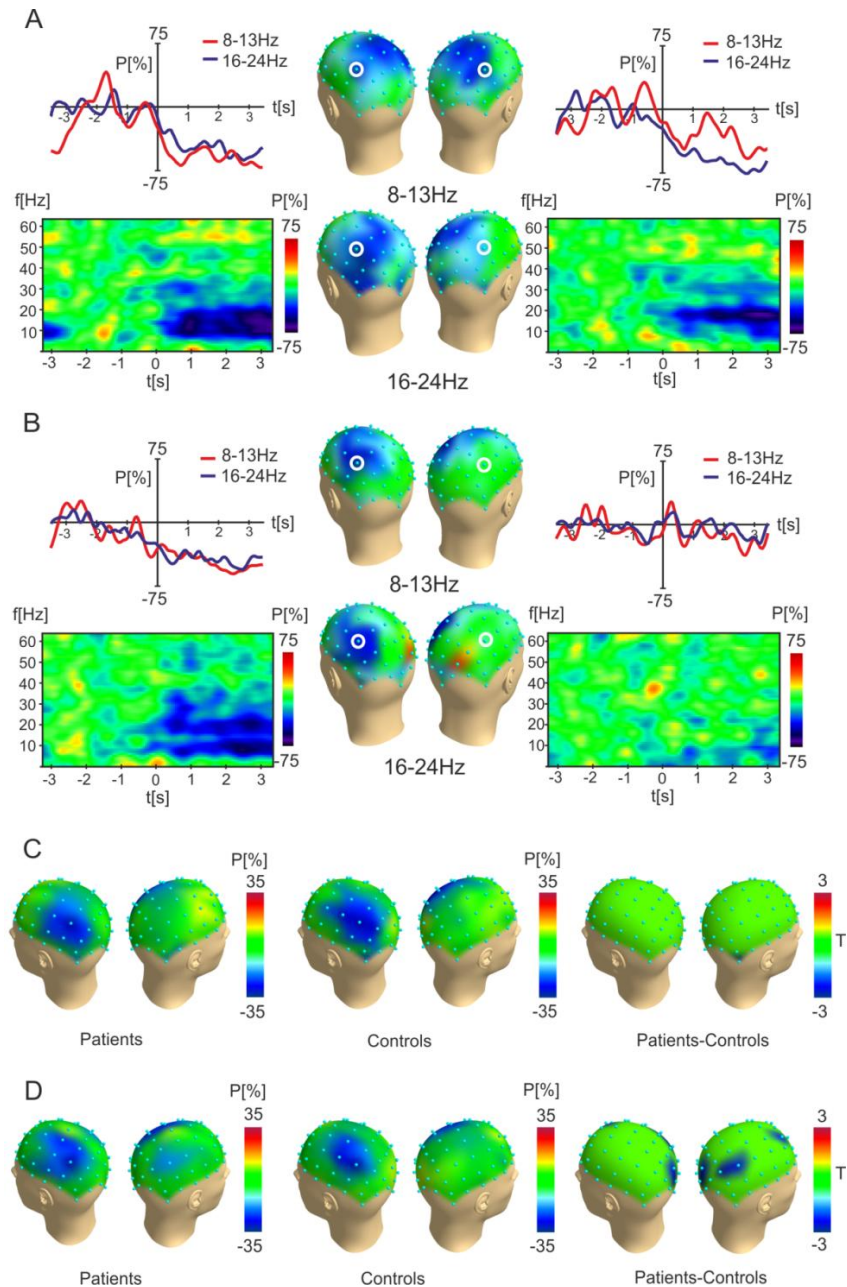
Fig. 5.1 Event-related desynchronisation analysis of oscillatory changes associated with brushing. **A** Alpha-band (8−13 Hz) and beta-band (16−24 Hz) amplitude changes and time-frequency spectrograms averaged over rest-brushing time course for one patient corresponding to electrode CP3 (left panel) and CP4 (right panel), overlying contralateral and ipsilateral central-parietal regions respectively. Three dimensional topographic maps (centre panel) illustrate (with white circles) the locations of electrodes CP3 and CP4 from two angles and alpha and beta-band relative band power changes during brushing across all scalp electrodes. **B** Alpha-band and beta-band amplitude changes and time-frequency spectrograms averaged over rest-brushing time course for one healthy control participant corresponding to electrode CP3 (left panel) and CP4 (right panel). Three dimensional topographic maps illustrate electrode locations and alpha and beta relative band power changes during brushing across all electrodes. **C** Topographic maps representing mean optimal alpha-band relative band power changes during brushing for patient and healthy control groups. The left panel image shows relative band power changes in patient group, centre; in healthy controls, right panel; a *t*-test contrast map thresholded to illustrate group differences in relative band power changes during brushing that achieve the level of statistical significance (corrected P < 0.05). **D** Topographic maps representing mean optimal beta relative band power changes during brushing for patient and healthy control groups.

Fig. 5.2 shows the grand average log-transformed power spectral densities computed for healthy control group and FMS group in electrode CP3 (contralateral central-parietal) and CP4 (ipsilateral central-parietal) in rest and brush epochs. In this analysis the presence of ERD during brushing in beta-band in ipsilateral hemisphere in patient group (upper-right panel) but not in control participants is again clear. A 2×2 mixed ANOVA was performed to compare resting spectral power between groups and hemispheres in alpha and beta-bands. No significant differences in variance were found in either frequency band for main effects or interactions ($P > 0.05$).
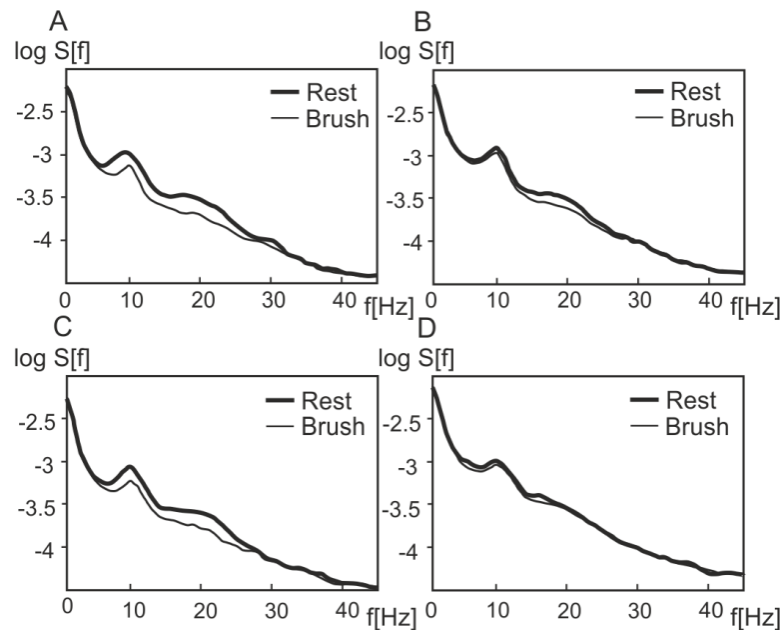
Fig. 5.2, Power spectral densities during resting and brushing EEG epochs. **A** The grand average log-transformed power spectral densities in electrode CP3 (contralateral central-parietal region) for rest (bold line) and brush (thin line) epochs in FMS patient group. **B** Electrode CP4 (ipsilateral central-parietal region) in FMS patient group. **C** Electrode CP3 in healthy control group, **D** Electrode CP4 in healthy control group.

The frequency component manifesting the strongest amplitude decreases in alpha and beta-bands during brushing was evaluated for each subject and condition. The mean peak alpha ERD frequency in patient group was 10.97 ± 1.58 Hz (mean ±

SD), and 10.44 ± 1.51 Hz in healthy control participants. Peak beta ERD frequency was 20.74 ± 2.81 Hz (mean ± SD) for patients and 20.73 ± 2.45 Hz for healthy control participants. A Student's independent *t*-test found no difference between groups for mean peak frequencies (P > 0.05). Mean optimal alpha and beta ERD during brushing was calculated for patients and healthy control subjects using subject specific alpha and beta frequencies to quantify individual ERD values before averaging for each group. The group averaged topographic maps, shown in Fig. 5.1C reveal a strong alpha-band ERD during brushing in contralateral electrodes in both patient and healthy control groups. An independent Student's *t*-test comparison of alpha-band ERD in each electrode (corrected for number of tests using permutation analysis) found no significant difference between groups; this is illustrated in the form of the difference *t*-map in Fig. 5.1C (upper panel, right image) which is trimmed to show only differences exceeding the 95% confidence level (P < 0.05). In the optimal beta-band, patient group topographic maps (Fig. 5.1D, left panel) show ERD in bilateral central-parietal electrodes. In the healthy control group, beta-band ERD was only present in contralateral central-parietal electrodes. Slight amplitude increase of beta-band power (ERS) was apparent in frontal and occipital electrodes. The topography of the contralateral ERD in patient group was more widespread than in healthy control subjects, extending down to electrodes located over the temporal lobe, indicating a more complex array of contralateral source activations in patients as well as the clear addition of ipsilateral activations. An independent Student's *t*-test comparison of optimal beta-band changes revealed three significant clusters of electrodes exhibiting greater ERD in patient group- compared to healthy controls (corrected for number of tests using permutation analysis). The clusters were located

in electrodes over ipsilateral frontal, ipsilateral central-parietal and medial occipital cortices (Fig. 5.1D, right panel).

Mean ERD was calculated in each cluster of electrodes for each participant, and averaged for patient and healthy control groups. Boxplots of optimal beta-band ERD/ERS changes for each cluster and group following the removal of outliers are shown in Fig. 5.3A. In the healthy control group weak ERS is evident in all three clusters. In patients a weak ERD was seen in ipsilateral frontal and medial occipital clusters and a moderate ERD in ipsilateral central-parietal cluster. After controlling for outliers only the ipsilateral central-parietal cluster demonstrated a robust ERD difference, statistically significant between patient and control groups. Patients exhibited a moderate-strong mean ERD (16.9%) in these electrodes compared to weak ERS in healthy controls (-5.9%), Student's independent t-test, t (35) = 2.93 (P < 0.001).

5.1.3 Correlations between beta-band ERD, brush pain ratings and clinical/psychological measures

Pearson's correlation analysis was performed to investigate relationships between beta-band ERD in patients and age, MTPS, STAI, BDI, FIQ and PCS scores. There was a significant correlation between the MTPS score and the size of ipsilateral central-parietal ERD in the patient group (r = 0.459, P = 0.048). Fig. 5.3B shows the data distributed along the regression line in a linear relationship. Spearman's correlation analysis was performed to investigate relationships between patient brush pain ratings and beta-band ERD, MTPS, STAI, BDI, FIQ and PCS scores. No significant correlations were found between subjective pain ratings and clinical or psychological measures (P > 0.05).The lack of a relationship between

experimental pain and psychological/clinical measures may be due to the relatively small number of participants in the patient group or the range of pain scores attributed during brushing. In future a scale with enhanced sensitivity (e.g. a 10 point visual analogue scale) could enhance the findings.

5.1.4 Beamformer analysis of band power changes during brushing

Beamformer analysis of optimal beta-band frequency amplitude changes during brushing in patients and healthy control participants revealed bilateral sources of activation in 15 of 19 patients, and 4 of 18 healthy control subjects. Univariate Student's *t*-test analysis of each group's individual volumetric beamformer maps was performed in SPM8. FMS patients demonstrated a widespread and complex array of beta-band power decreases analogous to the scalp ERD maps (Fig. 5.1D). Peak *t* values, indicating strongest sources of activation, were located in sources in contralateral primary somatosensory cortex (SI), contralateral parietal cortex (Brodmann Area, BA 40), bilateral insula cortices, ipsilateral secondary somatosensory cortex (SII), ipsilateral primary somatosensory cortex (SI) and occipital cortex (BA 18). Table 5.2A shows anatomical locations, MNI co-ordinates, *t*-maxima and *z*-maxima for the strongest source beta-band amplitude changes in patients. Fig. 5.4A illustrates the mean source amplitude changes in the patient group surpassing a corrected threshold of $P < 0.001$ ($t > 7.07$) in glass brains (upper panel) and MNI standardised anatomical brains.
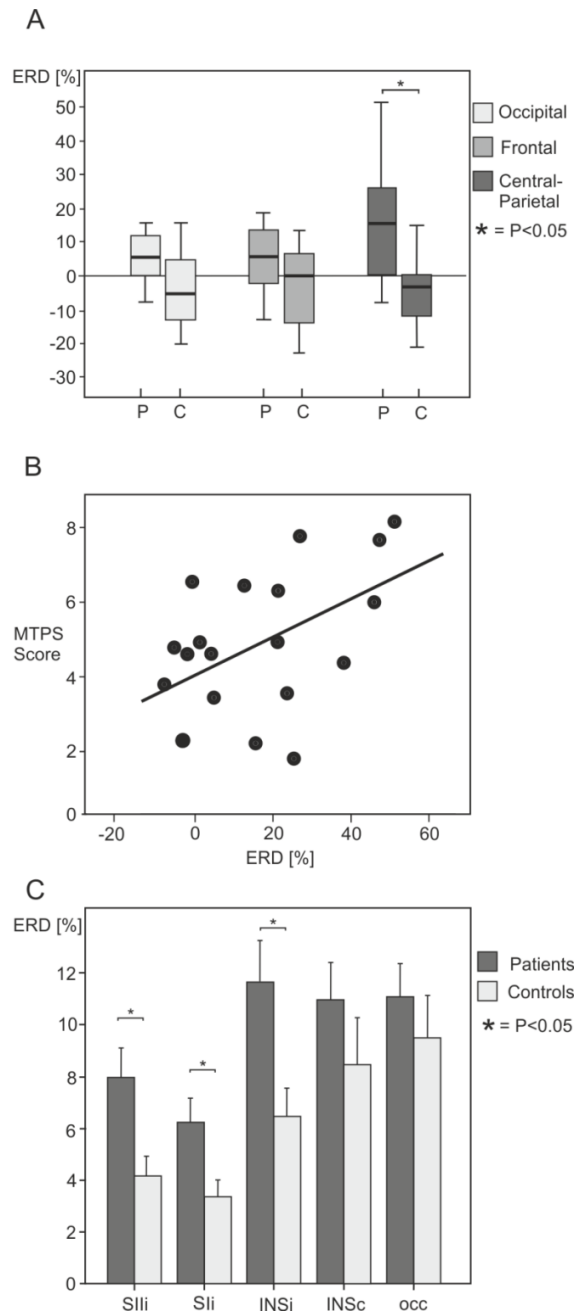
Fig. 5.3 Beta-band ERD: correlations with clinical measures and beamformer source analysis. **A** Boxplots indicating the distribution of mean optimal beta ERD/ERS in medial occipital, ipsilateral frontal and ipsilateral central-parietal clusters for patient (P) and healthy control (C) groups following removal of outliers. Each box plot represents the mean ERD halfway between upper and lower interquartile range, median values are indicated by the bold horizontal line. Significant differences between groups ($P < 0.05$) are indicated by an asterisk (*). **B** The scatter plot of patient group manual tender point examination scores and mean ERD associated with brushing in the ipsilateral central-parietal cluster of electrodes. The linear regression line is also shown. **C** Bar chart illustrating the mean ERD and standard error bars for each group in 8 mm sphere regions of interest evident in the source activations of FMS patient group but not in control subjects. Regions of interest included ipsilateral SI (SIi), ipsilateral SII (SIIi), ipsilateral insula (INSi), contralateral insula (INSc) and occipital cortex (occ). Significant differences between groups ($P < 0.05$) are indicated by an asterisk (*).

Table 5.2 Univariate analysis of source activation clusters associated with brushing. Peak source activation clusters from univariate Student's *t*-test analyses for patient (A) and healthy control group (B). All clusters surpass the threshold outlined in methods (corrected P < 0.001). Anatomical locations, MNI co-ordinates, hemisphere, *t*-maxima, *z*-maxima and (when appropriate) Brodmann areas are also given.

A Fibromyalgia syndrome patients

Anatomical location	MNI x,　y,　z	Hemisphere	T	Z	Brodmann area
SI	-47, -18, 54	Left	7.47	4.98	3
Inferior parietal lobule	-47, -57, 35	Left	8.98	5.47	40
Insula	-48, -16, 7	Left	7.63	5.11	-
Cuneus	-18, -80, 26	Left	8.21	5.23	18
Insula	47, -12, 5	Right	7.75	5.08	-
SII	45, -22, 18	Right	7.22	4.89	-
SI	62, -18, 26	Right	7.10	4.84	1

B Healthy control subjects

Anatomical location	MNI x,　y,　z	Hemisphere	T	Z	Brodmann area
SI	-43, -20, 56	Left	10.90	5.87	3
Superior parietal lobule	-45, -40, 52	Left	10.82	5.85	7
Pre-central gyrus	-21, 30, 52	Left	10.63	5.81	4
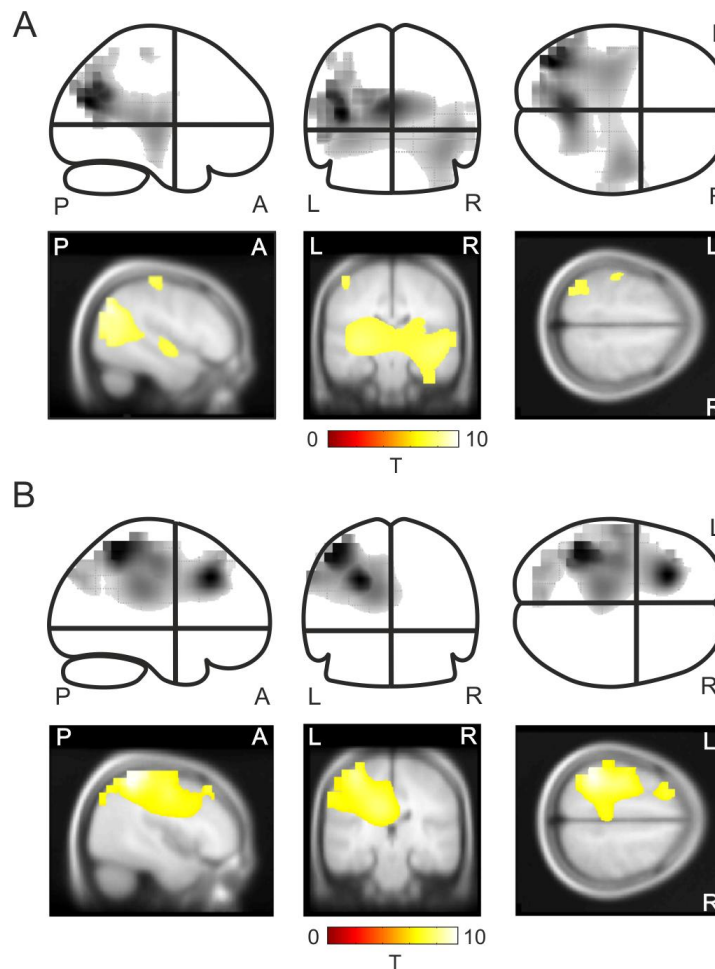
Fig. 5.4 Univariate analysis of source activation clusters from beamformer analyses. **A** Univariate Student's *t*-test source activation clusters for the averaged patient group beamformer analyses surpassing a corrected threshold of P < 0.001 (t > 7.07). Clusters are displayed in glass brains (upper panel) and MNI standardised anatomical brains (lower panel). **B** Univariate *t*-test source activation clusters for the healthy control group averaged beamformer analyses surpassing a threshold of P < 0.001 (t > 7.05).

In healthy control participants, univariate Student's *t*-test analysis revealed a unilateral array of beta-band power decreases with the strongest changes localised to contralateral SI cortices (BA 9). Table 5.2B shows anatomical locations, MNI co-ordinates, *t*-maxima and z-maxima for the strongest source beta-band amplitude changes in healthy subjects. Fig. 5.4B shows source amplitude changes in the healthy control group surpassing a threshold of corrected P < 0.001 (*t* > 7.05). Five cortical sources of beta-band ERD were evident in the patient group but not in the

control group. These sources were located in bilateral insulae, ipsilateral SII, ipsilateral SI, and occipital cortex. The t-maximum was identified for each cluster and the MNI co-ordinates used to align a spherical ROI 8 mm in diameter. Fig. 5.3C shows the mean source amplitude changes for each ROI in patient and healthy control groups. A two-way ANOVA for repeated measures (group × source) showed a significant group effect on source activation values ($F(1,35) = 4.82$, $P = 0.035$), and a significant effect for source ($F(4,35) = 14.94$, $P < 0.001$). Exploratory one-way ANOVA analysis to identify the sources responsible for the group effect revealed significant differences in variance between groups in ipsilateral SI ($F(1,35) = 6.75$, $P = 0.014$), SII ($F(1,35) = 7.36$, $P = 0.01$) and insula ($F(1,35) = 7.04$, $P = 0.012$). ROI located in medial occipital lobe and contralateral insula were not found to differ significantly in variance between groups ($P > 0.05$).

5.2 Cortical activations in FMS patients during observation of pain pictures

FMS patients demonstrate increased subjective displeasure and autonomic arousal (Bartley et al., 2009), and alterations to somatosensory-evoked potentials (Montoya et al., 2005) when viewing negative affective images. However, the cortical activations associated with observing pain have never been researched in FMS patients. To analyse whether neurophysiological processing is altered when observing pain pictures, ERP data associated with viewing of pain and non-pain pictures was analysed. Distributed source analysis was performed to investigate the source activations in time intervals with ERP components showing significant effects. It was hypothesised that FMS patients would attribute stronger pain to pain pictures relative to healthy control subjects. ERP analysis was conducted to understand the potential alterations to central processing during viewing of pain pictures in FMS patients.

5.2.1 Behavioural analysis of subjective picture ratings

Table 5.3 shows the mean pain ratings attributed to photographs during recordings, as well as the post-recording mean values for affective valence, and arousal for each type of photograph in FMS patient and healthy control groups. A two-way ANOVA for repeated measures revealed a significant main effect of picture type on the amount of pain participants attributed to images. Greater pain was attributed to pain pictures than to non-pain pictures ($F(1,35) = 361.19$, $P < 0.001$). A significant effect of group ($F(1,35) = 4.6$, $P = 0.039$) was evident and the group $\times$ picture type interaction effect was also significant ($F(1,35) = 4.52$, $P = 0.041$). Post-hoc Student's independent *t*-test analysis revealed a significant difference between the mean subjective pain ratings for pain pictures in patients (4.93 ± 1.25, mean $\pm$

SD) and controls (4.12 ± 0.91) (t (35) = 2.26, P = 0.03). However, no difference was seen between the mean pain ratings of non-pain pictures in patients (1.50 ± 0.47) compared with healthy control subjects (1.37 ± 0.34), t (35) = 0.92, P = 0.36. FMS patients attribute significantly stronger pain to pain photographs than healthy controls but not to non-pain pictures.

Subjective ratings of affective valence of each image showed a main effect of picture type (F(1,35) = 79.98, P < 0.001) with stronger valence attributed to pain pictures. No main effect of group was found (F(1,35) = 2.17, P = 0.15) but a significant group × picture type interaction effect was evident (F(1,35) = 5.82, P = 0.021). Post-hoc Student's t-test comparison of mean valence ratings of pain pictures in FMS patient group (4.36 ± 2.03) and healthy control group (3.17 ± 1.67) approached, but did not achieve significance, t (35) = 1.95, P = 0.059. There was no difference found between mean valence ratings of non-pain pictures in FMS patient (1.29 ± 0.40) and healthy control group (1.40 ± 0.78), t (35) = -0.55, P = 0.587. In order to confirm the existence and direction of the interaction effect a post-hoc Student's independent t-test of group differences in mean simple effects scores (subjective pain rating scores for pain pictures − non-pain pictures) was performed. Mean simple effects scores for FMS patients (3.08 ± 1.85) were significantly greater than in healthy group (1.77 ± 1.40), t (35) = 2.41, P = 0.021. The direction of t indicates that FMS patients attribute stronger valence to pain photographs than healthy controls, but not to non-pain photographs.

For subjective ratings of arousal in each picture, a main effect of picture type was found (F (1,35) = 77.55, P < 0.001), with pain pictures eliciting a stronger response than non-pain pictures, but no group effect was evident (F(1,35) = 1.18, P >

0.05). However, the group × picture type interaction effect was significant (F(1,35) = 4.42, P = 0.043). Post-hoc *t*-test comparisons of mean arousal ratings of pain pictures in FMS patient group (4.13 ± 2.02) and healthy control group (3.18 ± 1.59) was not significant (*t* (35) = 1.58, P = 0.122) and similarly no difference was found between mean arousal ratings of non-pain pictures in FMS patient (1.30 ± 0.56) and healthy control group (1.44 ± 0.81), *t* (35) = -0.61, P = 0.547. Mean simple effects scores for patients (2.83 ± 1.80) were significantly greater than for healthy controls (1.74 ± 1.31) *t* (35) = 2.10, P = 0.043. The direction reveals that FMS patients attribute stronger arousal to pain photographs, but not to non-pain photographs than healthy controls.

Table 5.3 Subjective ratings of pain, valence and arousal for observed pictures. Mean scores (± SD) for pain, valence and arousal attributed to pain and non-pain pictures in FMS patients and healthy control groups.

	FMS		Healthy	
	Pain	**Non-pain**	**Pain**	**Non-pain**
Pain	4.9 ± 1.2	1.5 ± 0.5	4.1 ± 1.2	1.4 ± 0.3
Valence	4.4 ± 2.0	1.3 ± 0.4	3.2 ± 1.7	1.4 ± 0.8
Arousal	4.1 ± 2.0	1.3 ± 0.6	3.2 ± 1.6	1.4 ± 0.8

5.2.2 ERP analysis

Fig. 5.5A shows the butterfly plot representing the mean visual evoked potential across all 717 time points, for all subjects and both types of photograph in 64 electrodes. Grand averaged GFP for all groups and conditions (Fig. 5.5B) and

GFP for each individual group and condition (Fig. 5.5C) are also illustrated. The black triangles indicate peaks in GFP which were used as the centre of 15 ms time windows for ERP amplitude and source analyses. In each time epoch up to 4 electrodes overlying each positive or negative deflection were exported for statistical analysis (Ibáñez et al., 2011).
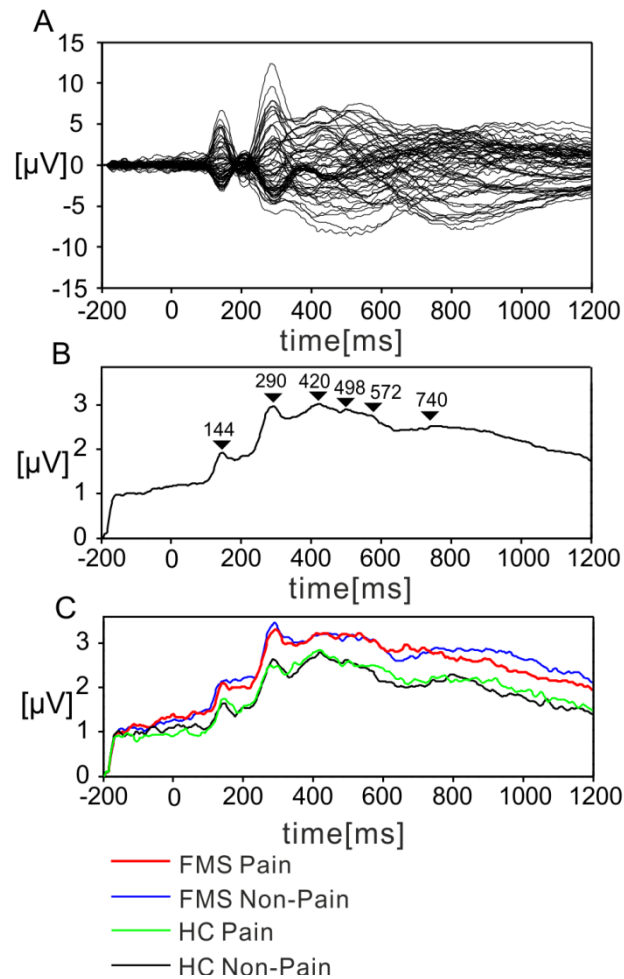
Fig. 5.5 Global field power of ERPs during observation of pictures. **A** The butterfly plot showing mean amplitudes for each electrode in all subjects and both types of photograph. **B** Grand-averaged global field power, black triangles indicate peak times used to centre 15 ms time window s of interest. **C** The global field power for each group and condition.

Two epochs indicated by peaks in global field power (412−427 ms and 732−747 ms) showed no significant effects using three-way ANOVA for repeated

measures analysis (group × picture type × electrode). In time epochs demonstrating significant effects, exploratory two-way ANOVA for repeated measures was performed in each electrode. Table 5.4 shows the individual electrodes showing significant main effect of group, picture type or interaction with amplitudes (mean ± SD), F statistics and significance values.

Table 5.4 Electrodes showing significant ANOVA effects for ERP components during observation of pictures. ERP amplitudes (mean ± SD) for pain and non-pain pictures are shown along with F statistics and significance values.

Time Epoch	FMS		Healthy		Electrode	ANOVA Effect	F	Sig
	Pain	Non-pain	Pain	Non-pain				
135 −150	2.37 ± 3.19	2.44 ± 2.85	2.80 ± 1.20	1.69 ± 1.72	PO7	Picture	4.79	.035
135 −150	-	-	-	-	PO7	Group × picture	6.19	.018
290−305	4.00 ± 2.81	4.23 ± 2.59	2.53 ± 2.17	2.44 ± 2.29	Oz	Group	4.5	.041
490−505	-4.10± 2.12	-3.26± 2.29	-2.00± 3.0	-2.08± 3.12	F5	Group	4.28	.046
565−580	-5.03± 2.95	-3.53± 2.79	-3.71± 3.42	-3.07± 3.64	F7	Picture	8.58	.006

In the early time epoch (135−150 ms) the three-way ANOVA for repeated measures revealed a significant group × picture type × electrode interaction effect ($F(1,33) = 2.93$, $P = 0.048$). Exploratory two-way ANOVA for repeated measures to investigate the specific electrodes responsible for the effect, showed that electrode PO7 (located over the left occipital lobe) demonstrated a significant effect of picture type ($F(1,35) = 4.79$, $P = 0.035$) and a significant group × picture type interaction effect ($F(1,35) = 6.19$, $P = 0.018$). Fig. 5.6A shows the isopotential maps representing the mean topography of ERP components for each group in each condition. The white circles indicate electrodes demonstrating significant ANOVA effects. Fig. 5.6B shows the mean ERP curves for each group and condition from significant electrodes.

Post-hoc Student's independent t-test analysis was performed to better understand the group × picture type interaction effect seen in electrode PO7. There was no significant group difference between mean amplitudes of the ERP component in the 135−150 ms time epoch in patients (2.37 ± 3.19) and controls (2.80 ± 1.20) when observing pain pictures ($t(35) = -0.55$, $P = 0.579$) or between patients (2.44 ± 2.85) and control subjects (1.69 ± 1.72) when viewing non-pain pictures ($t(35) = 0.26$, $P = 0.342$). However, independent t-test analysis of mean simple effects scores (pain image amplitude − non-pain image) for FMS (-0.07 ± 1.34) and healthy group (1.12 ± 1.57) confirmed the significant interaction effect ($t(35) = -2.491$, $P = 0.018$). The direction shows that the mean ERP amplitudes in this electrode and time epoch are greater for pain photographs than non-pain photographs in healthy control group but are similar across both types of picture in FMS patients.

In the 290−305 ms window a three-way ANOVA for repeated measures of ERP data revealed a significant effect of electrode ($F(5,35) = 43.43$, $P < 0.001$) and a group $\times$ electrode interaction effect ($F(1,35) = 4.59$, $P = 0.039$). Exploratory mixed two-way ANOVA for repeated measures showed that electrode Oz, located over the occipital lobe on the midline, showed a group difference in amplitude ($F(1,35) = 4.501$, $P = 0.041$). FMS patient group showed a larger mean positivity in this electrode compared to healthy control subjects during viewing of both types of image (Table 5.4, Fig.5.6A-B).

In the time epoch ranging from 490−505 ms, three-way ANOVA for repeated measures revealed a significant effect of electrode ($F(5,35) = 7.23$, $P < 0.001$) and a group $\times$ picture type interaction effect ($F(1,35) = 3.99$, $P = 0.050$). Exploratory two-way ANOVA for repeated measures showed that electrode F5 demonstrated a main effect of group on ERP amplitudes ($F(1,35) = 4.283$, $P = 0.046$). Patients showed a stronger negativity in this electrode compared to healthy controls for both types of image (Table 5.4, Fig.5.6A-B). In the 565−580 ms time window the three-way ANOVA for repeated measures of ERP amplitudes revealed a significant main effect of electrode ($F(3,35) = 13.89$, $P < 0.001$) and a significant effect of picture type ($F(1,35) = 6.56$, $P = 0.015$). Exploratory two-way ANOVA showed that electrode F7 demonstrated a strong effect of picture type ($F(1,35) = 8.576$, $P = 0.006$). Pain photographs were associated with a larger negativity seen in this electrode and time epoch for both groups (Table 5.4, Fig.5.6A-B).
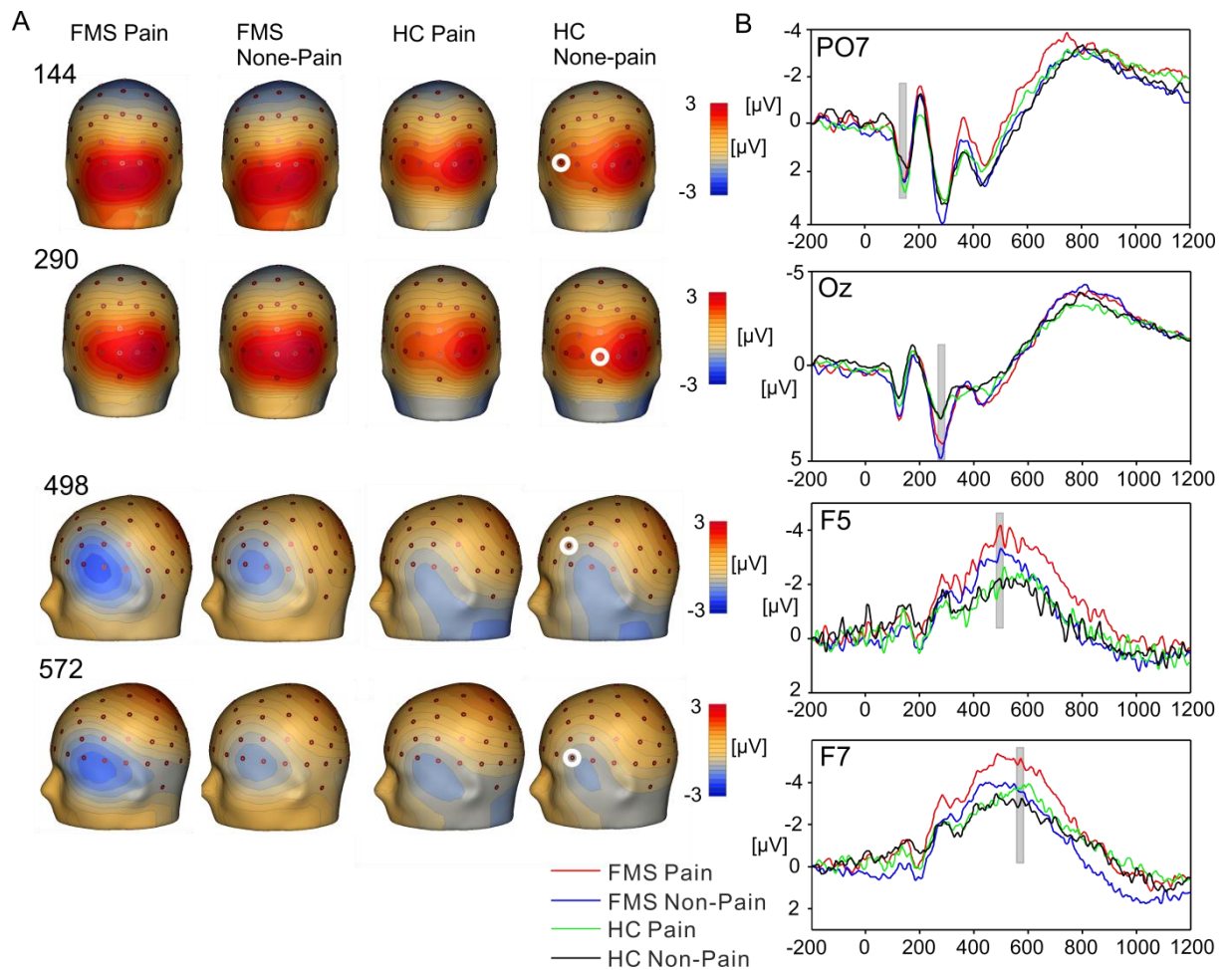
Fig. 5.6 Topography of ERP components and statistically significant electrode effects during observation of pictures. **A** The average isopotential maps for each group are shown for time points indicated by peaks in GFP. White circles indicate electrodes demonstrating significant difference in amplitude in the 15 ms time epochs centred on the peaks in GFP. **B** The averaged event-related potential curves for each group and condition are shown at select electrodes, indicated by white circles on the topographic head maps. HC = healthy control subjects. Red colour denotes FMS patients observing pain photographs, blue=FMS-non-pain, green= HC-pain photographs, black=HC-non-pain. Grey shaded areas signify the time epoch showing a significant ANOVA effect of ERP amplitude between groups, conditions or an interaction.

5.2.3 Source analysis

In time epochs showing significant effects in ERP data, source activations identified by univariate analysis of LAURA volumes were entered into a three-way ANOVA for repeated measures. Fig. 5.7A-D shows the results of univariate analyses of LAURA activations (thresholded at $t > 10$), and locations of t-maxima used for source analyses in time epochs showing significant ERP effects. Table 5.5 shows the LAURA source activations (mean $\pm$ SD) for each source demonstrating significant ANOVA effects as well as t-maxima, F values and significance. Locations of sources demonstrating effects are also indicated on Fig.5.7A-D by blue circles.

Mean LAURA source activations demonstrating strongest activations during the early time epoch (135−150 ms) were exported for each subject in each condition. A three-way ANOVA for repeated measures revealed a significant main effect of picture type ($F(1,35) = 14.11$, $P = 0.001$) and a significant photograph $\times$ source interaction effect ($F(4,35) = 16.58$, $P < 0.001$). Exploratory mixed two-way ANOVA to investigate specific sources responsible for these effects showed that the source located in the left occipital lobe, corresponding to Brodmann area 19, demonstrated a significant group $\times$ picture type interaction effect ($F(1,35) = 5.231$, $P = 0.027$). Post-hoc Student's independent t-test analysis was performed to investigate this interaction, no significant difference was seen between the mean source activation in this occipital source in patients (0.09 ± 0.06) and controls (0.10 ± 0.04) during pain pictures (t (35) = -0.45, $P = 0.656$), or between the activation during non-pain pictures in patients (0.11 ± 0.06) and controls (0.07 ± 0.03), t (35) = 1.47, $P = 0.149$. However, student's t-test analysis of mean simple effects scores (pain image source activation − non-pain image) for FMS (-0.01 ± 0.05) and healthy group (0.02 ± 0.04)

confirmed the significant interaction effect (t (35) = -2.30, P = 0.027), the direction demonstrates that pain photographs caused stronger activation in this source in the healthy group but not in FMS group (Table 5.5, Fig. 5.7A).

The three-way ANOVA for repeated measures analysis of LAURA source activations in the 290−305 ms time epoch revealed a significant group × source interaction effect (F(5,35) = 3.241, P = 0.028). Exploratory ANOVA analysis showed that the source located in the left hippocampal formation exhibited a significant group effect (F(1,35) = 5.35, P = 0.027). Mean activation in this source in FMS patient group when observing pain pictures (0.34 ± 0.21) and non-pain pictures (0.33 ± 0.15) was stronger than mean activation in healthy control subjects observing pain pictures (0.22 ± 0.10) or non-pain pictures (0.23 ± 0.10). Therefore, FMS patients demonstrate stronger left parahippocampal source activations during viewing of both types of images relative to healthy control subjects in this time epoch (Fig. 5.7B). In the 490−505 ms time window ANOVA analysis of LAURA source activations exports did not reveal a significant main effect for group, picture type or interaction (Fig. 5.7C). The three-way ANOVA for repeated measures of LAURA source activations in the 565−580 ms time epoch showed a main effect of picture type (F(1,35) = 5.26, P = 0.028) and main effect of source (F(1,35) = 44.34, P < 0.001). Post-hoc Student's independent t-test analysis indicated that a strong main effect of type of photograph was evident in mean source activation located in the left precentral gyrus (F(1,35) = 7.96, P = 0.008) with pain photographs eliciting a stronger activation in both groups (Table 5.5, Fig. 5.7D).

5.2.4 Covariate analysis of source activations with clinical measures and subjective picture ratings

ANCOVA analysis for repeated measures was performed in SPSS v.19 to test whether source group effects would be explained by BDI, FIQ, PCS scores or subjective pain score covariates. In the 290–305 ms epoch, the group main effect in the left parahippocampal source showed a significant covariance with FIQ scores ($F(1,32) = 5.22$, $P = 0.029$) and BDI scores ($F(1,32) = 5.88$, $P = 0.021$). To analyse whether self-report measures of perceived pain in images, image valence and arousal would covary with the source activation sources derived from LAURA maps, one-way ANCOVA for repeated measures were computed using BMDP2V program. A statistically significant covariate effect of pain ratings ($F(1,31) = 4.86$, $P = 0.035$) was found in the source activation located in the left parahippocampal gyrus in the time interval from 290–305 ms.
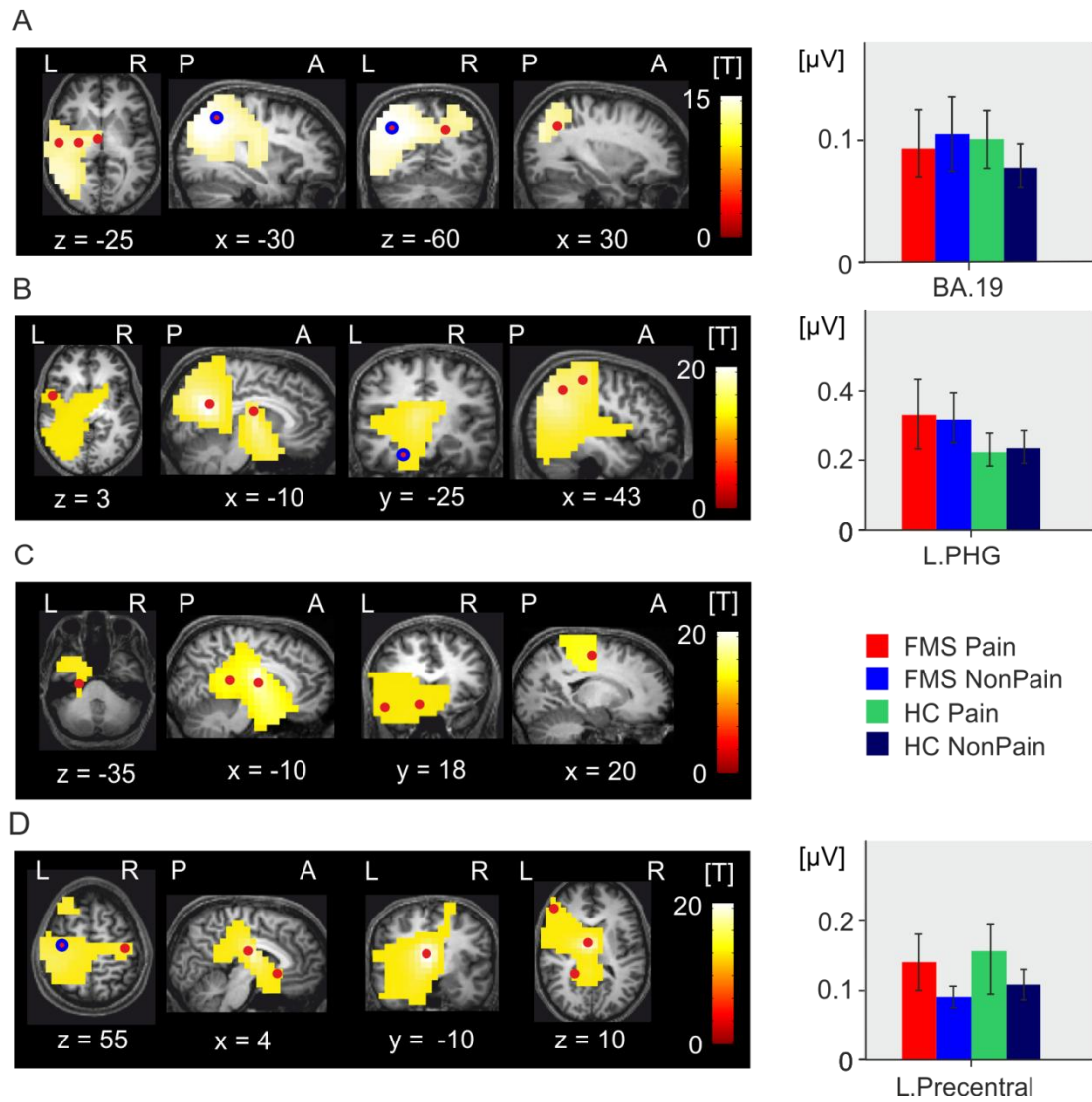
Fig. 5.7 Univariate analyses of LAURA source activations during observation of pictures. **A** Univariate *t*-test results showing LAURA source activations associated with viewing both types of photograph in the 135−150 ms time epoch, the threshold is set at *t* > 10. Peak activation source locations, exported for statistical analyses, are highlighted in red. Locations of source activations demonstrating main effects are indicated by blue circles. *Right panel,* bar charts demonstrating mean source activation values and error bars for each group and condition in each source showing a significant ANOVA effect in each time epoch of interest. **B** 290−305 ms, **C** 490 505 ms, **D** 565−580 ms.

Table 5.5 Source activations showing significant ANOVA effects during observation of pictures. Source activation values (mean ± SD), F

statistics and significance values are shown.

Time Epoch	FMS		Healthy		MNI [mm]						
	Pain	Non-pain	Pain	Non-pain	x	y	z	Location	ANOVA Effect	F	Sig
135−150	2.37 ± 3.19	2.44 ± 2.85	2.80 ± 1.20	1.69 ± 1.72	-38.5	-70	38.5	L.BA19	Group × Image	5.31	.027
290−305	0.34 ± 0.21	0.33 ± 0.15	0.22 ± 0.10	0.23 ± 0.10	-22.5	-24.5	-28.5	L.PHG	Group	5.35	.027
565−580	0.14 ± 0.01	0.09 ± 0.04	0.16 ± 0.10	0.11 ± 0.04	-55	-10.5	55	L.Precentral	Image	7.96	.008

L, left; R, right; BA, Brodmann area; PHG, parahippocampal gyrus

5.3 Resting-state functional connectivity alterations in FMS patients

Ongoing chronic pain may cause a time-dependent reorganisation of resting-state networks such as the DMN (Baliki et al., 2008), and previous studies indicate potential resting-state networks alterations in FMS patients (Napadow et al., 2010; Cifre et al., 2012). In order to expand on recent findings, a novel method of functional connectivity analysis (Whitfield-Gabrieli and Nieto-Castanon, 2012) was used to analyse resting-state fMRI data in FMS patients. Seed regions of interest (ROIs) were located in DMN and pain processing structures using co-ordinates identified by recent meta-analyses (Laird et al., 2009; Duerden and Albanese, 2011), and stringent exclusion criteria were employed to reduce sample heterogeneity. The functional connectivity method utilised allows for voxelwise analysis of connectivity between seed ROIs and voxels throughout the whole brain, as well as specific comparisons of connectivity between the selected ROIs. It was hypothesised that DMN and pain matrix functional connectivity would show alterations in FMS patients relative to healthy control subjects and that such abnormal connectivity would correlate with clinical measures of symptom severity.

5.3.1 Seed-to-voxel analysis

FMS patients, relative to healthy control participants, demonstrated significant connectivity differences between DMN seeds located in posterior cingulate cortex (PCC), left medial frontal gyrus (LMFG) and right inferior parietal lobule (RIPL) and a variety of cortical structures. Table 5.6 shows the *t*-maxima, MNI co-ordinates of the clusters showing altered connectivity with DMN structures, the number of voxels, and cluster-extent FDR corrected P values.

FMS patients exhibited reduced functional connectivity, relative to healthy controls, between the PCC seed in the DMN and the right parahippocampal gyrus (t (29) = -7.8, P = 0.011), and right inferior temporal gyrus (t (29) = −5.6, P = 0.011). FMS patient group also demonstrated enhanced connectivity, compared to healthy participants, between the right IPL seed and right hippocampus, (t (29) = 6.91, P = 0.034), the left MFG and left posterior parietal cortex (t (29) = 4.92, P = 0.024), and between the PCC seed and the left dorsolateral anterior cingulate cortex (t (29) = 6.18, P = 0.034). Seed-to-voxel analyses revealed no significant connectivity differences between FMS patients and healthy control subjects utilising pain processing structures as seeds. Results indicate that patients show abnormal connectivity between DMN seeds and structures located outside the DMN. Fig.5.8 shows the locations of DMN seeds (Fig.5.8 A), as well as the locations of clusters of voxels demonstrating altered functional connectivity to DMN seeds in the FMS patient group relative to healthy control group, and bar charts showing the relative functional connectivity strengths between seed regions and the clusters (Fig.5.8 B-D).
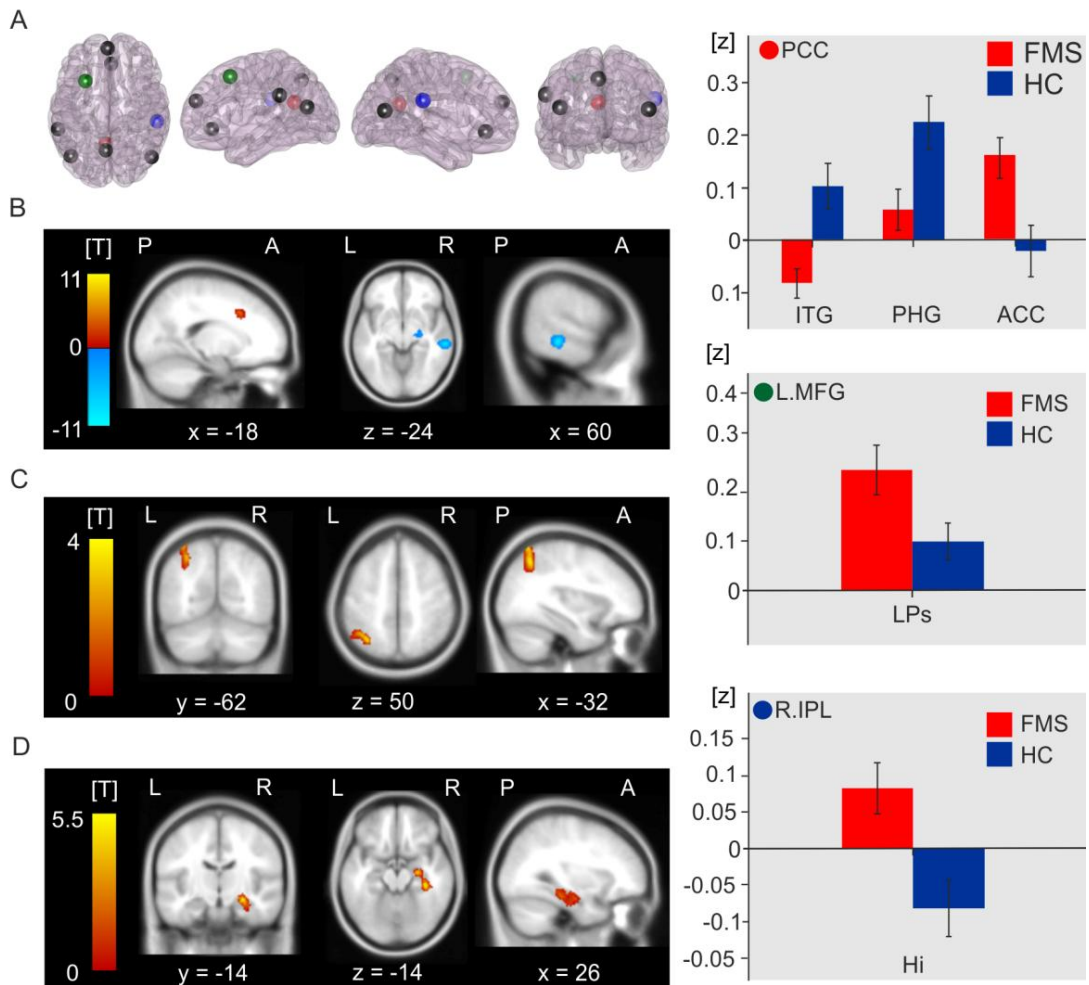
Fig.5.8 Functional connectivity alterations with default mode network structures. **A** The locations of all 9 DMN seeds as specified by Laird et al. (2009), the red sphere indicates the location of the PCC seed, green = left MFG and blue = right IPL seed, which showed significant group differences in functional connectivity with extrinsic structures. **B** Functional connectivity with the PCC seed. Red-yellow colour indicates a cluster showing increased connectivity with PCC in FMS patients relative to healthy controls, blue-light blue colour indicates clusters demonstrating reduced connectivity. *Top right panel;* bar chart of mean Fischer transformed correlation coefficients indicating relative functional connectivity with PCC seed for each significant cluster in FMS and healthy control groups. **C** Functional connectivity with the left MFG seed. Red-yellow colour indicates a cluster showing increased connectivity in FMS patients. *Middle right panel;* bar chart of mean Fischer transformed correlation coefficients indicating relative connectivity between left MFG seed and significant clusters in FMS and healthy control groups. **D** Functional connectivity with the right IPL seed. *Bottom right panel;* bar chart of mean Fischer transformed correlation coefficients indicating relative connectivity between right IPL seed and significant clusters in FMS and healthy control groups. ITG=inferior temporal gyrus; PHG=parahippocampal gyrus; ACC=anterior cingulate cortex; LPs=superior parietal lobule; Hi=hippocampal formation.

5.3.2 ROI-to-ROI analysis

ROI-ROI functional connectivity was compared between FMS patients and healthy control group using two-sided independent *t*-test analysis implemented in the CONN toolbox. There were no group differences in connectivity evident between any of the allocated DMN or pain processing seed ROIs (P > 0.05). This result is consistent with the seed-to-voxel analyses which only showed abnormal DMN seed connections with regions outside the accepted network which were not included in ROI-ROI analysis.

5.3.3 Correlations between functional connectivity parameters and clinical measures

Fisher-transformed correlation coefficients were extracted for each cluster of voxels showing significant group differences in functional connectivity between FMS patients and healthy control group. Pearson's correlation analysis was performed between functional connectivity coefficients and clinical measures including MTPS scores and symptom duration (years) in the FMS patient group. The correlation coefficients for functional connectivity between the cluster located in the right parahippocampal gyrus and the PCC seed, which demonstrated reduced connectivity in FMS patients relative to healthy control group, negatively correlated with the duration of symptoms in FMS patients (r = −0.50, p = 0.049). The degree of disruption to functional connectivity between PCC and parahippocampal gyrus was associated with longer symptom duration. Fig. 5.9 shows the scatterplot of correlation coefficients representing relative functional connectivity between PCC and parahippocampal gyrus and duration of symptoms in the FMS patient group.
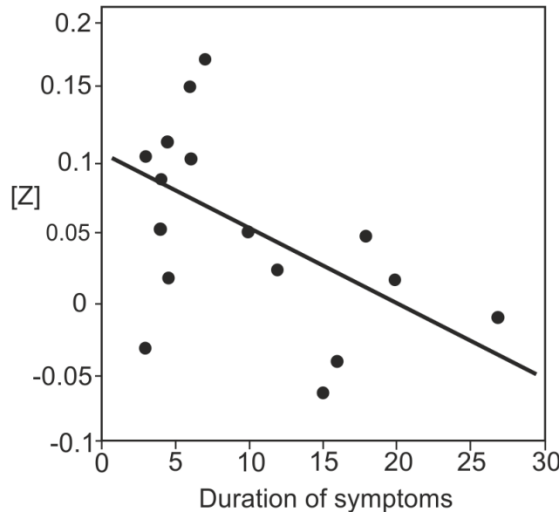
Fig. 5.9 Correlation between duration of symptoms and functional connectivity parameters. Scatter plot showing duration of symptoms (in years), and Fischer transformed connectivity correlation coefficients representing relative functional connectivity between precuneus and right parahippocampal gyrus in FMS patient group. The linear regression line is also shown.

TABLE 5.6 Seed-to-voxel analysis of resting-state functional connectivity alterations in FMS patients. Brain regions showing abnormal functional connectivity with DMN seed ROIs in FMS patients, relative to healthy control subjects, are shown with Cluster location, MNI co-ordinates (x,y,z) and *t*-maxima (cluster-level FDR corrected).

Seed	Contrast	Cluster	MNI [mm]	*k*	T	Clus P-FDR
PCC	HC>FMS	ITG	60 -30 -12	283	-7.8	0.011
	HC>FMS	PHG	14, -12 -24	264	-5.6	0.011
	FMS>HC	ACC	-18, 18 , 24	157	6.18	0.034
RIPL	FMS>HC	Hi	26 -14 -14	335	6.91	0.034
LMFG	FMS>HC	LPs	-32 -62 50	294	4.92	0.024

k = number of contiguous voxels; BA = Brodmann area; PCC =posterior cingulate cortex; MPFG= medial prefrontal gyrus; RIPL= right inferior parietal lobule; GTi=inferior temporal gyrus; PHG=parahippocampal gyrus; ACC=anterior cingulate cortex; Hi=hippocampal formation; LPs=superior parietal lobule.

5.4 Morphological alterations to subcortical and cortical structures in FMS patients

In the past, several studies have utilised voxel based morphometry analysis to investigate structural grey matter differences in FMS patients relative to healthy control subjects (Kuchinad et al., 2007; Schmidt-Wilcke et al., 2007; Luerding et al., 2008; Lutz et al., 2008; Burgmer et al., 2009; Valet et al., 2009; Robinson et al., 2011). However, there is little consistency in the VBM findings in FMS patients. To further investigate morphological alterations in FMS, a novel method of shape analysis of fifteen subcortical regions was carried out in a homogenous sample of FMS patients and healthy, age-matched controls subjects. In order to compare results with shape analyses, concurrent global and local grey matter alterations throughout the whole brain of FMS patients were also investigated using VBM. It was hypothesised that FMS patients would show subcortical abnormalities in shape and volume.

5.4.1 Subcortical shape analysis

Vertex analysis was performed to evaluate the shape and volume of 15 sub-cortical structures in FMS and healthy control groups. The brainstem showed a localised shape difference, which was significant following FDR correction for the number of vertices tested and Bonferroni-Šidák correction for multiple comparisons across 15 structures ($P = 0.01_{corr}$). In the FMS patient group the brainstem demonstrated an inward movement of vertices compared to healthy control participants on the left lateral medullary funiculus extending from the inferior tip of the brainstem to the level of the inferior olivary body (Naidich and Duvernoy, 2009) suggesting a volume reduction in this region. A slight outward movement of vertices just above this area in the left medulla at the level of the inferior olivary body was

also apparent. Fig. 5.9 shows the locations, directions and *F*-statistics of shape change of brainstem vertices in the FMS group relative to healthy control group. A correlation analysis was also performed in the FMS patient group to distinguish whether MTPS, FIQ and BDI scores were related to the alterations seen in FMS patient's brainstem shape. No correlations were identified between brainstem vertices positions and clinical or psychological measures in the FMS patient group ($P > 0.05$).
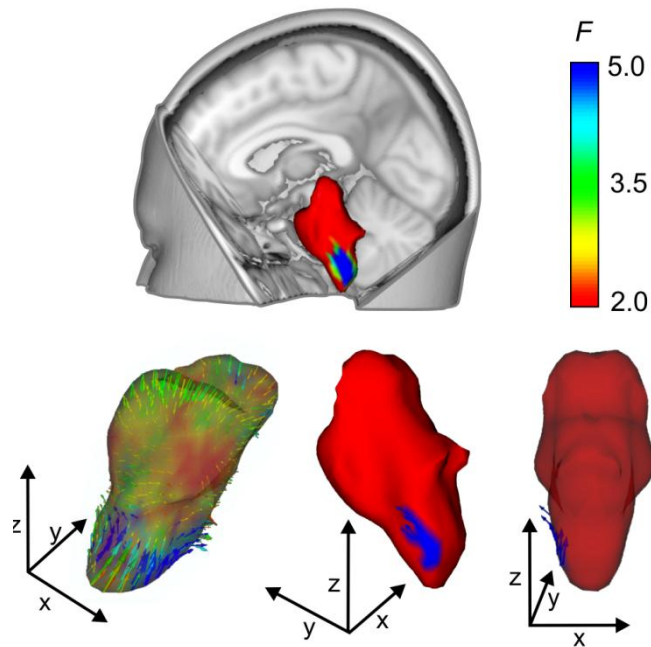

Fig. 5.10 Vertex analysis of brainstem shape alterations in the brainstem of the FMS patient group in comparison to healthy control participants. *Upper panel* shows the anatomical location of the brainstem and the local area exhibiting shape change in FMS patient group. *Lower left panel* indicates shape change in FMS patient group compared to healthy control group at an uncorrected level, this semi-transparent image shows direction of vectors, inward direction represents relative inward positions of vertices (in FMS subjects compared with healthy control subjects) indicative of volume reduction. Outward direction of vectors indicates relative shape increases in FMS patient group. Arrow colour and surface colour indicate the *F*-statistic of the change in the specific vertices (see colour bar). *Lower centre panel* shows the location of the difference in patient group following FDR correction, red colour indicates areas which did not differ significantly following FDR correction. *Lower right panel shows* a semi-transparent image following FDR correction indicating the direction of significant vectors showing alterations in the FMS group in comparison to healthy control participants.

A Student's independent *t*-test was performed to compare mean brainstem volumes (calculated during FIRST analysis) in FMS patient and healthy control groups. The mean total volume of the brainstem in FMS patient group was shown to be significantly reduced in comparison to healthy control participants (*t* (29) = 2.56, P = 0.016). Fig. 5.10A shows the bar charts and error bars for mean brainstem volumes in both groups. Pearson's correlation analysis was performed to evaluate the relationship between the reduction of brainstem volume and MTPS scores in the FMS patient group. A significant one-tailed correlation (r = -0.45, P = 0.039) was evident, indicating that patients exhibiting greater reductions in volume of the brainstem reported higher scores on the MTPS evaluation. Fig. 5.10B shows the scatterplot of individual brainstem volume and MTPS score data for the FMS patient group distributed along the regression line.
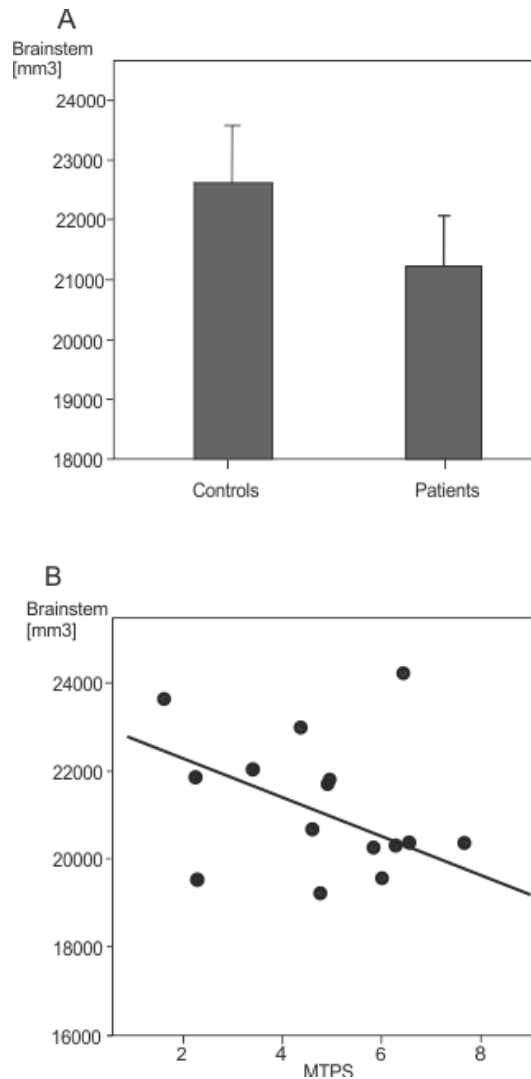
Fig. 5.11 Brainstem volume reductions and correlations with clinical measures. **A** Bar chart illustrating the mean total volume of the brainstem in FMS patient group and healthy control participants and error bars. **B** Scatter plot showing manual tender point scale (MTPS) scores and total volume of the brainstem (mm^3) as calculated during FIRST analyses. The linear regression line is also shown.

As Chiari I malformation may have contributed to the shape and volume changes in the brainstem, the distance between the basion-opisthion line and lower tip of cerebellar tonsils. Two FMS patients and one control subject showed a tonsillar position >5mm below the basion-opisthion line which meets the diagnostic criterion of Chiari I malformation (Watson et al., 2011). The mean distance between the basion-opisthion line to the tip of cerebellar tonsils was 0.58 ± 3.33 mm (mean $\pm$

SD) in FMS group and −0.12 ± 2.23 mm (mean ± SD) in control subjects (t (29) = −0.68, P = 0.50). Positive values denote measurements inferior to the basion-opisthion line and negative values indicate a measurement superior to the line. The individual measurements for each participant were entered as a covariate in MANCOVA design vertex analysis in FIRST. However, the cluster differentiating FMS and controls in the lower brainstem (Fig. 5.9A) was unchanged after entering cerebellar tonsils measurement as a covariate. Thus, it is unlikely that Chiari I malformation would account for the brainstem alterations seen in FMS patients.

5.4.2 Regional grey matter volume changes in FMS patients

Voxelwise comparison of local grey matter volumes across the whole brain revealed that FMS patients, relative to healthy control participants, exhibited two significant clusters of grey matter volume reduction located in the brainstem in the left ventral aspect of the basilar pons (in the pontine nuclei), and in the left precuneus (cluster extent P < 0.05 FWE-corrected). FMS patients also exhibited two clusters of grey matter volume increases located in bilateral primary somatosensory cortices. Fig. 5.11 shows the locations of clusters of voxels demonstrating grey matter alterations in FMS patient group relative to healthy control subjects displayed in glass brains and MNI standardised anatomical brains. Table 5.7 shows the MNI co-ordinates of clusters demonstrating grey matter volume decreases, table 5.8 shows increases in FMS patients relative to healthy control subjects. The anatomical locations of t-maxima as defined by the Harvard-Oxford atlas in FSL, peak T and Z values, number of voxels (k) and the cluster-level FWE corrected significance values are also shown. Pearson's correlation analysis was used to investigate potential linear relationships between regional grey matter volume changes and BDI, MTPS and FIQ

scores within the FMS patient group. Grey matter volume data was extracted for all clusters demonstrating increases or decreases for each patient in the FMS group and correlations with BDI, MTPS, FIQ score, age and duration of symptoms were calculated, no significant correlations were found.
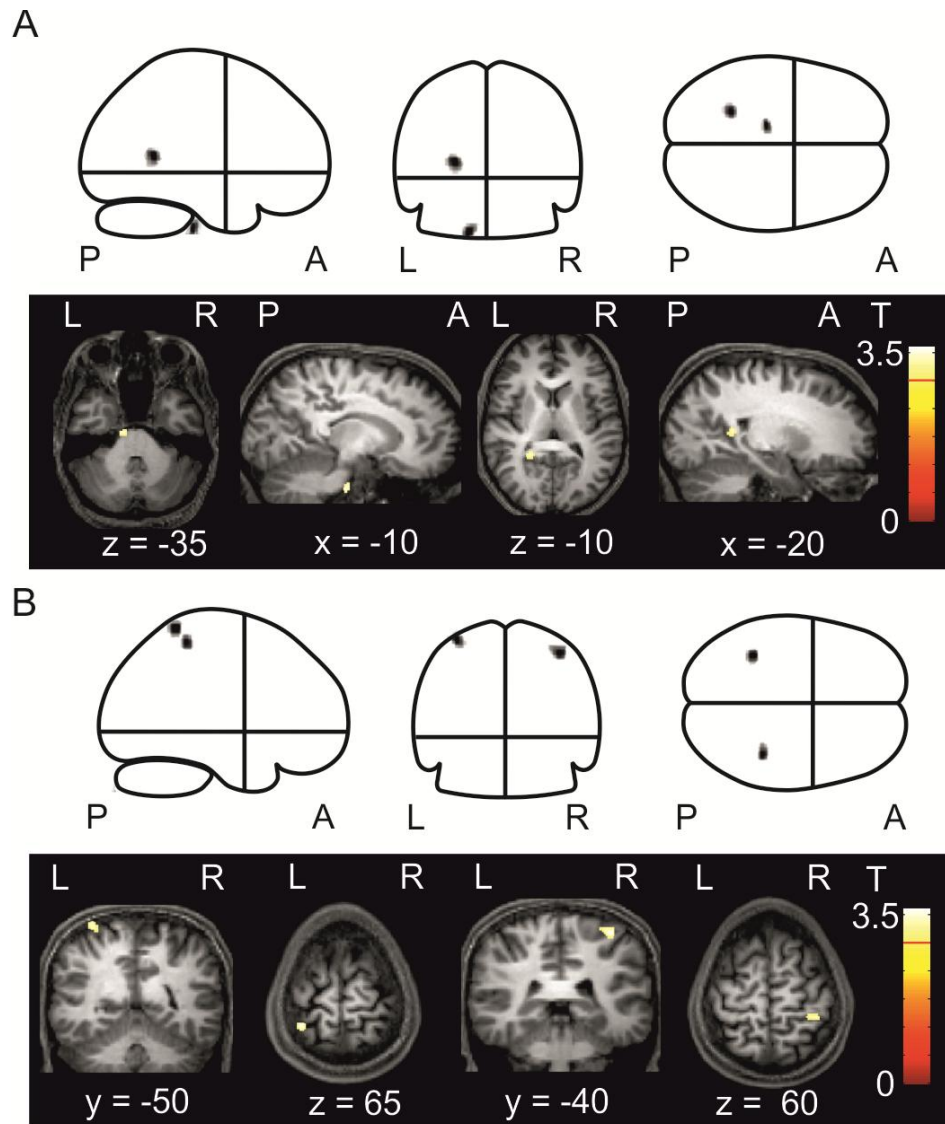
Fig. 5.12 Local grey matter volume alterations in FMS patients. **A** Local grey matter volume idereases as indicated by clusters representing spatially extended groups of voxels which differed significantly in grey matter volume at the uncorrected level (P < 0.001) in whole brain analysis and corrected level (P < 0.05) in cluster level analysis. Clusters are displayed in glass brains (*upper panel*) and MNI standardised anatomical brains (*lower panel*) x, y and z co-ordinates indicate slice dimensions in MNI space. **B** Local grey matter volume increases at the same significance level. Clusters are displayed in glass brains (*upper panel*) and MNI standardised anatomical brains (*lower panel*).

Table 5.7 Local grey matter volume decreases in FMS patient group relative to healthy control group.

Structure	MNI [mm]			k	Z	T	Sig.
	x	y	z				
Brainstem	-14	-21	-38	35	3.41	3.82	0.008
Precuneus	-23	-51	12	40	3.37	3.77	0.008

Table 5.8 Local grey matter density volume increases in FMS patient group relative to healthy control group.

Structure	MNI [mm]			k	Z	T	Sig.
	x	y	z				
Left SI	-36	-47	69	39	3.41	3.83	0.005
Right SI	39	-39	59	35	3.37	3.76	0.005

Anatomical structure location as defined by the Harvard-Oxford atlas. *t*-maxima locations according with MNI x, y, z co-ordinates in millimetres. L, left; R, right; k, number of voxels; T, peak *t* values; Z, peak z values; Sig., cluster-level FWE corrected P values; SI, primary somatosensory cortex.

5.4.3 Total intracranial volume statistics and correlations with clinical measures

A Student's independent samples *t*-test indicated no significant differences in mean total grey matter volume for FMS patients compared to healthy control subjects ($P > 0.05$). However, Pearson's correlation analysis showed a significant

correlation between total grey matter volume and MTPS scores in the FMS patient group (r = −0.63, P = 0.009). Patients with lower total grey matter volumes scored higher on the MTPS examination. Fig. 5.12 shows the data distributed along the regression line in a linear relationship.
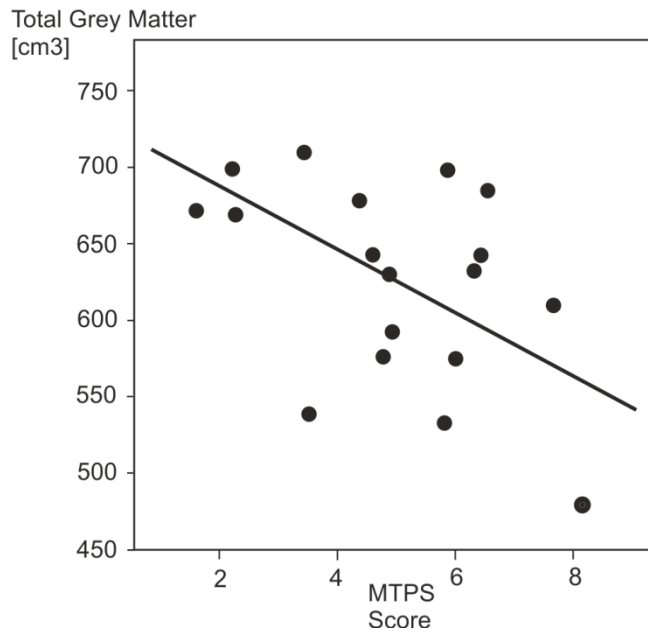
**Fig. 5.12** Correlations between total grey matter volume and MTPS scores. Scatter plot of MTPS scores and total grey matter volume (voxels, cm^3) as indicated by VBM8 analyses in the FMS patient group. The linear regression line is also shown.

5.5 Microstructural alterations to white matter in FMS patients

Studies of various chronic pain populations have revealed white matter alterations evident in pain processing structures (Geha et al., 2008; Gustin et al., 2010; Chen et al., 2011). Altered FA values were previously seen in structures such as the thalami of FMS patients (Sundgren et al., 2007; Lutz et al., 2008). This study investigated variations in the white matter integrity of FMS patients relative to healthy control subjects. DTI scans were quantitatively analysed by measuring fractional anisotropy (FA) to compare the integrity of white matter microstructure throughout the entire brain of FMS patients, relative to healthy control subjects. In addition, *a priori* regions of interest were identified in endogenous pain modulation and somatosensory processing systems and local directional diffusion information was used to probabilistically trace white matter pathways connecting the structures. It was hypothesised that alterations to FA values would occur within pain processing regions in FMS, and alterations to white matter connectivity would be evident between the structures associated with somatosensory processing and descending pain modulation.

5.5.1 Tract-based spatial statistics

TBSS was utilised to perform a voxelwise group comparison of FA values between FMS patients and healthy control subjects. Permutation analysis method was employed to account for multiple tests and TFCE was used to highlight clusters demonstrating significant differences without the need for a cluster forming threshold. Results revealed no significant clusters of voxels showing altered FA values in FMS patients relative to healthy controls. ($P > 0.05$). Mean FA values for the whole white matter skeleton for each subject were extracted using the study

specific FA mask. A Student's independent samples *t*-test indicated no significant difference in mean FA values throughout the white matter skeleton of FMS patients (0.37 ± 0.01), relative to healthy control group (0.38 ± 0.01), *t* (29) = -1.09, P = 0.282.

5.5.2, Probabilistic tractography analysis

Probabilistic tractography was performed to investigate potential connectivity alterations in specific tracts connecting *a priori* selected networks of particular interest in FMS. Table 5.10 shows the mean connectivity values and standard deviations between seed and target ROIs for FMS group and healthy control group. As functional alterations to processing of tactile stimuli (Gracely et al., 2002; Fallon et al., 2013) and endogenous pain modulation (Jensen et al., 2009; Jensen et al., 2012) were previously seen in FMS patients, cortical structures associated with somatosensory processing and endogenous pain modulation were selected for probabilistic tractography analysis based on previous studies (for a complete description of structure locations and basis for selection see Chapter 4.2.6.2).

Probabilistic tractography was performed using the rACC as a seed mask to investigate white matter connectivity to a target mask located in the brainstem, via two waypoint masks encompassing anterior thalami in each hemisphere. After exporting connectivity values and normalising by dividing by the waytotal a mixed two-way ANOVA for repeated measures (group × hemisphere) was performed. The results indicated a significant effect of hemisphere, (F(1,29) = 9.38, P = 0.005). Mean connectivity values between these structures in the right hemisphere of FMS patients (0.07 ± 0.03, mean ± SD) and healthy control subjects (0.10 ± 0.07) exceed connectivity between these structures in the left hemisphere in FMS patients (0.04 ±

0.03) and healthy controls (0.06 ± 0.05). The effect of group was not significant and neither was the group × hemisphere interaction effect (P > 0.05). Connectivity between somatosensory processing structures encompassing bilateral thalamus seed masks, a waypoint mask of the left and right thalamocortical tract, and target masks in bilateral SI cortices was also investigated. A two-way ANOVA for repeated measures revealed no significant effect of group, hemisphere or interaction for mean connectivity values (Table 5.9) between these structures (P > 0.05).

A concise summary of the main findings of each study in the thesis can be seen in Chapter 7.1.
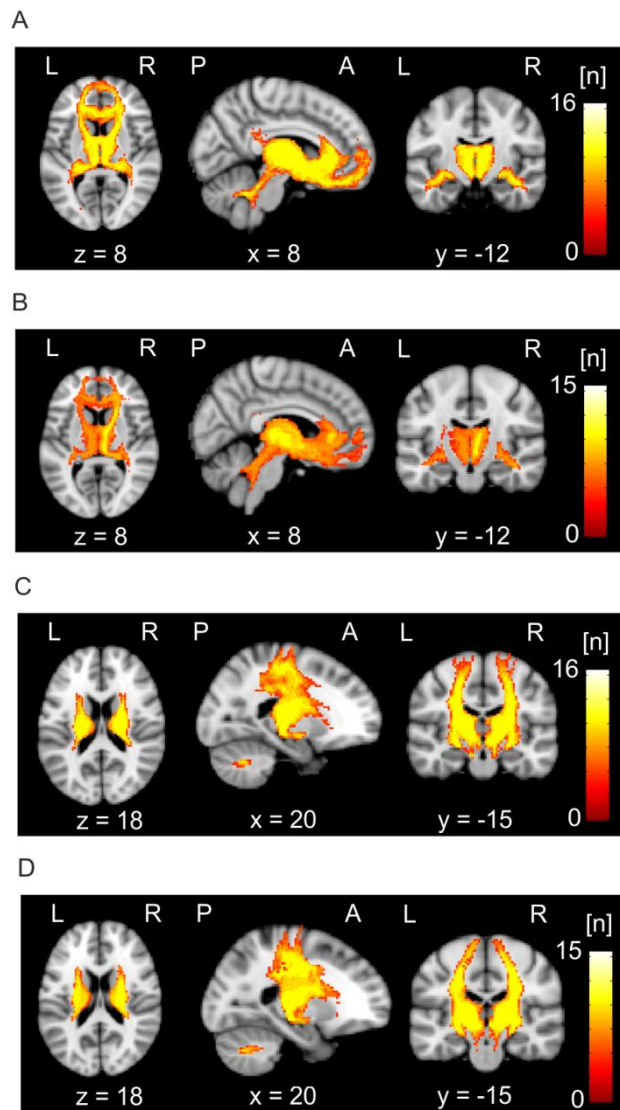
Fig. 5.13, Probabilistic tractography in somatosensory and endogenous pain modulatory structures of FMS patients. **A** Binarised tracts showing connectivity between rACC and brainstem, via anterior thalami, in FMS patients. Image is thresholded to show tracts evident in at least 50% of group members (>8 for FMS patient group, > 7 for healthy control group). **B** Connectivity between rACC and brainstem, via anterior thalami, in healthy control participants. **C** Binarised tracts showing connectivity between bilateral thalami and SI in FMS patients. **D** Binarised tracts showing connectivity between bilateral thalami and SI in healthy control participants.

Table 5.9 Relative connectivity between somatosensory and endogenous pain modulation structures identified using probabilistic tractography. Mean Fischer transformed connectivity coefficient values between seed and target ROIs in FMS Patients and Healthy Control Groups.

	FMS Patients		Healthy Control	
	Right	**Left**	**Right**	**Left**
rACC – brainstem	0.07 ± 0.03	0.04 ± 0.03	0.10 ± 0.07	0.06 ± 0.05
Thalamus-SI	0.12 ± 0.05	0.13 ± 0.06	0.11 ± 0.05	0.13 ± 0.05

rACC = rostral anterior cingulate cortex; SI = primary somatosensory cortex.

Chapter Six

Study discussions

6.1 Brain responses in FMS patients during somatosensory stimulations

The novel finding of the study of cortical oscillatory changes during brushing was the presence of beta-band ERD in the ipsilateral central-parietal region during brushing in FMS patients, but not in healthy control subjects. Larger beta-band ERD in FMS patients compared to control subjects co-occurred with greater pain scores during brushing period, although these two measures were not correlated ($P > 0.05$). However, the strength of ipsilateral beta-band ERD correlated with the patients MTPS scores suggesting that abnormal functioning during the processing of innocuous somatosensory stimulation could be significant in relation to clinical severity of FMS. Furthermore, the study pointed to insula and SII as cortical regions manifesting a stronger suppression of beta-band oscillations during brushing in FMS patients than in healthy participants.

Healthy control subjects demonstrated a contralateral beta-band ERD in central-parietal electrodes during brushing with the strongest source activation cluster localised by beamformer analysis to contralateral primary somatosensory cortex, confirming previous findings in healthy subjects (Cheyne et al., 2003). In contrast to healthy participants, FMS patients showed a more widespread beta-band ERD in contralateral electrodes and additional foci of beta-band ERD over ipsilateral

central-parietal electrodes. Beta-band ERD overlying somatosensory cortices accompanies tactile stimuli (Cheyne et al., 2003; Stancak et al., 2003; Gaetz and Cheyne, 2006) and is generally stronger overlying the contralateral hemisphere (Stancak, 2006). Further, beta-band ERD is indicative of activation in underlying cortical structures (Pfurtscheller and Lopes da Silva, 1999). The augmented pattern of beta-band ERD seen in FMS patients was modelled by distributed clusters of source activity in bilateral insular, SII and SI cortices. Ipsilateral cortical activations, occurring in SII and insula and corresponding to brush evoked allodynia, were demonstrated in neuropathic pain patients using fMRI (Peyron et al., 2004) and PET studies (Witting et al., 2006), and in fMRI studies of chronic regional pain syndrome patients (Mailhöfner et al., 2006). Thus, ERD topographic maps and source activations in the present study accord with previous imaging studies of chronic pain conditions.

Bilateral insula and somatosensory activations were previously demonstrated in fMRI studies of FMS patients using noxious mechanical pressure, cold and thermal stimuli (Gracely et al., 2002; Cook et al., 2004; Staud et al., 2008). This thesis demonstrates for the first time the presence of ipsilateral insula and SII activations in FMS patients during mechanic-tactile stimulation using electrophysiological measures of cortical activation. The activations in ipsilateral insula cortex during brushing may be particularly relevant to the clinical symptomatology of FMS. Augmented functional connectivity was previously shown between DMN structures and insula cortex in FMS patients at rest (Napadow et al., 2010). Magnetic resonance spectroscopy was used to demonstrate enhanced levels of glutamate in insula cortices in FMS patients, and glutamate levels also correlated with experimental pain reports (Harris et al., 2009). Decreased levels of gamma-

aminobutyric acid (GABA) have also been demonstrated in right anterior insula of FMS patients suggesting reduced inhibitory neurotransmission in this structure (Foerster et al., 2011). Neurotransmitter alterations may relate to the additional insula activation found during brushing in the current study. The insula cortex has been frequently reported as an important functional region in processing of clinical and experimental pain (Apkarian et al., 2005). Contralateral posterior insula has previously been identified as a structure which is particularly important in sensory-discriminative processing of pain and it is also highly interconnected with SI and SII cortices (Peltz et al., 2011). Inappropriate activation of insula cortices (particularly ipsilateral insula cortices) during innocuous stimulation may play a role in allodynia occurring in response to dynamic tactile stimulation in FMS patients.

Results allude to enhanced propagation of somatosensory afferent impulses into ipsilateral cortical structures in FMS patients which are not seen in healthy participants. However, it is not possible to infer whether ipsilateral cortical activation during tactile stimulation in FMS patients would be related to an increased excitability of the somatosensory cortices that may have been primed by long periods of muscle and joint pain, or to altered wiring of neuronal circuits such as thalamo-cortical or callosal neurons. The present finding corresponds with a previous functional imaging study of FMS patients in which innocuous pressure stimulation elicited allodynia and bilateral SII activations (Gracely et al., 2002). It was previously proposed that ipsilateral somatosensory and insula cortex activations during brushing may result from neuroplasticity alterations (Peyron et al., 2004). Prolonged nociceptive input in chronic pain disorders can lead to maladaptive neuroplasticity changes to nociceptive, motor and somatosensory systems (Seifert and Maihöfner, 2011). Pre-existing neuroanatomical pathways may become

potentiated or disinhibited as a result of severe injury or chronic pain (Kaas et al., 1999), and such neuroplasticity changes can cause abnormal activation patterns in functional imaging studies. However, when considering the evidence for central processing abnormalities in FMS, the possibility of altered functioning in the peripheral nervous system contributing to findings should not be overlooked (Petersel et al., 2011).

Ipsilateral activations in SI, SII and insular cortices and allodynia pain have previously been demonstrated during brushing of the paretic limb of hemispherectomised patients (Olausson et al., 2001) suggesting that neuroplasticity changes can lead to ipsilateral pain matrix activations during brush evoked allodynia. FMS patients may undergo re-organisation of spino-thalamo-cortical projections to ipsilateral somatosensory and insular cortices. Such neuroplasticity changes in FMS could occur as a result of prolonged afferent nociceptive input which then exacerbates the experience of chronic pain. Alternatively, a predisposition to develop the specific structural brain changes seen in structural neuroimaging studies may constitute the primary pathophysiology of FMS (Schmidt-Wilcke and Clauw, 2011). However, the present use of a cross-sectional study design means it is difficult to infer any progressive aspect of alterations and this limitation should be considered when interpreting the findings.

In conclusion, the findings of the first study show that FMS patients demonstrate functional alterations to processing of innocuous somatosensory stimulation such as brushing. The degree of ipsilateral beta-band ERD at central-parietal electrodes in FMS patients correlated with clinical MTPS scores indicating that functional alterations to the processing of innocuous somatosensory stimulation in FMS patients may contribute to clinical symptom severity. It appears that

ipsilateral activations in FMS relate to the clinical manifestations of FMS rather than the presence of disorder alone. Augmented ipsilateral ERD may represent a physiological correlate of central sensitisation in FMS patients, possibly as a result of potentiation or disinhibition of subcortico-cortical projections to ipsilateral cortical regions such as insula, SI and SII. Such functional alterations could be a result of the stream of afferent somatosensory information manifesting in FMS chronic pain, or, alternatively, may exist as a predisposing factor to the development of FMS. This finding is particularly promising and highlights the potential for utilisation of ERD method in conjunction with, for example, standardised pressure stimulation as a research tool to investigate clinical aspects of FMS such as ongoing symptom progress or the effectiveness of therapeutic interventions. Further research should also elaborate on the specific pathophysiological causes of functional alterations in FMS in response to innocuous somatosensory stimulation.

6.2 Cortical activations in FMS patients during observation of pain pictures

Behavioural results from the analysis of observation of pictures revealed that FMS patients attribute greater pain, valence and arousal to pain pictures, but not to non-pain pictures, relative to healthy control participants. This finding accords with a previous study which showed that FMS patients exhibit increased aversion to negative affective photographs (Bartley et al., 2009). FMS patients also demonstrated alterations to ERP components during observation of pain pictures and source activations located in occipital cortex and parahippocampal gyrus displayed abnormal activation patterns when observing pain pictures. The parahippocampal source activation difference in FMS patients covaried with psychological and clinical measures.

In the healthy control group, pain pictures- relative to non-pain pictures- were associated with increased amplitude of the positive component overlying occipital electrodes 135−150 ms after stimulus onset; this was not found in FMS patient group. Analysis of LAURA distributed source localisation volumes revealed a source activation located in the occipital lobe (BA19), which similarly demonstrated augmented source activation during pain pictures in healthy control subjects but not in FMS patients. Enhanced short latency components (110−180 ms post-stimulus) were previously observed in occipital electrodes during observation of pain pictures relative to non-pain pictures (Proverbio et al., 2009), and augmented occipital activations were also seen during viewing of pain scenes in fMRI studies (Lamm et al., 2007; Akitsuki and Decety, 2009). It was previously proposed that occipital lobe structures play an active role in early discrimination of pain and non-pain scenes (Fan and Han, 2008). The data suggests that these early cortical processes, localised in occipital cortices, may be compromised in FMS patients when observing pain pictures. It is also noteworthy that other factors, such as visual attention, may effect subjective ratings and neural responses to pain perceived in others (Gu and Han, 2007). It is also feasible that the alterations seen in early ERP components in FMS patients may also relate to enhanced visual attention to perceived pain stimuli.

In the time period 290−305 ms after stimulus onset, FMS patients exhibited an augmented positive component over occipital regions relative to healthy volunteers when observing pain pictures. LAURA source analysis in this time window also revealed stronger source activations in the left parahippocampal gyrus of FMS patients when observing pain pictures. A significant covariation effect was found between source activation differences in the parahippocampal gyrus and clinical symptom severity (FIQ scores), depression (BDI scores) and the amount of

pain attributed to pain pictures. Parahippocampal activations were previously shown to play a role in emotional modulation of experimental pain (Ploghaus et al., 2001; Stancak et al., 2012a) and source activations in this region were augmented in females, relative to males, when observing pain pictures, which was purportedly linked to increased empathic response to perceived pain in females (Proverbio et al., 2009). The hippocampal formation also forms part of a network underlying memory function, and is vital for the appropriate formation of pain memories (Buckner et al., 2008; Spreng et al., 2009). Painful stimuli are encoded as pain memories in order to adapt future behaviour to prevent painful outcomes (Vogt, 2005), and this process of learning may be dysfunctional in chronic pain states (Albanese et al., 2007). Covariance analyses indicate that augmented parahippocampal gyrus source activation may relate to enhanced pain scores attributed to pain pictures by FMS patients as well as clinical symptom severity and negative affective disturbance. Therefore, this region may play an important functional role in the interaction between psychological disturbance and clinical symptoms in FMS.

FMS patients demonstrated an enhanced negative component over left frontal electrodes in the 490−505 ms window. However, LAURA source analyses indicated no sources demonstrating an effect in this time epoch. Pain pictures were associated with an augmented negative component over left frontal scalp electrodes in the 565−580 ms window and LAURA analysis revealed a source in the left precentral gyrus demonstrating an effect of picture type. As the picture effect on activation in this source was similar in both groups, it is unlikely to be affected in FMS. Components in similar time epochs were previously shown to differentiate between pain and non-pain pictures (Han et al., 2008). An fMRI study also identified stronger precentral gyri activations during viewing of pain relative to non-pain scenes which

was inferred as relating to enhanced motor-readiness during viewing of pain (Akitsuki and Decety, 2009). Thus, FMS appears to affect discriminatory and psychological aspects of processing observation of pain pictures, but not perception-action response mechanisms.

To conclude, FMS patients attribute more pain to pain scenes, and rate them as more unpleasant and arousing compared to healthy people. FMS patients also show alterations to ERP components and distributed source localisation activations when observing pain pictures. Reduced early occipital component amplitudes and sources activations localised to BA19 may indicate dysfunction to early discriminatory processing of pain pictures in FMS patients. Later occipital component amplitudes and augmented source activations in the parahippocampal gyrus of FMS patients relative to healthy control subjects may infer exaggerated processing during viewing of pain in FMS patients which could relate to the attribution of enhanced pain in pain pictures, as well as psychological and clinical factors.

6.3 Resting-state functional connectivity alterations in FMS patients

Functional connectivity analysis of resting-state fMRI data revealed that FMS patients exhibit alterations to connectivity between DMN structures and various cortical regions located outside the network. No connectivity differences were identified between DMN structures in the FMS group. Correlation analysis showed that DMN connectivity reductions with right parahippocampal formation in FMS patients were associated with longer duration of symptoms. Analysis of seed ROIs in pain processing structures revealed no significant functional connectivity

differences between the ROIs or with external structures in FMS patients relative to healthy people.

FMS patients exhibited reduced functional connectivity, relative to the healthy control group, between the PCC seed in the DMN and a cluster located in the right parahippocampal gyrus. The PCC is often activated during experimental pain stimuli and is considered part of the 'pain matrix' (Apkarian et al., 2005). This region has been shown to be important in multiple aspects of pain processing such as anticipation of pain (Watson et al., 2009), or the encoding of pain memories to prevent future inappropriate behaviour which will lead to painful outcomes (Vogt et al., 1996; Vogt, 2005).These aspects of pain processing may be affected as part of the complex psychological profile of FMS. The disruption to connectivity between PCC and parahippocampal gyrus correlated with the duration of symptoms in FMS patients, which may indicate a time-dependent alteration although this cannot be specifically attributed to FMS pain. The right IPL structure in the DMN demonstrated increased connectivity with the right hippocampal formation in FMS patients relative to control group. Connectivity between DMN structures and hippocampal formation, including entorhinal cortex and parahippocampal gyri is regularly seen in functional imaging studies, and the region was previously proposed as a structure of the DMN (Greicius et al., 2004; Buckner et al., 2008). Similarly, functional connectivity analysis utilising a seed located in hippocampal formation was shown to demonstrate functional correlations with regions which closely mimic DMN activations (Vincent et al., 2006).

The overlap between DMN and hippocampal activation led to the proposal that DMN activity may be related to networks underlying memory function (Buckner et al., 2008; Spreng et al., 2009). This theory is supported by evidence that resting-

state functional connectivity between PCC and hippocampal formation predicts performance on a memory task in healthy people (Wang et al., 2010), and reductions in connectivity between PCC and hippocampal formation were previously identified in patients with Alzheimer's disease (Kenna et al., 2012; Schwindt et al., 2012). Grey matter reductions were seen in the hippocampal formation (including parahippocampal gyri) of FMS patients (Kuchinad et al., 2007; Wood et al., 2009), and it was previously postulated that morphological abnormalities in this region in FMS patients may contribute to cognitive and perceptual deficits in the disorder (Wood et al., 2009). Reduced functional connectivity between PCC in the DMN and parahippocampal gyri may be related to the cognitive dysfunction seen in FMS.

The PCC in the DMN also showed reduced connectivity with right inferior temporal gyrus in FMS patients. Reduced connectivity between the DMN and inferior temporal gyrus was previously identified in chronic pain patients with diabetic neuropathy, and this was attributed to a reorganisation of resting brain networks which may contribute to spontaneous pain (Cauda et al., 2009). FMS patients also demonstrated increased functional connectivity between the left middle frontal gyrus in the DMN and the left superior parietal lobule. As this cluster is adjacent to the left inferior parietal lobule DMN seed, the augmented connectivity is likely to reflect an expansion of the DMN into this region in FMS patient group. This finding accords with hyperperfusion in the left superior parietal lobule that was previously seen in FMS patients during rest (Usui et al., 2010), and which the investigators attributed to reorganisation of the DMN.

FMS patients also demonstrated increased functional connectivity between posterior and anterior cingulate cortex. Similar increases in connectivity between DMN and ACC were previously seen in chronic back pain patients, which was

conjectured to represent dysfunction of the endogenous pain modulatory system in chronic pain (Loggia et al., 2012). The ACC plays an important role in endogenous pain modulation (Bingel et al., 2006), and is also a structure of the DMN (Fox et al., 2005; Laird et al., 2009; Whitfield-Gabrieli and Ford, 2012). Therefore, the augmented connectivity evident between PCC and ACC may relate to dysfunctional endogenous pain modulation in FMS patients due to tonic ongoing pain when at rest.

No connectivity differences were found between pain processing structures in FMS patients relative to healthy control participants. Although one previous study identified aberrant functional connectivity between pain processing regions (Cifre et al., 2012) it should be noted that this analysis only utilised 9 FMS patients, and also used patients of both sexes. In the present study a superior sample size was employed. DMN seed connectivity did show connectivity differences with pain processing structures such as ACC and inferior temporal gyrus, which may relate to aberrant processing of tonic pain in FMS. However, no abnormal connectivity was identified between DMN structures and anterior insula cortex as seen in previous studies (Napadow et al., 2010; Napadow et al., 2012). These previous findings originate from the same research group, and heterogeneity of the FMS patient sample available to this group may explain the results. A similar finding was recently highlighted in temporomandibular disorder patients by the same research group (Ichesco et al., 2012).

The DMN can be disrupted by chronic pain (Baliki et al., 2008), and our findings suggest that FMS alters DMN functional connectivity with brain regions such as parahippocampal gyrus, ACC and inferior temporal gyrus. Connectivity alterations may reflect ongoing time-dependent reorganisation of resting-state networks, and could also have implications for cognitive dysfunction, spontaneous

pain processing and dysfunctional endogenous pain modulation in FMS. However, the failure to reproduce some of the previous findings in FMS patients highlights the difficulties of performing functional connectivity analysis in a complex disorder. FMS shares a complex epidemiological profile with several other somatoform disorders and FMS patient groups routinely demonstrate a wide-range of heterogeneity. As a result of this complexity it may not be possible to identify specific functional connectivity alterations that specifically relate to FMS pathophysiology, especially when analysing a relatively small sample. However, larger sample sizes, which would enable utilisation of sub-grouping based on heterogeneity in the population, could eventually lead to improved understanding of functional connectivity alterations in FMS.

6.4 Morphological alterations to cortical and subcortical structures in FMS patients

Morphological analysis of subcortical structures revealed significant shape alterations and volume reduction in the brainstem of FMS patients. VBM analysis also demonstrated local grey matter reductions in the brainstem as well as in the left precuneus of FMS patients. The volume reduction of the brainstem of FMS patients showed a significant correlation with MTPS scores, indicating that alterations to this structure may play an important role in the pathogenesis or maintenance of FMS symptoms. In addition, total grey matter volume in FMS patients also demonstrated a negative correlation with MTPS scores which further suggests that grey matter reduction in FMS is relevant to symptom severity.

In the FMS patient group a reduction or 'sucking in' of shape was evident, located in the left lateral medullary funiculus extending from the inferior tip of the brainstem (as defined by FIRST segmentation) to the level of the inferior olivary

body. Patients also exhibited significant reduction in mean total volume of this structure. The inward shape alterations evident on the surface of the medulla in FMS patients indicate volume reduction in the nuclei underlying this region. Specifically, a reduction in the reticular formation nuclei could lead to the shape alterations seen on the brainstem surface. Reticular formation nuclei are involved in many systems relevant to fibromyalgia syndrome such as homeostatic regulation, postural maintenance, and sleep cycle control (Naidich and Duvernoy, 2009). This formation also contains the rostral ventromedial medulla which is the major relay point between periaqueductal grey and dorsal horn neurons. Together these structures constitute the primary descending pain modulation pathway in the brain (Lovick, 2008; Naidich and Duvernoy, 2009).

Behavioural and psychophysical studies have demonstrated that descending pain modulation is impaired in FMS (Kosek and Hansson, 1997; Julien et al., 2005). Functional MRI studies of FMS patients undergoing noxious stimulation indicate reduced activity in regions of the brainstem associated with descending pain inhibitory mechanisms (Jensen et al., 2009), and reduced connectivity between the brainstem and pain processing regions (Jensen et al., 2012). A VBM study also recently reported grey matter reductions in the brainstem of FMS patients (May, 2009). Functional imaging of the brainstem and spinal cord during innocuous and painful touch suggests that dysfunctional descending pain modulation may explain allodynia pain (Ghazni et al., 2010), and such dysfunction may facilitate the development of chronic pain in FMS and other chronic pain disorders (Staud, 2011a). Shape alterations and volumetric reductions may relate to dysfunctional pain modulation in FMS but it should also be noted that a recent VBM study of chronic fatigue syndrome, a disorder closely related to FMS (Barnden et al., 2011), also

suggests structural grey matter reductions in the brainstem (although these changes appear to involve different sub-regions of brainstem). In a complex syndrome such as FMS it is difficult to infer specific clinical implications of local abnormalities in a cross sectional study.

Reduction of the lower brainstem in FMS patients, seen in shape analysis, cannot be attributed to Chiari I malformation which has been suggested to be associated with FMS syndrome (Thimineur et al., 2002; Heffez, 2011; Watson et al., 2011). However, it was reported that symptoms seen in FMS, such as widespread muscle tenderness, occur in 21.6% of patients after a neck injury compared to only 1.7% patients with injuries to the lower extremities (Buskila et al., 1997). A combination of multiple anatomical factors in the cervico-spinal region might have contributed to the shape change in lower brainstem in FMS patients seen in the study.

VBM analysis identified decreased local grey matter volumes in the brainstem and left precuneus of FMS patients compared to healthy controls. Brainstem reductions in patients were localised to the left ventral aspect of the basilar pons, indicating a region containing the pontine nuclei (Naidich and Duvernoy, 2009). This group of nuclei are involved in regulation of motor activity and the relaying of information between the motor cortices and the cerebellum. Lesions in this region cause motor deficiencies, such as complete or partial hemiplegia (paralysis) and dysarthria (clumsiness) (Schmahmann et al., 2004). FMS affects dynamic balance control and is associated with clumsiness and a high prevalence of falls which may result from alterations to somato-motor inputs to the CNS (Jones et al., 2011). Postural correction requires fast subconscious correction of

muscle movements, peripheral input is relayed, via the brainstem, to the cerebellum where efferent corrections are generated (Peterka and Loughlin, 2004; Horak, 2006). Therefore, grey matter reductions in pontine nuclei, may relate to the postural deficits in FMS and this should be addressed in future studies.

The left precuneus also demonstrated grey matter reductions in FMS patients relative to healthy control subjects. Precuneus is important in endogenous pain modulation and shares connectivity with periaqueductal grey and posterior cingulate cortex during experimental pain relief (Zyloney et al., 2010). Enhanced resting cerebral blood flow has been demonstrated in the precuneus of FMS patients and this hyperperfusion predicted the success of gabapentin treatment (Usui et al., 2010). The precuneus is also a structure of the DMN (Greicius et al., 2003). DMN connectivity can be disrupted by long term chronic pain (Baliki et al., 2008). As described earlier (Chapter 5.3, 6.3), FMS patients exhibit functional connectivity alterations between DMN structures and extrinsic structures such as parahippocampal gyrus. Previous studies have also identified abnormal connectivity with DMN structures in FMS (Napadow et al., 2010). Thus, grey matter reductions in the precuneus of FMS patients may contribute to dysfunctional descending pain modulation generated in the posteromedial cortex and/or disrupted DMN connectivity.

FMS patients exhibited bilateral increases in grey matter in somatosensory cortices. Primary somatosensory cortices are important in the discriminative aspects of pain processing (Lee et al., 2008). FMS patients have demonstrated increased activity in somatosensory cortices, compared to healthy controls, during noxious mechanical pressure (Gracely et al., 2002), and a VBM study in healthy people reported increased grey matter in somatosensory cortices following daily

experimental pain stimulation (Teutsch et al., 2008). However, grey matter reductions appear to be more relevant to the symptoms of FMS. The total grey matter volume in FMS patients correlated negatively with MTPS scores, indicating that patients with greater global grey matter volume reduction exhibited increased clinical symptom severity.

Subcortical morphological alterations in FMS patients are pertinent to the disorders pathophysiology and merit further investigation. The novel technique of shape analysis used in the present study indicates morphological abnormalities in structures relevant to endogenous pain modulation and other aspects of FMS. Future studies could prioritise scan parameters and analysis techniques to further elucidate subcortical alterations in FMS, and also to investigate whether such alterations could correlate with functional abnormalities such as decreased functioning of descending nociceptive control system during experimental pain, dysfunctional postural control or sleep disturbance. Possible structural and functional alterations to the pain modulatory system in FMS may also be affected by current treatments (such as pharmacological interventions, self-management programs, or non-invasive brain stimulation techniques), and a longitudinal investigation could improve understanding of both the pathophysiology of FMS and the mechanisms of such interventions.

6.5 Microstructural alterations to white matter in FMS patients

Analysis of DTI data revealed no significant white matter alterations in FMS patients in either voxelwise comparisons of FA values or probabilistic tractography comparison of connectivity between specific ROIs. These negative findings are still important as they indicate that structural and anatomical abnormalities evident in

FMS are unlikely to occur in white matter. Therefore, grey matter structural alterations are the more probable explanation for any evident morphological changes such as the brainstem alterations described in the previous study. DTI method is suitable for assessing the effects of clinical pathologies which challenge the integrity of white matter such as multiple sclerosis (Iwasawa et al., 1997). It was previously proposed that this DTI analysis may possess improved sensitivity to subtle morphological changes in FMS patients (Lutz et al., 2008). It is known that the majority of white matter developmental changes occur in the first few years of life (Hermoye et al., 2006; Huang et al., 2006), although certain white matter pathways, particularly in prefrontal cortices, can continue development into adolescence or early adulthood (Giorgio et al., 2008).However, the lack of findings in FMS patients using DTI methods suggests that the syndrome does not affect the development or integrity of white matter microstructure in the brain.

As described in Chapter 1.2.3, FMS is a heterogeneous syndrome, and patient samples may show varying proportions of comorbidities such as depression or anxiety (Thieme et al., 2004), irritable bowel syndrome, post-traumatic stress disorder and temporomandibular disorder (Clauw, 2009). This wide heterogeneity means that the structural alterations found in anatomical imaging studies are difficult to interpret. A previous study identified multiple FA changes in FMS patients (Lutz et al., 2008). However, the authors also identified a high frequency of post-traumatic stress disorder and anxiety in their patient population, and this was proposed as a potentially confounding factor in white matter findings (Lutz et al., 2008). White matter abnormalities were previously associated with several disorders often comorbid with FMS such as post-traumatic stress (Abe et al., 2006), anxiety (Ayling et al., 2012), and temporomandibular disorder (Moayedi et al., 2012). Therefore, a

high frequency of any of these disorders in an FMS sample could illicit white matter findings that are not actually specific to FMS.

A previous DTI study of FMS patients found reduced FA in the right thalamus in FMS patients relative to a healthy group (Sundgren et al., 2007). However, the same study reported no differences in the apparent diffusion coefficient (indicating that the total amount of water diffusion in the region was normal). These results have been interpreted to suggest that FA differences in the thalami of FMS may be caused by neuronal reorganisation rather than axonal degeneration (Gracely and Ambrose, 2011). Disorganisation of white matter may be better investigated utilising probabilistic tractography to investigate the connections between appropriate structures that are relevant to the symptoms seen in FMS. In this study probabilistic tractography was utilised to investigate specific alterations to connectivity between structures in the somatosensory processing (including the thalami) and pain modulatory systems. This method was previously used to assess white matter tract connectivity associated with endogenous pain modulation during experimental placebo analgesia (Stein et al., 2012). However, despite utilising ROIs similar to those in previous successful investigations, no connectivity probability differences were found between FMS patients and healthy control group in either system. This would indicate that, although dysfunction in either of these systems could contribute to FMS symptoms, any problem is unlikely to be a result of alterations to white matter tract structure or connectivity. Therefore neuronal re-organisation is unlikely to account for the functional ERD alterations previously shown during processing of innocuous somatosensory stimuli in FMS patients (Chapter 5.1, 6.1).

In conclusion, it would appear that white matter microstructure and integrity in FMS patients is comparable to normal populations. Although previous studies have identified white matter alterations in FMS, it is important to appreciate no differences in FA have ever been reported at a corrected voxelwise level (Sundgren et al., 2007; Lutz et al., 2008). The present findings highlight the relative importance of grey matter alterations to morphological abnormalities associated with FMS, and also suggest that the development of white matter pathways is unaffected by FMS. Probabilistic tractography results show that abnormal white matter connectivity alterations are unlikely to contribute to somatosensory processing or endogenous pain modulation dysfunction in FMS.

Chapter Seven

General discussion

7.1. Summary of findings

The first study of this thesis investigated whether allodynia pain caused by innocuous somatosensory stimuli in FMS patients was associated with cortical excitability alterations. It was hypothesised that FMS patients would report subjective pain as a result of brushing stimuli, and exhibit alterations in alpha and beta band power changes, relative to healthy control subjects. Mean subjective pain ratings reported during brushing were significantly higher in FMS patients than in the healthy control group. An independent *t*-test comparison of ERD during brushing revealed a cluster of electrodes over ipsilateral (right) central-parietal region which demonstrated beta-band ERD in the patient group only. It was shown that beta-band ERD in this cluster of electrodes correlated with clinical MTPS scores, indicating that functional alterations in the processing of innocuous somatosensory stimulation in FMS patients may contribute to clinical symptom severity. Using beamformer analysis, the beta-band ERD in FMS patients was modelled by clusters of source activity in bilateral insular, SII and SI cortices compared to contralateral sources in healthy control group.

The second study utilised ERP analysis of EEG data recorded during viewing of pictures depicting pain in others or graphically similar images not containing pain. The aim of this investigation was to evaluate potential changes to cortical processes

underlying the observation of pain in FMS patients, and to elucidate how alterations could relate to FMS symptoms. It was hypothesised that FMS patients, relative to healthy control subjects, would attribute stronger pain ratings to pain pictures and manifest alterations in ERP components, which could be localised to pain or emotional processing structures. Results showed that FMS patients subjectively rated pain pictures as containing greater pain, and also rated them as more unpleasant and arousing than healthy people. The FMS patient group exhibited alterations in early occipital component amplitudes and source activations localised to BA19 compared to healthy control subjects. This finding may relate to dysfunctional early processing of pain pictures in FMS patients. Later occipital component amplitudes and source activations in the parahippocampal gyrus were augmented in FMS patients compared to controls during observation of pain pictures. The degree of augmentation covaried with the amount of pain attributed to pain pictures, and psychological and clinical factors.

Using resting-state fMRI scans, the third study investigated functional connectivity between the brain structures involved in pain processing and the DMN in FMS patients. It was hypothesised that FMS patients would show altered functional connectivity between DMN and pain processing structures, and also with structures extrinsic to these networks. FMS patients, relative to healthy control participants, exhibited functional connectivity alterations between DMN structures and various brain regions outside the network such as the hippocampal formation (including parahippocampal gyrus), anterior cingulate cortex, superior parietal lobule and inferior temporal gyrus. No abnormal functional connectivity was identified with pain processing structures. Correlation analysis revealed that reduced functional connectivity between the PCC in the DMN and the right parahippocampal gyrus in

FMS patients was associated with longer duration of symptoms. The present findings suggest that FMS alters DMN connectivity with brain regions such as the hippocampal formation, and particularly the parahippocampal gyrus. Although no alterations in functional connectivity between DMN structures were identified, altered DMN connectivity with parahippocampal gyrus is particularly interesting as it was previously considered as a structure of the DMN (Greicius et al., 2004; Buckner et al., 2008). Disrupted functional connectivity between PCC and parahippocampal gyrus was associated with a longer duration of symptoms, which may reflect ongoing time-dependent reorganisation of resting-state networks in FMS.

The fourth study employed a novel method of geometric shape analysis of subcortical structures and VBM analysis to evaluate morphological alterations to subcortical structures and cortical grey matter in FMS patients. High-resolution T1-weighted anatomical scans were analysed, and it was hypothesised that FMS patients would show subcortical shape and volume abnormalities relative to healthy people. FMS patients demonstrated shape alterations to the left lateral aspect of the medulla in the brainstem and the mean total volume of the brainstem was found to be significantly reduced in the patient group. The degree of volume reduction in this structure correlated with clinical MTPS scores, which infers that morphological alterations in this region are related to clinical symptom severity. This finding may be indicative of deficiencies in underlying nuclei or structures involved in descending nociceptive control in FMS patients, which would explain the relationship with sensitivity to mechanical pressure. VBM analyses also revealed that patients exhibited local grey matter volume reductions in the brainstem (pons), as well as left precuneus. Increases in grey matter volumes were seen in bilateral primary somatosensory cortices of FMS patients.

The final study of the thesis investigated whether FMS patients demonstrate alterations to white matter anatomy using analysis of DTI scans. FA values throughout the whole brain were compared using TBSS analysis, and probabilistic tractography analysis was performed to investigate alterations to specific tracts connecting structures involved in somatosensory processing and endogenous pain modulation. It was hypothesised that patients would show reduced white matter integrity in pain processing structures in the brain, as well as alterations to the tracts connecting structures involved in endogenous pain modulation and somatosensory processing. Neither analysis method yielded significant results. It would therefore appear that white matter microstructure and integrity in FMS patients is comparable to normal populations. This negative finding highlights the relative importance of grey matter alterations to pathophysiology and morphological alterations associated with FMS. Results could also enhance our understanding of the manner and timeframe for development of FMS, as it appears unlikely to affect white matter pathway development during formative years.

7.2. Themes

The investigation of ERD changes associated with brushing described in this thesis was the first study to publish electrophysiological data regarding abnormal cortical processes during innocuous somatosensory stimuli in FMS patients (Fallon et al., 2013). Recently, this finding was followed by a study utilising MEG and innocuous somatosensory stimulation, which demonstrated abnormal event-related field potentials overlying somatosensory processing regions of FMS patients (Maestu et al., 2013). These studies show that FMS patients demonstrate alterations to processing of innocuous somatosensory stimuli, which may provide further insight into central sensitisation in FMS. Investigations of somatosensory processing may

eventually help to elucidate the mechanisms of allodynia pain in FMS. Prior to this thesis, psychophysical studies have primarily focused on abnormal processing of painful stimuli in FMS (Gracely et al., 2002; Cook et al., 2004; Staud et al., 2008). However, the role of somatosensory processing should not be underestimated in the disorder. Allodynia pain is particularly problematic in FMS and relates to other symptoms such as sleep disturbance (Chiu et al., 2005). It was previously proposed that pain and abnormal processing of somatosensory stimuli may relate to the pathophysiological mechanisms underlying the development of the syndrome (Staud et al., 2009; Staud, 2010). The findings of this thesis suggest that, rather than merely considering FMS as a dysfunction of pain processing, it is also important to consider the dysfunctional processing of somatosensory afferents to fully understand the mechanisms involved in FMS pain. This notion is further supported by recent findings which suggest peripheral pathophysiological causes such as small fibre neuropathy may contribute to FMS pain (Serra, 2012).

Event-related potential analysis of EEG data recorded during viewing of pain photographs revealed augmented source activations in FMS patients which were localised to the left parahippocampal gyrus. The enhanced source activation also covaried with the amount of pain attributed to images and measures of clinical and psychological disturbance. Alternatively, the resting-state functional connectivity analysis revealed reduced functional connectivity between the PCC in the DMN and the right parahippocampal gyrus. The degree of this disruption may relate to ongoing symptoms of FMS, as indicated by a significant correlation with years of symptom duration. Taken together, these findings appear to indicate that functional alterations localised to the parahippocampal gyri may be important to various clinical and psychological aspects of FMS.

The parahippocampal gyrus makes up part of the hippocampal formation (Insausti and Amaral, 2004), and it is functionally important in episodic and semantic memory retrieval (Binder et al., 2009; Langston et al., 2010). Cognitive function is impaired in FMS, and patients exhibit deficits in attention and working memory (Park et al., 2001; Leavitt and Katz, 2006; Dick et al., 2008). Dysfunction of the parahippocampal gyri in FMS may relate to such cognitive deficits. Although it is not considered to be a region of the 'pain matrix' (Apkarian et al., 2005), parahippocampal activations are commonly seen during experimental pain (Bingel et al., 2002; Veldhuijzen et al., 2009; Villemure and Bushnell, 2009; Berna et al., 2010), and activations in this region may encode emotional context during experimental pain (Ploghaus et al., 2001; Stancak et al., 2012a). Therefore, the parahippocampal gyrus may be functionally relevant to both memory and the emotional aspects of pain processing in FMS. Abnormal functioning of the parahippocampal gyri could relate to psychological aspects of pain in FMS such as empathic processing during observation of pain. Alternatively, such dysfunction may relate to abnormal resting-state connectivity with the structure, which appears to deteriorate over time and could relate to cognitive disturbance in FMS.

This thesis identified morphological alterations in the grey matter and subcortical structures of FMS patients relative to a healthy control group, but no alterations in white matter microstructure. Previously, a wide-range of cortical grey matter changes in FMS were identified using VBM methods (Kuchinad et al., 2007; Schmidt-Wilcke et al., 2007; Luerding et al., 2008; Lutz et al., 2008; Burgmer et al., 2009; Valet et al., 2009; Robinson et al., 2011), with little consistency or replication of findings. Therefore, the subcortical shape and volume alterations seen in the brainstem, identified using a novel shape analysis technique, may prove to be a

particularly relevant finding in FMS. Brainstem morphological alterations were previously shown in chronic pain patients, including those with FMS (May, 2009). The brainstem contains nuclei which underlie a wide range of processes such as homeostatic regulation, postural maintenance, and sleep cycle control (Naidich and Duvernoy, 2009), as well as structures which are vital for endogenous pain modulation (Lovick, 2008; Naidich and Duvernoy, 2009). Brainstem malformations could therefore be related to the symptoms seen in FMS. Morphological alterations to the brainstem could also be a causal factor in the various cortical alterations seen in previous VBM studies. Underlying subcortical alterations could interact with comorbidities and environmental factors to bring about the variety of cortical grey matter alterations seen in previous studies. FMS symptoms also show similarities with those seen in Chiari I malformation (Holman, 2008), and the syndrome is more than 10 times more likely to occur following a neck injury than after trauma to lower extremities (Buskila et al., 1997). The current findings accord with a potential cervico-spinal aspect of FMS pathophysiology. Whilst it would be inaccurate to suggest that FMS patients simply suffer from Chiari I malformation, it is possible that brainstem malformation, or abnormal pressure on the brainstem, could affect at least a proportion of patients.

White matter findings from previous studies in FMS patients are sparse and the findings of the final study of the thesis revealed no white matter anatomical alterations in FMS patients. However, this negative finding is still important as it suggests that the pathogenesis of FMS is unlikely to relate to abnormal development of white matter pathways, which primarily occurs during infancy and childhood (Hermoye et al., 2006; Huang et al., 2006). Therefore, grey matter and subcortical

alterations are more likely to contribute to any morphological pathophysiology of FMS.

7.3. Clinical applications of the findings

The finding of abnormal ERD in FMS patients during somatosensory stimulation highlights the potential for ERD method to be utilised as a research tool to investigate clinical aspects of FMS. Longitudinal paradigms could utilise ERD method to evaluate ongoing progression of symptoms or the effectiveness of therapeutic interventions in FMS. Recently ERD method was proposed as a potential diagnostic tool for autism (Bernier et al., 2007). It is not unfeasible to consider that, given an appropriate database of ERD findings in FMS patients, such a method could be used to contribute to diagnoses of a complex syndrome such as FMS. Previously, it was proposed that different emotional and affective disorders are more prevalent in specific subgroups of FMS patients, resulting in heterogeneous patient groups which may respond better to various psychological treatments (Thieme et al., 2004). In the past, psychological constructs such as pain catastrophising scores were utilised to target specific subgroups of FMS patients with individual psychological therapies, and this resulted in improved treatment efficacy (Thieme and Gracely, 2009). Hypervigilance to pain is also evident in FMS (Crombez et al., 2004), and patients exhibit augmented aversion when observing pain in others (Bartley et al., 2009). This thesis demonstrated that cortical activations associated with the observation of pain are altered in FMS patients. This finding suggests that empathy for observed pain is a psychological construct that could be assessed to improve sensitivity to psychological heterogeneity in FMS patients in order to better target psychological therapeutic interventions.

The morphological findings in the current thesis revealed brainstem abnormalities in FMS patients relative to healthy people. This result accords with previous data which suggests a potential relationship between FMS and cervico-spinal pathology such as Chiari I malformation (Holman, 2008). At least a subgroup of FMS patients may be affected by cervico-spinal morphological alterations and this could be important for future diagnosis and treatment methods. If it was possible to identify FMS patients with potential brainstem alterations, they would likely require an individual targeted treatment plan, for example, it was previously shown that some FMS patients exhibit reductions in symptoms following surgical treatments usually utilised for Chiari I type malformation patients (Heffez et al., 2004; Heffez et al., 2007). The structural alterations seen in the brainstem may be affected by specific current therapeutic interventions. A longitudinal investigation of brainstem morphology could improve understanding of the pathophysiology of FMS, or the mechanisms of suitable interventions.

7.4. Limitations

The primary limitation of the thesis pertains to the relatively small sample size. Nineteen FMS patients (total of 37 subjects) took part in EEG studies, and 16 patients (total of 31 subjects) in MRI studies. The sample size reflects the practical difficulties associated with recruiting FMS patients who met the strict age and medication criteria in the time period required to complete the thesis. Less stringent inclusion and exclusion criteria may have allowed for a larger sample. However, increasing the heterogeneity of the sample could also adversely affect results (May, 2011). The sample sizes utilised are comparable to those employed in previous MR and EEG studies of FMS patients (Kuchinad et al., 2007; Sundgren et al., 2007;

Napadow et al., 2010; de Tommaso et al., 2011). However, it should be noted that comparisons of samples of this size may not possess the statistical power to elucidate weak, subtle effects using neuroimaging methods.

Due to the relatively small sample size, it was not possible to cluster the FMS patient group to investigate whether specific brain alterations would pertain more to a particular sub-group, who exhibit a specific symptom profile or comorbidity. This could be improved upon in future studies provided that the timeframe and budget allowed for the recruitment of a larger FMS sample. Clustering based on psychological and clinical symptoms, or frequent comorbidities, could utilise the heterogeneity in FMS as a constructive (rather than destructive) factor. Such an approach could help to elucidate the various pathophysiological mechanisms that may drive the wide range of symptoms in FMS. A final limitation that should be considered is the cross-sectional nature of each study. It would be particularly interesting to evaluate whether the observed brain alterations remain stable over time, fluctuate with symptom severity, or deteriorate in a time-dependent manner. Unfortunately, repeated scanning of the patient sample was again not realistically possible for practical reasons. Longitudinal studies would have required a larger budget for MR scanning, and would have proven more susceptible to a higher drop-out rate. It was also considered that such study designs were not viable in the timeframe of a 3-year funded research degree.

7.5. Future research

Alterations to the processing of innocuous somatosensory afferents appear to manifest in FMS. This finding should be further researched to fully elucidate the functional alterations to somatosensory processing, and to improve the

understanding of how such alterations could contribute to the pathogenesis or maintenance of FMS symptoms. The implementation of ERD technique in FMS patients is a novel step forward, and opens the door for studies utilising ERD with a variety of innocuous stimuli, or varying intensities of stimuli. The technique could also be used in a longitudinal, within-subjects design to test the efficacy of therapeutic interventions.

FMS patients demonstrated augmented aversion and arousal, and attributed more pain when observing pain pictures relative to healthy control subjects. This finding was accompanied with alterations in ERP components and source activation patterns. The results enhance the current understanding of the psychological aspects of FMS, and particularly the structures which may exhibit altered functioning in FMS patients during observation of pain cues. The role of the parahippocampal gyrus in alterations to emotional modulation and ongoing tonic pain in FMS is particularly interesting. Functional alterations in this structure appear to relate to both psychological disturbance and clinical symptoms in FMS. Future research is required to further elucidate the causes of increased aversion to observed pain stimuli in FMS. The original paradigm could be expanded to include aversive stimuli without pain relevance, in order to investigate whether pain-specific cues cause the abnormal activations seen in the findings. Alternatively, to further investigate the emotional modulation of pain in FMS, it would be useful perform to ERP analysis of EEG data recorded whilst manipulating affective stimuli and applying experimental pain stimuli to FMS patients.

With regards to the structural findings of this thesis, future functional and anatomical imaging research should prioritise brainstem alterations in FMS. For example, functional cervico-spinal MRI analysis could be performed to evaluate

potential anatomical differences in FMS patients specifically in the brainstem and spinal-cord. Future anatomical studies should also investigate whether subcortical alterations in the brainstem may correlate with relative functional abnormalities such as decreased descending nociceptive control or deteriorate in a time dependent fashion.

The current findings indicate that there were no white matter anatomical alterations in the FMS patient group relative to healthy people. This can be considered as one of the more important findings of the thesis, as the results suggest that less focus should be applied to this area in the future, at least until new techniques which may offer a different perspective or improved sensitivity are developed.

7.6. Concluding comments

To conclude, this thesis employed new methods and experimental paradigms to bring a fresh approach to brain imaging of FMS patients. Novel findings of the thesis include the presence of abnormal somatosensory processing during innocuous stimuli, alterations to processing of pain viewed in others, changes to resting-state functional networks and morphological abnormalities in FMS patients. The thesis expands upon previous neuroimaging findings in FMS patients and advocates new areas for further research such as the abnormal processing of innocuous somatosensory afferents, the functional role of specific brain structures such as parahippocampal gyri, and the pathophysiological importance of morphological alterations in the brainstem. The findings of this thesis offer new insight into structural and functional alterations in this complex syndrome. It is hoped that future

research will continue to enhance the comprehension of FMS, and eventually lead to improved therapeutic interventions and a superior quality of life for patients.

References

Abe O, Yamasue H, Kasai K, Yamada H, Aoki S, Iwanami A, Ohtani T, Masutani Y, Kato N, Ohtomo K (2006) Voxel-based diffusion tensor analysis reveals aberrant anterior cingulum integrity in posttraumatic stress disorder due to terrorism. Psychiatry research 146:231-242.

Ablin K, Clauw DJ (2009) From fibrositis to functional somatic syndromes to a bell-shaped curve of pain and sensory sensitivity: evolution of a clinical construct. Rheumatic diseases clinics of North America 35:233-251.

Akitsuki Y, Decety J (2009) Social context and perceived agency affects empathy for pain: An event-related fMRI investigation. NeuroImage 47:722-734.

Albanese MC, Duerden EG, Rainville P, Duncan GH (2007) Memory traces of pain in human cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:4612-4620.

Andersen P, Andersson SA (1968) Physiological basis of the alpha rhythm. New York: Appleton-Century-Crofts.

Apkarian AV, Bushnell MC, Treede RD, Zubieta JK (2005) Human brain mechanisms of pain perception and regulation in health and disease. Eur J Pain 9:463-484.

Arendt-Nielsen L, Yarnitsky D (2009) Experimental and clinical applications of quantitative sensory testing applied to skin, muscles and viscera. The journal of pain : official journal of the American Pain Society 10:556-572.

Arnold LM, Clauw DJ (2010) Fibromyalgia syndrome: practical strategies for improving diagnosis and patient outcomes. Am J Med 123:S2.

Arnold LM, Hudson JI, Keck PE, Auchenbach MB, Javaras KN, Hess EV (2006) Comorbidity of fibromyalgia and psychiatric disorders. The Journal of clinical psychiatry 67:1219-1225.

Arnold LM, Hudson JI, Hess EV, Ware AE, Fritz DA, Auchenbach MB, Starck LO, Keck PE, Jr. (2004) Family study of fibromyalgia. Arthritis Rheum 50:944-952.

Ashburner J, Friston KJ (2000) Voxel-based morphometry--the methods. NeuroImage 11:805-821.

Ashburner J, Friston KJ (2001) Why voxel-based morphometry should be used. NeuroImage 14:1238-1243.

Ashburner J, Friston KJ (2005) Unified segmentation. NeuroImage 26:839-851.

Atzeni F, Cazzola M, Benucci M, Di Franco M, Salaffi F, Sarzi-Puttini P (2011) Chronic widespread pain in the spectrum of rheumatological diseases. Best Pract Res Clin Rheumatol 25:165-171.

Aurlien H, Gjerde IO, Aarseth JH, Eldoen G, Karlsen B, Skeidsvoll H, Gilhus NE (2004) EEG background activity described by a large computerized database. Clin Neurophysiol 115:665-673.

Ayling E, Aghajani M, Fouche JP, van der Wee N (2012) Diffusion tensor imaging in anxiety disorders. Current psychiatry reports 14:197-202.

Babiloni F, Cincotti F, Babiloni C, Carducci F, Mattia D, Astolfi L, Basilisco A, Rossini PM, Ding L, Ni Y, Cheng J, Christine K, Sweeney J, He B (2005) Estimation of the cortical functional connectivity with the multimodal integration of high-resolution EEG and fMRI data by directed transfer function. NeuroImage 24:118-131.

Baliki MN, Geha PY, Apkarian AV, Chialvo DR (2008) Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:1398-1403.

Banic B, Petersen-Felix S, Andersen OK, Radanov BP, Villiger PM, Arendt-Nielsen L, Curatolo M (2004) Evidence for spinal cord hypersensitivity in chronic pain after whiplash injury and in fibromyalgia. Pain 107:7-15.

Barnden LR, Crouch B, Kwiatek R, Burnet R, Mernone A, Chryssidis S, Scroop G, Del Fante P (2011) A brain MRI study of chronic fatigue syndrome: evidence of brainstem dysfunction and altered homeostasis. NMR in biomedicine 24:1302-1312.

Bartley EJ, Rhudy JL, Williams AE (2009) Experimental assessment of affective processing in fibromyalgia. The journal of pain : official journal of the American Pain Society 10:1151-1160.

Basser PJ, Mattiello J, LeBihan D (1994) MR diffusion tensor spectroscopy and imaging. Biophysical journal 66:259-267.

Baumstark KE, Buckelew SP, Sher KJ, Beck N, Buescher KL, Hewett J, Crews TM (1993) Pain behavior predictors among fibromyalgia patients. Pain 55:339-346.

Beaulieu C (2002) The basis of anisotropic water diffusion in the nervous system - a technical review. NMR in biomedicine 15:435-455.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psych 4:561-571.

Behrens TE, Berg HJ, Jbabdi S, Rushworth MF, Woolrich MW (2007) Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? NeuroImage 34:144-155.

Behrens TE, Woolrich MW, Jenkinson M, Johansen-Berg H, Nunes RG, Clare S, Matthews PM, Brady JM, Smith SM (2003) Characterization and propagation of uncertainty in diffusion-weighted MR imaging. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 50:1077-1088.

Behzadi Y, Restom K, Liau J, Liu TT (2007) A component based noise correction method (CompCor) for BOLD and perfusion based fMRI. NeuroImage 37:90-101.

Belliveau JW, Kennedy DN, Jr., McKinstry RC, Buchbinder BR, Weisskoff RM, Cohen MS, Vevea JM, Brady TJ, Rosen BR (1991) Functional mapping of the human visual cortex by magnetic resonance imaging. Science 254:716-719.

Benjamini Y, Hochberg Y (1995) Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. J Roy Stat Soc B Met 57:289-300.

Bennett RM, Jones J, Turk DC, Russell IJ, Matallana L (2007) An internet survey of 2,596 people with fibromyalgia. BMC Musculoskelet Disord 8:27.

Berg P, Scherg M (1994) A multiple source approach to the correction of eye artifacts. Electroencephalogr Clin Neurophysiol 90:229-241.

Berger A, Dukes E, Martin S, Edelsberg J, Oster G (2007) Characteristics and healthcare costs of patients with fibromyalgia syndrome. International journal of clinical practice 61:1498-1508.

Berna C, Leknes S, Holmes EA, Edwards RR, Goodwin GM, Tracey I (2010) Induction of depressed mood disrupts emotion regulation neurocircuitry and enhanves pain unpleasantness. Biol Psych 67:1083-1090.

Bernatsky S, Dobkin PL, De Civita M, Penrod JR (2005) Co-morbidity and physician use in fibromyalgia. Swiss medical weekly 135:76-81.

Bernier R, Dawson G, Webb S, Murias M (2007) EEG mu rhythm and imitation impairments in individuals with autism spectrum disorder. Brain Cognition 64:228-237.

Binder JR, Desai RH, Graves WW, Conant LL (2009) Where is the semantic system? A critical review and meta-analysis of 120 functional neuroimaging studies. Cereb Cortex 19:2767-2796.

Binder JR, Frost JA, Hammeke TA, Bellgowan PS, Rao SM, Cox RW (1999) Conceptual processing during the conscious resting state. A functional MRI study. J Cogn Neurosci 11:80-95.

Bingel U, Lorenz J, Schoell E, Weiller C, Buchel C (2006) Mechanisms of placebo analgesia: rACC recruitment of a subcortical antinociceptive network. Pain 120:8-15.

Bingel U, Quante M, Knab R, Bromm B, Weiller C, Buchel C (2002) Subcortical structures involved in pain processing: evidence from single-trial fMRI. Pain:313-221.

Biswal B, Yetkin FZ, Haughton VM, Hyde JS (1995) Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 34:537-541.

Bondy B, Spaeth M, Offenbaecher M, Glatzeder K, Stratz T, Schwarz M, de Jonge S, Kruger M, Engel RR, Farber L, Pongratz DE, Ackenheil M (1999) The T102C polymorphism of the 5-HT2A-receptor gene in fibromyalgia. Neurobiology of disease 6:433-439.

Boonen A, van den Heuvel R, van Tubergen A, Goossens M, Severens JL, van der Heijde D, van der Linden S (2005) Large differences in cost of illness and wellbeing between patients with fibromyalgia, chronic low back pain, or ankylosing spondylitis. Annals of the rheumatic diseases 64:396-402.

Bradley LA (2009) Pathophysiology of fibromyalgia. Am J Med 122:S22-30.

Bradley MM, Lang PJ (1994) Measuring emotion: the Self-Assessment Manikin and the Semantic Differential. Journal of behavior therapy and experimental psychiatry 25:49-59.

Branco JC, Bannwarth B, Failde I, Abello Carbonell J, Blotman F, Spaeth M, Saraiva F, Nacci F, Thomas E, Caubere JP, Le Lay K, Taieb C, Matucci-Cerinic M (2010) Prevalence of fibromyalgia: a survey in five European countries. Seminars in arthritis and rheumatism 39:448-453.

Brookes MJ, Vrba J, Robinson SE, Stevenson CM, Peters AM, Barnes GR, Hillebrand A, Morris PG (2008) Optimising experimental design for MEG beamformer imaging. NeuroImage 39:1788-1802.

Buckalew N, Haut MW, Morrow L, Weiner D (2008) Chronic pain is associated with brain volume loss in older adults: preliminary evidence. Pain Med 9:240-248.

Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain's default network: anatomy, function, and relevance to disease. Annals of the New York Academy of Sciences 1124:1-38.

Burckhardt CS (2005) Educating patients: self-management approaches. Disability and rehabilitation 27:703-709.

Burckhardt CS, Clark SR, Bennett RM (1991) The fibromyalgia impact questionnaire: development and validation. The Journal of rheumatology 18:728-733.

Burgmer M, Gaubitz M, Konrad C, Wrenger M, Hilgart S, Heuft G, Pfleiderer B (2009) Decreased gray matter volumes in the cingulo-frontal cortex and the amygdala in patients with fibromyalgia. Psychosom Med 71:566-573.

Buskila D (2009) Developments in the scientific and clinical understanding of fibromyalgia. Arthritis research & therapy 11:242.

Buskila D, Neumann L (1997) Fibromyalgia syndrome (FM) and nonarticular tenderness in relatives of patients with FM. The Journal of rheumatology 24:941-944.

Buskila D, Neumann L, Hazanov I, Carmi R (1996) Familial aggregation in the fibromyalgia syndrome. Seminars in arthritis and rheumatism 26:605-611.

Buskila D, Neumann L, Vaisberg G, Alkalay D, Wolfe F (1997) Increased rates of fibromyalgia following cervical spine injury. A controlled study of 161 cases of traumatic injury. Arthritis Rheum 40:446-452.

Cakit BD, Taskin S, Nacir B, Unlu I, Genc H, Erdem HR (2010) Comorbidity of fibromyalgia and cervical myofascial pain syndrome. Clin Rheumatol 29:405-411.

Cauda F, Sacco K, Duca S, Cocito D, D'Agata F, Geminiani GC, Canavero S (2009) Altered resting state in diabetic neuropathic pain. PloS one 4:e4542.

Chandran A, Schaefer C, Ryan K, Baik R, McNett M, Zlateva G (2012) The comparative economic burden of mild, moderate, and severe fibromyalgia: results from a retrospective chart review and cross-sectional survey of working-age U.S. adults. Journal of managed care pharmacy : JMCP 18:415-426.

Chatrian GE, Petersen MC, Lazarte JA (1959) The blocking of the rolandic wicket rhythm and some central changes related to movement. Electroenceph Clin Neurophysiol 11:497-510.

Chen JY, Blankstein U, Diamant NE, Davis KD (2011) White matter abnormalities in irritable bowel syndrome and relation to individual factors. Brain research 1392:121-131.

Chen S, Xia W, Li L, Liu J, He Z, Zhang Z, Yan L, Zhang J, Hu D (2006) Gray matter density reduction in the insula in fire survivors with posttraumatic stress disorder: a voxel-based morphometric study. Psychiatry research 146:65-72.

Chenevert TL, Brunberg JA, Pipe JG (1990) Anisotropic diffusion in human white matter: demonstration with MR techniques in vivo. Radiology 177:401-405.

Cheng Y, Lin C-P, Liu H-L, Hsu Y-Y, Lim K-E, Hung D, Decety J (2007) Expertise Modulates the Perception of Pain in Others. Current Biology 17:1708-1713.

Cheyne D, Gaetz W, Garnero L, Lachaux JP, Ducorps A, Schwartz D, Varela FJ (2003) Neuromagnetic imaging of cortical oscillations accompanying tactile stimulation. Cogn Brain Res 17:599-611.

Chiu YH, Silman AJ, Macfarlane GJ, Ray D, Gupta A, Dickens C, Morriss R, McBeth J (2005) Poor sleep and depression are independently associated with a reduced pain threshold. Results of a population based study. Pain 115:316-321.

Cifre I, Sitges C, Fraiman D, Munoz MA, Balenzuela P, Gonzalez-Roldan A, Martinez-Jauand M, Birbaumer N, Chialvo DR, Montoya P (2012) Disrupted functional connectivity of the pain network in fibromyalgia. Psychosom Med 74:55-62.

Clauw DJ (2009) Fibromyalgia: an overview. Am J Med 122:S3-S13.

Clauw DJ, Chrousos GP (1997) Chronic pain and fatigue syndromes: overlapping clinical and neuroendocrine features and potential pathogenic mechanisms. Neuroimmunomodulation 4:134-153.

Cook DB, Lange G, Ciccone DS, Liu WC, Steffener J, Natelson BH (2004) Functional imaging of pain in patients with primary fibromyalgia. The Journal of rheumatology 31:364-378.

Coxon JP, Van Impe A, Wenderoth N, Swinnen SP (2012) Aging and inhibitory control of action: cortico-subthalamic connection strength predicts stopping performance. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:8401-8412.

Crombez G, Eccleston C, Van den Broeck A, Goubert L, Van Houdenhove B (2004) Hypervigilance to pain in fibromyalgia: the mediating role of pain intensity and catastrophic thinking about pain. Clin J Pain 20:98-102.

de Lange FP, Kalkman JS, Bleijenberg G, Hagoort P, van der Meer JW, Toni I (2005) Gray matter volume reduction in the chronic fatigue syndrome. NeuroImage 26:777-781.

de Souza JB, Potvin S, Goffaux P, Charest J, Marchand S (2009) The deficit of pain inhibition in fibromyalgia is more pronounced in patients with comorbid depressive symptoms. Clin J Pain 25:123-127.

de Tommaso M, Federici A, Santostasi R, Calabrese R, Vecchio E, Lapadula G, Iannone F, Lamberti P, Livrea P (2011) Laser-evoked potentials habituation in fibromyalgia. The journal of pain : official journal of the American Pain Society 12:116-124.

Decety J, Lamm C (2006) Human empathy through the lens of social neuroscience. TheScientificWorldJournal 6:1146-1163.

Decety J, Yang C-Y, Cheng Y (2010) Physicians down-regulate their pain empathy response: An event-related brain potential study. NeuroImage 50:1676-1682.

Delorme A, Makeig S (2004) EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods 134:9-21.

Dick BD, Verrier MJ, Harker KT, Rashiq S (2008) Disruption of cognitive function in fibromyalgia syndrome. Pain 139:610-616.

Dubner R, Ren K (1999) Endogenous mechanisms of sensory modulation. Pain Suppl 6:S45-53.

Duerden EG, Albanese MC (2011) Localization of pain-related brain activation: A meta-analysis of neuroimaging data. Hum Brain Mapp.

Duncan CC, Barry RJ, Connolly JF, Fischer C, Michie PT, Naatanen R, Polich J, Reinvang I, Van Petten C (2009) Event-related potentials in clinical research: guidelines for eliciting, recording, and quantifying mismatch negativity, P300, and N400. Clin Neurophysiol 120:1883-1908.

Eccleston C, Crombez G (1999) Pain demands attention: a cognitive-affective model of the interruptive function of pain. Psychol Bull 125:356-366.

Eippert F, Bingel U, Schoell ED, Yacubian J, Klinger R, Lorenz J, Buchel C (2009) Activation of the opioidergic descending pain control system underlies placebo analgesia. Neuron 63:533-543.

Ekstrom A (2010) How and when the fMRI BOLD signal relates to underlying neural activity: the danger in dissociation. Brain research reviews 62:233-244.

Fair DA, Schlaggar BL, Cohen AL, Miezin FM, Dosenbach NU, Wenger KK, Fox MD, Snyder AZ, Raichle ME, Petersen SE (2007) A method for using blocked and event-related fMRI data to study "resting state" functional connectivity. NeuroImage 35:396-405.

Fallon N, Chiu YH, Li X, Nurmikko TJ, Stancak A (2013) Ipsilateral cortical activation in fibromyalgia patients during brushing correlates with symptom severity. Clin Neurophysiol 124:154-163.

Fan Y, Han S (2008) Temporal dynamic of neural mechanisms involved in empathy for pain: An event-related brain potential study. Neuropsychologia 46:160-173.

Fietta P, Manganelli P (2007) Fibromyalgia and psychiatric disorders. Acta bio-medica : Atenei Parmensis 78:88-95.

Fisch BJ (1999) Fisch and Spehlmann's EEG primer : basic principles of digital and analog EEG, 3rd rev. and enlarged Edition. Amsterdam ; Oxford: Elsevier.

Foerster BR, Petrou M, Edden RA, Phd PC, Schmidt-Wilcke T, Lowe SE, Phd SE, Clauw DJ, Harris RE (2011) Reduced insular gamma-aminobutyric acid in fibromyalgia. Arthritis Rheum.

Formaggio E, Storti SF, Avesani M, Cerini R, Milanese F, Gasparini A, Acler M, Pozzi Mucelli R, Fiaschi A, Manganotti P (2008) EEG and FMRI coregistration to investigate the cortical oscillatory activities during finger movement. Brain topography 21:100-111.

Forseth KO, Gran JT (1992) The prevalence of fibromyalgia among women aged 20-49 years in Arendal, Norway. Scandinavian journal of rheumatology 21:74-78.

Forstmann BU, Anwander A, Schafer A, Neumann J, Brown S, Wagenmakers EJ, Bogacz R, Turner R (2010) Cortico-striatal connections predict control over speed and accuracy in perceptual decision making. Proc Natl Acad Sci U S A 107:15916-15920.

Foss JM, Apkarian AV, Chialvo DR (2006) Dynamics of pain: Fractal dimension of temporal variability of spontaneous pain differentiates between pain states. J Neurophysiol 95:730-736.

Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME (2005) The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A 102:9673-9678.

Fox PT, Raichle ME (1986) Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. Proc Natl Acad Sci U S A 83:1140-1144.

Freedman RR (1986) EEG power spectra in sleep-onset insomnia. Electroencephalogr Clin Neurophysiol 63:408-413.

Gaetz W, Cheyne D (2006) Localization of sensorimotor cortical rhythms induced by tactile stimulation using spatially filtered MEG. NeuroImage 30:899-908.

Gastaut MH (1952) Etude Electrocorticographique De La Reactivite Des Rythmes Rolandiques. Rev Neurol 87:176-182.

Ge HY, Nie H, Graven-Nielsen T, Danneskiold-Samsoe B, Arendt-Nielsen L (2011) Descending pain modulation and its interaction with peripheral sensitization following sustained isometric muscle contraction in fibromyalgia. Eur J Pain.

Geddes LA, Baker LE (1967) The specific resistance of biological material--a compendium of data for the biomedical engineer and physiologist. Medical & biological engineering 5:271-293.

Geha PY, Baliki MN, Harden RN, Bauer WR, Parrish TB, Apkarian AV (2008) The brain in chronic CRPS pain: abnormal gray-white matter interactions in emotional and autonomic regions. Neuron 60:570-581.

Ghazni NF, Cahill CM, Stroman PW (2010) Tactile sensory and pain networks in the human spinal cord and brain stem mapped by means of functional MR imaging. AJNR American journal of neuroradiology 31:661-667.

Gibson SJ, Littlejohn GO, Gorman MM, Helme RD, Granges G (1994) Altered heat pain thresholds and cerebral event-related potentials following painful CO2 laser stimulation in subjects with fibromyalgia syndrome. Pain 58:185-193.

Giorgio A, Watkins KE, Douaud G, James AC, James S, De Stefano N, Matthews PM, Smith SM, Johansen-Berg H (2008) Changes in white matter microstructure during adolescence. NeuroImage 39:52-61.

Goldenberg DL (1987) Fibromyalgia syndrome. An emerging but controversial condition. JAMA : the journal of the American Medical Association 257:2782-2787.

Goncalves S, de Munck JC, Heethaar RM, Lopes da Silva FH, van Dijk BW (2000) The application of electrical impedance tomography to reduce systematic errors in the EEG inverse problem--a simulation study. Physiological measurement 21:379-393.

Goodro M, Sameti M, Patenaude B, Fein G (2012) Age effect on subcortical structures in healthy adults. Psychiatry research 203:38-45.

Gracely RH, Ambrose KR (2011) Neuroimaging of fibromyalgia. Best Pract Res Clin Rheumatol 25:271-284.

Gracely RH, Lynch SA, Bennett GJ (1992) Painful neuropathy: altered central processing maintained dynamically by peripheral input. Pain 51:175-194.

Gracely RH, Petzke F, Wolf JM, Clauw DJ (2002) Functional magnetic resonance imaging evidence of augmented pain processing in fibromyalgia. Arthritis Rheum 46:1333-1343.

Gracely RH, Geisser ME, Giesecke T, Grant MA, Petzke F, Williams DA, Clauw DJ (2004) Pain catastrophizing and neural responses to pain among persons with fibromyalgia. Brain : a journal of neurology 127:835-843.

Gran JT (2003) The epidemiology of chronic generalized musculoskeletal pain. Best Pract Res Clin Rheumatol 17:547-561.

Grave de Peralta Menendez R, Gonzalez Andino SL (2002) Comparison of algorithms for localization of focal sources: Evaluation with simulated data and analysis of experimental data. Int J Biomagnet 4.

Grave de Peralta Menendez R, Gonzalez Andino S, Lantz G, Michel CM, Landis T (2001) Noninvasive localization of electromagnetic epileptic activity. I. Method descriptions and simulations. Brain topography 14:131-137.

Grave de Peralta Menendez R, Murray MM, Michel CM, Martuzzi R, Gonzalez Andino SR (2004) Electrical neuroinaging based on biophysical constraints. NeuroImage 21:527-539.

Graven-Nielsen T, Arendt-Nielsen L (2010) Assessment of mechanisms in localized and widespread musculoskeletal pain. Nature reviews Rheumatology 6:599-606.

Greicius MD, Krasnow B, Reiss AL, Menon V (2003) Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 100:253-258.

Greicius MD, Srivastava G, Reiss AL, Menon V (2004) Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A 101:4637-4642.

Greicius MD, Flores BH, Menon V, Glover GH, Solvason HB, Kenna H, Reiss AL, Schatzberg AF (2007) Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. Biol Psychiatry 62:429-437.

Gross J, Kujala J, Hämäläinen M, Timmermann L, Schnitzler A, Salmelin R (2001) Dynamic imaging of coherent sources: Studying neural interactions in the human brain. Proc Natl Acad Sci U S A 98:694-699.

Gu X, Han S (2007) Attention and reality constraints on the neural processes of empathy for pain. NeuroImage 36:256-267.

Gustin SM, Wrigley PJ, Siddall PJ, Henderson LA (2010) Brain anatomy changes associated with persistent neuropathic pain following spinal cord injury. Cereb Cortex 20:1409-1419.

Gwilym SE, Filippini N, Douaud G, Carr AJ, Tracey I (2010) Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. Arthritis Rheum 62:2930-2940.

Hadjipavlou G, Dunckley P, Behrens TE, Tracey I (2006) Determining anatomical connectivities between cortical and brainstem pain processing regions in humans: a diffusion tensor imaging study in healthy controls. Pain 123:169-178.

Hämäläinen MS, Ilmoniemi RJ (1994) Interpreting magnetic fields of the brain: minimum norm estimates. Medical & biological engineering & computing 32:35-42.

Han S, Fan Y, Mao L (2008) Gender difference in empathy for pain: An electrophysiological investigation. Brain research 1196:85-93.

Hari R, Parkkonen L, Nangini C (2010) The brain in time: insights from neuromagnetic recordings. Annals of the New York Academy of Sciences 1191:89-109.

Harkness EF, Macfarlane GJ, Nahit E, Silman AJ, McBeth J (2004) Mechanical injury and psychosocial factors in the work place predict the onset of widespread body pain: a two-year prospective study among cohorts of newly employed workers. Arthritis Rheum 50:1655-1664.

Harris RE, Sundgren PC, Craig AD, Kirshenbaum E, Sen A, Napadow V, Clauw DJ (2009) Elevated insular glutamate in fibromyalgia is associated with experimental pain. Arthritis Rheum 60:3146-3152.

Heffez DS (2011) "Is Chiari-I malformation associated with fibromyalgia?" revisited. Neurosurgery 69:E507; author reply E507-508.

Heffez DS, Ross RE, Shade-Zeldow Y, Kostas K, Morrissey M, Elias DA, Shepard A (2007) Treatment of cervical myelopathy in patients with the fibromyalgia syndrome: outcomes and implications. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 16:1423-1433.

Heffez DS, Ross RE, Shade-Zeldow Y, Kostas K, Shah S, Gottschalk R, Elias DA, Shepard A, Leurgans SE, Moore CG (2004) Clinical evidence for cervical myelopathy due to Chiari malformation and spinal stenosis in a non-randomized group of patients with the diagnosis of fibromyalgia. European spine journal : official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society 13:516-523.

Hendee WR, Morgan CJ (1984) Magnetic resonance imaging. Part I--physical principles. The Western journal of medicine 141:491-500.

Hermoye L, Saint-Martin C, Cosnard G, Lee SK, Kim J, Nassogne MC, Menten R, Clapuyt P, Donohue PK, Hua K, Wakana S, Jiang H, van Zijl PC, Mori S (2006) Pediatric diffusion tensor imaging: normal database and observation of the white matter maturation in early childhood. NeuroImage 29:493-504.

Holliday KL, McBeth J (2011) Recent advances in the understanding of genetic susceptibility to chronic pain and somatic symptoms. Current rheumatology reports 13:521-527.

Holman AJ (2008) Positional cervical spinal cord compression and fibromyalgia: a novel comorbidity with important diagnostic and treatment implications. The journal of pain : official journal of the American Pain Society 9:613-622.

Horak FB (2006) Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? Age and ageing 35 Suppl 2:ii7-ii11.

Howarth C, Hutton C, Deichmann R (2006) Improvement of the image quality of T1-weighted anatomical brain scans. NeuroImage 29:930-937.

Hsu MC, Harris RE, Sundgren PC, Welsh RC, Fernandes CR, Clauw DJ, Williams DA (2009) No consistent difference in gray matter volume between individuals with fibromyalgia and age-matched healthy subjects when controlling for affective disorder. Pain 143:262-267.

Hua K, Zhang J, Wakana S, Jiang H, Li X, Reich DS, Calabresi PA, Pekar JJ, van Zijl PC, Mori S (2008) Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. NeuroImage 39:336-347.

Huang H, Zhang J, Wakana S, Zhang W, Ren T, Richards LJ, Yarowsky P, Donohue P, Graham E, van Zijl PC, Mori S (2006) White and gray matter development in human fetal, newborn and pediatric brains. NeuroImage 33:27-38.

Hudson JI, Pope HG, Jr. (1996) The relationship between fibromyalgia and major depressive disorder. Rheumatic diseases clinics of North America 22:285-303.

Hudson JI, Hudson MS, Pliner LF, Goldenberg DL, Pope HG, Jr. (1985) Fibromyalgia and major affective disorder: a controlled phenomenology and family history study. Am J Psychiatry 142:441-446.

Hughes G, Martinez C, Myon E, Taieb C, Wessely S (2006) The impact of a diagnosis of fibromyalgia on health care resource use by primary care patients in the UK: an observational study based on clinical practice. Arthritis Rheum 54:177-183.

Iadarola MJ, Coghill RC (1999) Imaging of pain: recent developments. Current opinion in anaesthesiology 12:583-589.

Ibáñez A, Hurtado E, Lobos A, Escobar J, Trujillo N, Baez S, Huepe D, Manes F, Decety J (2011) Subliminal presentation of other faces (but not own face) primes behavioral and evoked cortical processing of empathy for pain. Brain research 1398:72-85.

Ichesco E, Quintero A, Clauw DJ, Peltier S, Sundgren PM, Gerstner GE, Schmidt-Wilcke T (2012) Altered functional connectivity between the insula and the cingulate cortex in patients with temporomandibular disorder: a pilot study. Headache 52:441-454.

Insausti R, Amaral DG (2004) Hippocampal formation. In: The Human Nervous System., 2nd Edition (Paxinos G, Mai KJ, eds), pp 871-914. Amsterdam: Ensevier, Inc.

Iwasawa T, Matoba H, Ogi A, Kurihara H, Saito K, Yoshida T, Matsubara S, Nozaki A (1997) Diffusion-weighted imaging of the human optic nerve: a new approach to evaluate optic neuritis in multiple sclerosis. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 38:484-491.

Jackson PL, Meltzoff AN, Decety J (2005) How do we perceive the pain of others? A window into the neural processes involved in empathy. NeuroImage 24:771-779.

Jackson PL, Brunet E, Meltzoff AN, Decety J (2006) Empathy examined through the neural mechanisms involved in imagining how I feel versus how you feel pain. Neuropsychologia 44:752-761.

Jacobs JV, Henry SM, Nagle KJ (2010) Low back pain associates with altered activity of the cerebral cortex prior to arm movements that require postural adjustment. Clin Neurophysiol 121:431-440.

Jasper H (1958) Report of committee on methods of clinical exam in EEG. Electroencephalogr Clin Neurophysiol 10:370-375.

Jasper HH, Andrews HL (1938) Electro-encephalography. III. Normal differentiation of occipital and precentral regions in man. Arch Neuro Psychiatr 39:96-115.

Jbabdi S, Sotiropoulos SN, Savio AM, Grana M, Behrens TE (2012) Model-based analysis of multishell diffusion MR data for tractography: How to get over fitting problems. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 68:1846-1855.

Jenkinson M, Smith S (2001) A global optimisation method for robust affine registration of brain images. Medical image analysis 5:143-156.

Jenkinson M, Beckmann CF, Behrens TE, Woolrich MW, Smith SM (2012) Fsl. NeuroImage 62:782-790.

Jensen KB, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Giesecke T, Mainguy Y, Gracely R, Ingvar M (2009) Evidence of dysfunctional pain inhibition in Fibromyalgia reflected in rACC during provoked pain. Pain 144:95-100.

Jensen KB, Loitoile R, Kosek E, Petzke F, Carville S, Fransson P, Marcus H, Williams SC, Choy E, Mainguy Y, Vitton O, Gracely RH, Gollub R, Ingvar M, Kong J (2012) Patients With Fibromyalgia Display Less Functional Connectivity In The Brain's Pain Inhibitory Network. Molecular pain 8:32.

Jonas BS, Brody D, Roper M, Narrow WE (2003) Prevalence of mood disorders in a national sample of young American adults. Social psychiatry and psychiatric epidemiology 38:618-624.

Jones A, Watson A (2007) Functional imaging of pain: a cortical model of allodynia in postroke pain and fibromyalgia. In: Central neuropathic pain : focus on poststroke pain (Henry JL, ed), pp 183-210. Seattle: IASP Press.

Jones AK, Huneke NT, Lloyd DM, Brown CA, Watson A (2012) Role of functional brain imaging in understanding rheumatic pain. Current rheumatology reports 14:557-567.

Jones DK, Symms MR, Cercignani M, Howard RJ (2005) The effect of filter size on VBM analyses of DT-MRI data. NeuroImage 26:546-554.

Jones KD, King LA, Mist SD, Bennett RM, Horak FB (2011) Postural control deficits in people with fibromyalgia: a pilot study. Arthritis research & therapy 13:R127.

Julien N, Goffaux P, Arsenault P, Marchand S (2005) Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. Pain 114:295-302.

Jung TP, Makeig S, Humphries C, Lee TW, McKeown MJ, Iragui V, Sejnowski TJ (2000) Removing electroencephalographic artifacts by blind source separation. Psychophysiology 37:163-178.

Juottonen K, Gockel M, Silen T, Hurri H, Hari R, Forss N (2002) Altered central sensorimotor processing in patients with complex regional pain syndrome. Pain 98:315-323.

Kaas JH, Florence SL, Jain N (1999) Subcortical contributions to massive cortical reorganizations. Neuron 22:657-660.

Kamp A, Pfurtscheller G, Edlinger G, Lopes da Silva F (2005) Technological basis of EEG recordings. In: Electroencephalography: basic principles, clinical applications and related fields, 5th Edition (Niedermeyer E, ed), pp 127-138. Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Kenna H, Hoeft F, Kelley R, Wroolie T, Demuth B, Reiss A, Rasgon N (2012) Fasting plasma insulin and the default mode network in women at risk for Alzheimer's disease. Neurobiology of aging.

Kerns RD, Turk DC, Rudy TE (1985) The West Haven-Yale Multidimensional Pain Inventory (WHYMPI). Pain 23:345-356.

Kersh BC, Bradley LA, Alarcon GS, Alberts KR, Sotolongo A, Martin MY, Aaron LA, Dewaal DF, Domino ML, Chaplin WF, Palardy NR, Cianfrini LR, Triana-Alexander M (2001) Psychosocial and health status variables independently predict health care seeking in fibromyalgia. Arthritis Rheum 45:362-371.

Kim SG, Ogawa S (2012) Biophysical and physiological origins of blood oxygenation level-dependent fMRI signals. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 32:1188-1206.

King CD, Wong F, Currie T, Mauderli AP, Fillingim RB, Riley JL, 3rd (2009) Deficiency in endogenous modulation of prolonged heat pain in patients with Irritable Bowel Syndrome and Temporomandibular Disorder. Pain 143:172-178.

Klein SB, Thorne BM (2007) Biological psychology. New York, N.Y.: Worth.

Klem GH, Luders HO, Jasper HH, Elger C (1999) The ten-twenty electrode system of the International Federation. The International Federation of Clinical Neurophysiology. Electroencephalography and clinical neurophysiology Supplement 52:3-6.

Koenig T, Melie-Garcia L (2010) A method to determine the presence of averaged event-related fields using randomization tests. Brain topography 23:233-242.

Kong J, Gollub RL, Rosman IS, Webb JM, Vangel MG, Kirsch I, Kaptchuk TJ (2006) Brain activity associated with expectancy-enhanced placebo analgesia as measured by functional magnetic resonance imaging. The Journal of neuroscience : the official journal of the Society for Neuroscience 26:381-388.

Kosek E, Hansson P (1997) Modulatory influence on somatosensory perception from vibration and heterotopic noxious conditioning stimulation (HNCS) in fibromyalgia patients and healthy subjects. Pain 70:41-51.

Kosek E, Ordeberg G (2000) Lack of pressure pain modulation by heterotopic noxious conditioning stimulation in patients with painful osteoarthritis before, but not following, surgical pain relief. Pain 88:69-78.

Kosek E, Ekholm J, Hansson P (1996) Sensory dysfunction in fibromyalgia patients with implications for pathogenic mechanisms. Pain 68:375-383.

Krienen FM, Buckner RL (2009) Segregated fronto-cerebellar circuits revealed by intrinsic functional connectivity. Cereb Cortex 19:2485-2497.

Kuchinad A, Schweinhardt P, Seminowicz DA, Wood PB, Chizh BA, Bushnell MC (2007) Accelerated brain gray matter loss in fibromyalgia patients: premature aging of the brain? The Journal of neuroscience : the official journal of the Society for Neuroscience 27:4004-4007.

Kurtze N, Svebak S (2001) Fatigue and patterns of pain in fibromyalgia: correlations with anxiety, depression and co-morbidity in a female county sample. Br J Med Psychol 74:523-537.

Kurtze N, Gundersen KT, Svebak S (1998) The role of anxiety and depression in fatigue and patterns of pain among subgroups of fibromyalgia patients. Br J Med Psychol 71 (Pt 2):185-194.

Labyt E, Devos D, Bourriez JL, Cassim F, Destee A, Guieu JD, Defebvre L, Derambure P (2003) Motor preparation is more impaired in Parkinson's disease when sensorimotor integration is involved. Clin Neurophysiol 114:2423-2433.

Lachaine J, Beauchemin C, Landry PA (2010) Clinical and economic characteristics of patients with fibromyalgia syndrome. Clin J Pain 26:284-290.

Laird AR, Eickhoff SB, Li K, Robin DA, Glahn DC, Fox PT (2009) Investigating the functional heterogeneity of the default mode network using coordinate-based meta-analytic modeling. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:14496-14505.

Lamm C, Batson CD, Decety J (2007) The Neural Substrate of Human Empathy: Effects of Perspective-taking and Cognitive Appraisal. Journal of Cognitive Neuroscience 19:42-58.

Lamm C, Decety J, Singer T (2011) Meta-analytic evidence for common and distinct neural networks associated with directly experienced pain and empathy for pain. NeuroImage 54:2492-2502.

Lang S, Yu T, Markl A, Muller F, Kotchoubey B (2011) Hearing others' pain: neural activity related to empathy. Cognitive, affective & behavioral neuroscience 11:386-395.

Langston RF, Stevenson CH, Wilson CL, Saunders I, Wood ER (2010) The role of hippocampal subregions in memory for stimulus associations. Behavioural brain research 215:275-291.

Lautenbacher S, Rollman GB (1997) Possible deficiencies of pain modulation in fibromyalgia. Clin J Pain 13:189-196.

Lawrence RC, Helmick CG, Arnett FC, Deyo RA, Felson DT, Giannini EH, Heyse SP, Hirsch R, Hochberg MC, Hunder GG, Liang MH, Pillemer SR, Steen VD, Wolfe F (1998) Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum 41:778-799.

Lawrence RC, Felson DT, Helmick CG, Arnold LM, Choi H, Deyo RA, Gabriel S, Hirsch R, Hochberg MC, Hunder GG, Jordan JM, Katz JN, Kremers HM, Wolfe F, National Arthritis Data W (2008) Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum 58:26-35.

Leavitt F, Katz RS (2006) Distraction as a key determinant of impaired memory in patients with fibromyalgia. The Journal of rheumatology 33:127-132.

Lee MC, Zambreanu L, Menon DK, Tracey I (2008) Identifying brain activity specifically related to the maintenance and perceptual consequence of central sensitization in humans. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:11642-11649.

Lee YC, Nassikas NJ, Clauw DJ (2011) The role of the central nervous system in the generation and maintenance of chronic pain in rheumatoid arthritis, osteoarthritis and fibromyalgia. Arthritis research & therapy 13:211.

Lehmann D (1987) Principles of spatial analysis. In: Handbook of Electroencephalography and Clinical Neurophysiology: Methods of Analysis of Brain Electrical and Magnetic Signals (Gevins AS, Remond A, eds), pp 309-354. Amsterdam: Elsevier.

Li W, Han S (2010) Perspective taking modulates event-related potentials to perceived pain. Neuroscience letters 469:328-332.

Loggia ML, Kim J, Gollub RL, Vangel MG, Kirsch I, Kong J, Wasan AD, Napadow V (2012) Default mode network connectivity encodes clinical pain: An arterial spin labeling study. Pain.

Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A (2001) Neurophysiological investigation of the basis of the fMRI signal. Nature 412:150-157.

Lopes da Silva F (2005) Event-Related Potentials: Methodology and Quantification. In: Electroencephalography: basic principles, clinical applications and related fields, 5th Edition (Niedermeyer E, ed), pp 991-1002. Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Lopes da Silva F, Van Rotterdam A (2005) Biophysical aspects of EEG and MEG generation. In: Electroencephalography: basic principles, clinical applications and related fields, 5th Edition (Niedermeyer E, ed), pp 17-29. Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Lopes da Silva FH (2006) Event-related neural activities: what about phase? Progress in brain research 159:3-17.

Lorenz J, Grasedyck K, Bromm B (1996) Middle and long latency somatosensory evoked potentials after painful laser stimulation in patients with fibromyalgia syndrome. Electroenceph Clin Neurophysiol 100:165-168.

Lovick TA (2008) Pro-nociceptive action of cholecystokinin in the periaqueductal grey: A role in neuropathic and anxiety-induced hyperalgesic states. Neurosci Biobehav R 32:852-862.

Luck SJ (2005) An introduction to the event-related potential technique. Cambridge, Mass. ; London: MIT Press.

Luck SJ, Woodman GF, Vogel EK (2000) Event-related potential studies of attention. Trends Cogn Sci 4:432-440.

Luerding R, Weigand T, Bogdahn U, Schmidt-Wilcke T (2008) Working memory performance is correlated with local brain morphology in the medial frontal and anterior cingulate cortex in fibromyalgia patients: structural correlates of pain-cognition interaction. Brain : a journal of neurology 131:3222-3231.

Lutz J, Jager L, de Quervain D, Krauseneck T, Padberg F, Wichnalek M, Beyer A, Stahl R, Zirngibl B, Morhard D, Reiser M, Schelling G (2008) White and gray matter abnormalities in the brain of patients with fibromyalgia: a diffusion-tensor and volumetric imaging study. Arthritis Rheum 58:3960-3969.

Maestu C, Cortes A, Vazquez JM, del Rio D, Gomez-Arguelles JM, del Pozo F, Nevado A (2013) Increased brain responses during subjectively-matched mechanical pain stimulation in fibromyalgia patients as evidenced by MEG. Clin Neurophysiol 124:752-760.

Maihöfner C, Handwerker HO (2005) Differential coding of hyperalgesia in the human brain: a functional MRI study. NeuroImage 28:996-1006.

Maihöfner C, Handwerker HO, Birklein F (2006) Functional imaging of allodynia in complex regional pain syndrome. Neurology 66:711-717.

Mailhöfner C, Handwerker HO, Birklein F (2006) Functional imaging of allodynia in complex regional pain syndrome. Neurology 66:711-717.

Mäkelä M, Heliövaara M (1991) Prevalence of primary fibromyalgia in the Finnish population. Bmj 303:216-219.

Makris N, Worth AJ, Sorensen AG, Papadimitriou GM, Wu O, Reese TG, Wedeen VJ, Davis TL, Stakes JW, Caviness VS, Kaplan E, Rosen BR, Pandya DN, Kennedy DN (1997) Morphometry of in vivo human white matter association pathways with diffusion-weighted magnetic resonance imaging. Annals of neurology 42:951-962.

Malinen S, Vartiainen N, Hlushchuk Y, Koskinen M, Ramkumar P, Forss N, Kalso E, Hari R (2010) Aberrant temporal and spatial brain activity during rest in patients with chronic pain. Proc Natl Acad Sci U S A 107:6493-6497.

Mandeville JB, Rosen BR (2002) Functional MRI. In: Brain mapping, 2nd Edition (Toga AW, Mazziotta JC, eds), pp 315-349. Amsterdam ; London: Academic.

Mantini D, Perrucci MG, Del Gratta C, Romani GL, Corbetta M (2007) Electrophysiological signatures of resting state networks in the human brain. Proc Natl Acad Sci U S A 104:13170-13175.

Maris E, Oostenveld R (2007) Nonparametric statistical testing of EEG- and MEG-data. J Neurosci Methods 164:177-190.

Martin MY, Bradley LA, Alexander RW, Alarcon GS, Triana-Alexander M, Aaron LA, Alberts KR (1996) Coping strategies predict disability in patients with primary fibromyalgia. Pain 68:45-53.

Martinez MP, Sanchez AI, Miro E, Medina A, Lami MJ (2011) The relationship between the fear-avoidance model of pain and personality traits in fibromyalgia patients. Journal of clinical psychology in medical settings 18:380-391.

Mason P (2005) Ventromedial medulla: pain modulation and beyond. The Journal of comparative neurology 493:2-8.

May A (2009) [Chronic pain alters the structure of the brain]. Schmerz 23:569-575.

May A (2011) Structural brain imaging: a window into chronic pain. Neuroscientist 17:209-220.

McBeth J, Mulvey MR (2012) Fibromyalgia: mechanisms and potential impact of the ACR 2010 classification criteria. Nature reviews Rheumatology 8:108-116.

Mease PJ, Clauw DJ, Arnold LM, Goldenberg DL, Witter J, Williams DA, Simon LS, Strand CV, Bramson C, Martin S, Wright TM, Littman B, Wernicke JF, Gendreau RM, Crofford LJ (2005) Fibromyalgia syndrome. The Journal of rheumatology 32:2270-2277.

Menendez RGD, Andino SG, Lantz G, Michel CM, Landis T (2001) Noninvasive localization of electromagnetic epileptic activity. I. Method descriptions and simulations. Brain topography 14:131-137.

Metting van Rijn AC, Peper A, Grimbergen CA (1990) High-quality recording of bioelectric events. Part 1. Interference reduction, theory and practice. Medical & biological engineering & computing 28:389-397.

Meyer-Lindenberg A, Gallhofer B (1998) Somatized depression as a subgroup of fibromyalgia syndrome. Z Rheumatol 57 Suppl 2:92-93.

Miller R (2007) Theory of the normal waking EEG: from single neurones to waveforms in the alpha, beta and gamma frequency ranges. International journal of psychophysiology : official journal of the International Organization of Psychophysiology 64:18-23.

Moayedi M, Weissman-Fogel I, Salomons TV, Crawley AP, Goldberg MB, Freeman BV, Tenenbaum HC, Davis KD (2012) White matter brain and trigeminal nerve abnormalities in temporomandibular disorder. Pain 153:1467-1477.

Moisset X, Bouhassira D (2007) Brain imaging of neuropathic pain. NeuroImage 37 Suppl 1:S80-88.

Montoya P, Sitges C, Garcia-Herrera M, Izquierdo R, Truyols M, Blay N, Collado D (2005) Abnormal affective modulation of somatosensory brain processing among patients with fibromyalgia. Psychosom Med 67:957-963.

Mori S, Zhang JY (2006) Principles of diffusion tensor imaging and its applications to basic neuroscience research. Neuron 51:527-539.

Mork PJ, Vasseljen O, Nilsen TI (2010) Association between physical exercise, body mass index, and risk of fibromyalgia: longitudinal data from the Norwegian Nord-Trondelag Health Study. Arthritis Care Res 62:611-617.

Moseley ME, Cohen Y, Mintorovitch J, Chileuitt L, Shimizu H, Kucharczyk J, Wendland MF, Weinstein PR (1990a) Early detection of regional cerebral ischemia in cats: comparison of diffusion- and T2-weighted MRI and spectroscopy. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 14:330-346.

Moseley ME, Cohen Y, Kucharczyk J, Mintorovitch J, Asgari HS, Wendland MF, Tsuruda J, Norman D (1990b) Diffusion-weighted MR imaging of anisotropic water diffusion in cat central nervous system. Radiology 176:439-445.

Murias M, Webb SJ, Greenson J, Dawson G (2007) Resting state cortical connectivity reflected in EEG coherence in individuals with autism. Biol Psychiatry 62:270-273.

Naidich TP, Duvernoy HM (2009) Duvernoy's Atlas of the human brain stem and cerebellum [electronic resource]. Wien: Springer.

Nakamura A, Yamada T, Goto A, Kato T, Ito K, Abe Y, Kachi T, Kakigi R (1998) Somatosensory homunculus as drawn by MEG. NeuroImage 7:377-386.

Napadow V, Kim J, Clauw DJ, Harris RE (2012) Decreased intrinsic brain connectivity is associated with reduced clinical pain in fibromyalgia. Arthritis Rheum.

Napadow V, LaCount L, Park K, As-Sanie S, Clauw DJ, Harris RE (2010) Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. Arthritis Rheum 62:2545-2555.

Narashiman PT, Jacobs RE (2002) Neuroanatomical micromagnetic resonance imaging. In: Brain mapping, 2nd Edition (Toga AW, Mazziotta JC, eds), pp 399-426. Amsterdam ; London: Academic.

Netter P, Hennig J (1998) The fibromyalgia syndrome as a manifestation of neuroticism? Z Rheumatol 57 Suppl 2:105-108.

Neuper C, Pfurtscheller G (2001) Evidence for distinct beta resonance frequencies in human EEG related to specific sensorimotor cortical areas. Clin Neurophysiol 112:2084-2097.

Nicassio PM, Schoenfeld-Smith K, Radojevic V, Schuman C (1995) Pain coping mechanisms in fibromyalgia: relationship to pain and functional outcomes. The Journal of rheumatology 22:1552-1558.

Niedermeyer E (2005) The normal EEG of the waking adult. In: Electroencephalography: basic principles, clinical applications and related fields, 5th Edition (Niedermeyer E, ed), pp 167-192. Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Nunez PL, Silberstein RB (2000) On the relationship of synaptic activity to macroscopic measurements: does co-registration of EEG with fMRI make sense? Brain topography 13:79-96.

Nunez PL, Srinivasan R, Westdorp AF, Wijesinghe RS, Tucker DM, Silberstein RB, Cadusch PJ (1997) EEG coherency. I: Statistics, reference electrode, volume conduction, Laplacians, cortical imaging, and interpretation at multiple scales. Electroencephalogr Clin Neurophysiol 103:499-515.

Offenbaecher M, Bondy B, de Jonge S, Glatzeder K, Kruger M, Schoeps P, Ackenheil M (1999) Possible association of fibromyalgia with a polymorphism in the serotonin transporter gene regulatory region. Arthritis Rheum 42:2482-2488.

Ogawa S, Lee TM, Kay AR, Tank DW (1990a) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci U S A 87:9868-9872.

Ogawa S, Lee TM, Nayak AS, Glynn P (1990b) Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 14:68-78.

Okifuji A, Turk DC, Sinclair JD, Starz TW, Marcus DA (1997) A standardized manual tender point survey. I. Development and determination of a threshold point for the identification of positive tender points in fibromyalgia syndrome. The Journal of rheumatology 24:377-383.

Olausson H, Marchand S, Bittar RG, Bernier J, Ptito A, Bushnell MC (2001) Central pain in a hemispherectomized patient. Eur J Pain 5:209-217.

Oostenveld R, Fries P, Maris E, Schoffelen JM (2011) FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci 2011:156869.

Pae CU, Masand PS, Marks DM, Krulewicz S, Peindl K, Mannelli P, Patkar AA (2009) History of depressive and/or anxiety disorders as a predictor of treatment response: a post hoc analysis of a 12-week, randomized, double-blind, placebo-controlled trial of paroxetine controlled release in patients with fibromyalgia. Progress in neuro-psychopharmacology & biological psychiatry 33:996-1002.

Pajevic S, Pierpaoli C (1999) Color schemes to represent the orientation of anisotropic tissues from diffusion tensor data: application to white matter fiber tract mapping in the human brain. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 42:526-540.

Palomino RA, Nicassio PM, Greenberg MA, Medina EP, Jr. (2007) Helplessness and loss as mediators between pain and depressive symptoms in fibromyalgia. Pain 129:185-194.

Park DC, Glass JM, Minear M, Crofford LJ (2001) Cognitive function in fibromyalgia patients. Arthritis Rheum 44:2125-2133.

Patenaude B, Smith SM, Kennedy DN, Jenkinson M (2011) A Bayesian model of shape and appearance for subcortical brain segmentation. NeuroImage 56:907-922.

Peltz E, Seifert F, DeCol R, Dorfler A, Schwab S, Maihofner C (2011) Functional connectivity of the human insular cortex during noxious and innocuous thermal stimulation. NeuroImage 54:1324-1335.

Penrod JR, Bernatsky S, Adam V, Baron M, Dayan N, Dobkin PL (2004) Health services costs and their determinants in women with fibromyalgia. The Journal of rheumatology 31:1391-1398.

Perrot S, Dickenson AH, Bennett RM (2008) Fibromyalgia: harmonizing science with clinical practice considerations. Pain practice : the official journal of World Institute of Pain 8:177-189.

Peterka RJ, Loughlin PJ (2004) Dynamic regulation of sensorimotor integration in human postural control. J Neurophysiol 91:410-423.

Petersel DL, Dror V, Cheung R (2011) Central amplification and fibromyalgia: disorder of pain processing. Journal of neuroscience research 89:29-34.

Petrovic P, Ingvar M, Stone-Elander S, Petersson KM, Hansson P (1999) A PET activation study of dynamic mechanical allodynia in patients with mononeuropathy. Pain 83:459-470.

Petzke F, Clauw DJ, Ambrose K, Khine A, Gracely RH (2003) Increased pain sensitivity in fibromyalgia: effects of stimulus type and mode of presentation. Pain 105:403-413.

Peyron R, Schneider F, Faillenot I, Convers P, Barral FG, Garcia-Larrea L, Laurent B (2004) An fMRI study of cortical representation of mechanical allodynia in patients with neuropathic pain. Neurology 63:1838-1846.

Peyron R, Garcia-Larrea L, Gregoire MC, Costes N, Convers P, Lavenne F, Mauguiere F, Michel D, Laurent B (1999) Haemodynamic brain responses to acute pain in humans: sensory and attentional networks. Brain : a journal of neurology 122 (Pt 9):1765-1780.

Pfefferbaum A, Sullivan EV, Hedehus M, Lim KO, Adalsteinsson E, Moseley M (2000) Age-related decline in brain white matter anisotropy measured with spatially corrected echo-planar diffusion tensor imaging. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 44:259-268.

Pfurtscheller G (1981) Central beta rhythm during sensorimotor activities in man. Electroenceph Clin Neurophysiol 51:253-264.

Pfurtscheller G (1992) Event-related synchronization (ERS): an electrophysiological correlate of cortical areas at rest. Electroencephalogr Clin Neurophysiol 83:62-69.

Pfurtscheller G, Aranibar A (1977) Event-related cortical desynchronization detected by power measurements of scalp EEG. Electroencephalogr Clin Neurophysiol 42:817-826.

Pfurtscheller G, Lopes da Silva FH (1999) Event-related EEG/MEG synchronization and desynchronization: basic principles. Clin Neurophysiol 110:1842-1857.

Pfurtscheller G, Neuper C, Kalcher J (1993) 40-Hz oscillations during motor behavior in man. Neuroscience letters 164:179-182.

Phillips K, Clauw DJ (2011) Central pain mechanisms in chronic pain states--maybe it is all in their head. Best Pract Res Clin Rheumatol 25:141-154.

Pierpaoli C, Basser PJ (1996) Toward a quantitative assessment of diffusion anisotropy. Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine 36:893-906.

Platz T, Kim IH, Engel U, Kieselbach A, Mauritz KH (2002) Brain activation pattern as assessed with multi-modal EEG analysis predict motor recovery among stroke patients with mild arm paresis who receive the Arm Ability Training. Restorative neurology and neuroscience 20:21-35.

Ploghaus A, Narain C, Beckmann CF, Clare S, Bantick S, Wise R, Matthews PM, Rawlins JN, Tracey I (2001) Exacerbation of pain by anxiety is associated with activity in a hippocampal network. J Neurosci 21:9896-9903.

Ploner M, Gross J, Timmermann L, Pollok B, Schnitzler A (2006) Pain suppresses spontaneous brain rhythms. Cereb Cortex 16:537-540.

Prescott E, Jacobsen S, Kjoller M, Bulow PM, Danneskiold-Samsoe B, Kamper-Jorgensen F (1993) Fibromyalgia in the adult Danish population: II. A study of clinical features. Scandinavian journal of rheumatology 22:238-242.

Preston SD, de Waal FB (2002) Empathy: Its ultimate and proximate bases. The Behavioral and brain sciences 25:1-20; discussion 20-71.

Price DD, Bennett GJ, Rafii A (1989) Psychophysical observations on patients with neuropathic pain relieved by a sympathetic block. Pain 36:273-288.

Price DD, Long S, Wilsey B, Rafii A (1998) Analysis of peak magnitude and duration of analgesia produced by local anesthetics injected into sympathetic ganglia of complex regional pain syndrome patients. Clin J Pain 14:216-226.

Proverbio AM, Adorni R, Zani A, Trestianu L (2009) Sex differences in the brain response to affective scenes with or without humans. Neuropsychologia 47:2374-2388.

Raichle ME, Snyder AZ (2007) A default mode of brain function: a brief history of an evolving idea. NeuroImage 37:1083-1090; discussion 1097-1089.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL (2001) A default mode of brain function. Proc Natl Acad Sci U S A 98:676-682.

Raphael KG, Janal MN, Nayak S, Schwartz JE, Gallagher RM (2006) Psychiatric comorbidities in a community sample of women with fibromyalgia. Pain 124:117-125.

Ren K, Dubner R (2002) Descending modulation in persistent pain: an update. Pain 100:1-6.

Robinson ME, Craggs JG, Price DD, Perlstein WM, Staud R (2011) Gray matter volumes of pain-related brain areas are decreased in fibromyalgia syndrome. The journal of pain : official journal of the American Pain Society 12:436-443.

Robinson RL, Birnbaum HG, Morley MA, Sisitsky T, Greenberg PE, Claxton AJ (2003) Economic cost and epidemiological characteristics of patients with fibromyalgia claims. The Journal of rheumatology 30:1318-1325.

Ruiz-Perez I, Plazaola-Castano J, Caliz-Caliz R, Rodriguez-Calvo I, Garcia-Sanchez A, Ferrer-Gonzalez MA, Guzman-Ubeda M, del Rio-Lozano M, Lopez-Chicheri Garcia I (2009) Risk factors for fibromyalgia: the role of violence against women. Clin Rheumatol 28:777-786.

Salenius S, Schnitzler A, Salmelin R, Jousmäki V, Hari R (1997) Modulation of human cortical rolandic rhythms during natural sensorimotor tasks. NeuroImage 5:221-228.

Salmelin R, Hari R (1994) Spatiotemporal characteristics of sensorimotor neuromagnetic rhythms related to thumb movement. Neuroscience 60:537-550.

Sameti M, Smith S, Patenaude B, Fein G (2011) Subcortical volumes in long-term abstinent alcoholics: associations with psychiatric comorbidity. Alcoholism, clinical and experimental research 35:1067-1080.

Sandrini G, Rossi P, Milanov I, Serrao M, Cecchini AP, Nappi G (2006) Abnormal modulatory influence of diffuse noxious inhibitory controls in migraine and chronic tension-type headache patients. Cephalalgia : an international journal of headache 26:782-789.

Sarzi-Puttini P, Atzeni F, Mease PJ (2011) Chronic widespread pain: from peripheral to central evolution. Best Pract Res Clin Rheumatol 25:133-139.

Schaefer C, Chandran A, Hufstader M, Baik R, McNett M, Goldenberg D, Gerwin R, Zlateva G (2011) The comparative burden of mild, moderate and severe fibromyalgia: results from a cross-sectional survey in the United States. Health and quality of life outcomes 9:71.

Schmahmann JD, Ko R, MacMore J (2004) The human basis pontis: motor syndromes and topographic organization. Brain : a journal of neurology 127:1269-1291.

Schmidt-Wilcke T, Clauw DJ (2011) Fibromyalgia: from pathophysiology to therapy. Nature reviews Rheumatology 7:518-527.

Schmidt-Wilcke T, Leinisch E, Ganssbauer S, Draganski B, Bogdahn U, Altmeppen J, May A (2006) Affective components and intensity of pain correlate with structural differences in gray matter in chronic back pain patients. Pain 125:89-97.

Schmidt-Wilcke T, Luerding R, Weigand T, Jurgens T, Schuierer G, Leinisch E, Bogdahn U (2007) Striatal grey matter increase in patients suffering from fibromyalgia--a voxel-based morphometry study. Pain 132 Suppl 1:S109-116.

Schmidt-Wilcke T, Leinisch E, Straube A, Kampfe N, Draganski B, Diener HC, Bogdahn U, May A (2005) Gray matter decrease in patients with chronic tension type headache. Neurology 65:1483-1486.

Schneider S, Strüder HK (2012) EEG: Theoretical Background and Practical Aspects. In: Functional neuroimaging in exercise and sport sciences [electronic resource], pp 197-212. New York, NY: Springer.

Schweinhardt P, Sauro KM, Bushnell MC (2008) Fibromyalgia: a disorder of the brain? Neuroscientist 14:415-421.

Schwindt GC, Chaudhary S, Crane D, Ganda A, Masellis M, Grady CL, Stefanovic B, Black SE (2012) Modulation of the Default-Mode Network Between Rest and Task in Alzheimer's Disease. Cereb Cortex.

Seifert F, Maihöfner C (2011) Functional and structural imaging of pain-induced neuroplasticity. Current opinion in anaesthesiology 24:515-523.

Seifert F, Jungfer I, Schmelz M, Maihofner C (2008) Representation of UV-B-induced thermal and mechanical hyperalgesia in the human brain: a functional MRI study. Hum Brain Mapp 29:1327-1342.

Seminowicz DA, Labus JS, Bueller JA, Tillisch K, Naliboff BD, Bushnell MC, Mayer EA (2010) Regional gray matter density changes in brains of patients with irritable bowel syndrome. Gastroenterology 139:48-57 e42.

Sephton SE, Studts JL, Hoover K, Weissbecker I, Lynch G, Ho I, McGuffin S, Salmon P (2003) Biological and psychological factors associated with memory function in fibromyalgia syndrome. Health psychology : official journal of the Division of Health Psychology, American Psychological Association 22:592-597.

Serra J (2012) Fibromyalgia: Update on the Relative Contribution of Peripheral vs.

Central Mechanisms. In: World Congress on Pain. Milan: IASP.

Shehzad Z, Kelly AM, Reiss PT, Gee DG, Gotimer K, Uddin LQ, Lee SH, Margulies DS, Roy AK, Biswal BB, Petkova E, Castellanos FX, Milham MP (2009) The resting brain: unconstrained yet reliable. Cereb Cortex 19:2209-2229.

Shulman GL, Corbetta M, Fiez JA, Buckner RL, Miezin FM, Raichle ME, Petersen SE (1997) Searching for activations that generalize over tasks. Hum Brain Mapp 5:317-322.

Singer T, Seymour B, O'Doherty J, Kaube H, Dolan RJ, Frith CD (2004) Empathy for Pain Involves the Affective but not Sensory Components of Pain. Science 303:1157-1162.

Singer T, Seymour B, O'Doherty JP, Stephan KE, Dolan RJ, Frith CD (2006) Empathic neural responses are modulated by the perceived fairness of others. Nature 439:466-469.

Singh KD, Barnes GR, Hillebrand A, Forde EM, Williams AL (2002) Task-related changes in cortical synchronization are spatially coincident with the hemodynamic response. NeuroImage 16:103-114.

Smith SM (2002) Fast robust automated brain extraction. Human brain mapping 17:143-155.

Smith SM, Nichols TE (2009) Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. NeuroImage 44:83-98.

Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, Behrens TE (2006) Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. NeuroImage 31:1487-1505.

Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TEJ, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J, Vickers J, Zhang YY, De Stefano N, Brady JM, Matthews PM (2004) Advances in functional and structural MR image analysis and implementation as FSL. NeuroImage 23:S208-S219.

Smythe HA (1975) Non-articular rheumatism and the fibrosists syndrome In: Arthritis and Allied Conditions (Hollander JL, ed), pp 874–884 Philadelphia: Lea & Febiger.

Smythe HA, Moldofsky H (1977) Two contributions to understanding of the "fibrositis" syndrome. Bull Rheum Dis 28:928-931.

Sorensen J, Bengtsson A, Backman E, Henriksson KG, Bengtsson M (1995) Pain analysis in patients with fibromyalgia. Effects of intravenous morphine, lidocaine, and ketamine. Scandinavian journal of rheumatology 24:360-365.

Speckmann E-J, Caspers H, Andersen P (1979) Origin of cerebral field potential : international symposium, Muenster, Germany [October 10-11, 1977]. Stuttgart: Thieme.

Speckmann EJ, Elger C (2005) Introduction to the neurophysiological basis of the EEG and DC potentials. In: Electroencephalography: basic principles, clinical applications and related fields, 5th Edition (Niedermeyer E, ed), pp 17-29. Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Spielberger CD, Gorusch RL, Lushene RE (1970) Manual for the State-Trait Anxiety Inventory (Self-evaluation questionnaire). Palo Alto: Consulting Psychologists Press.

Spreng RN, Mar RA, Kim AS (2009) The common neural basis of autobiographical memory, prospection, navigation, theory of mind, and the default mode: a quantitative meta-analysis. J Cogn Neurosci 21:489-510.

Srinivasan R, Winter WR, Nunez PL (2006) Source analysis of EEG oscillations using high-resolution EEG and MEG. Progress in brain research 159:29-42.

Stancak A (2006) Cortical oscillatory changes occurring during somatosensory and thermal stimulation. Progress in brain research 159:237-252.

Stancak A, Ward H, Fallon N (2012a) Modulation of pain by emotional sounds: A laser-evoked potential study. Eur J Pain.

Stancak A, Johnstone J, Fallon N (2012b) Effects of motor response expectancy on cortical processing of noxious laser stimuli. Behavioural brain research 227:215-223.

Stancak A, Svoboda J, Rachmanova R, Vrana J, Kralik J, Tintera J (2003) Desynchronization of cortical rhythms following cutaneous stimulation: effects of stimulus repetition and intensity, and of the size of corpus callosum. Clin Neurophysiol 114:1936-1947.

Stancak A, Jr., Pfurtscheller G (1996) Event-related desynchronisation of central beta-rhythms during brisk and slow self-paced finger movements of dominant and nondominant hand. Brain research Cognitive brain research 4:171-183.

Stancak A, Jr., Pfurtscheller G (1997) Effects of handedness on movement-related changes of central beta rhythms. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 14:419-428.

Staud R (2010) Is it all central sensitization? Role of peripheral tissue nociception in chronic musculoskeletal pain. Current rheumatology reports 12:448-454.

Staud R (2011a) Evidence for shared pain mechanisms in osteoarthritis, low back pain, and fibromyalgia. Current rheumatology reports 13:513-520.

Staud R (2011b) Peripheral pain mechanisms in chronic widespread pain. Best Pract Res Clin Rheumatol 25:155-164.

Staud R (2012) Abnormal endogenous pain modulation is a shared characteristic of many chronic pain conditions. Expert review of neurotherapeutics 12:577-585.

Staud R, Spaeth M (2008) Psychophysical and neurochemical abnormalities of pain processing in fibromyalgia. CNS spectrums 13:12-17.

Staud R, Nagel S, Robinson ME, Price DD (2009) Enhanced central pain processing of fibromyalgia patients is maintained by muscle afferent input: a randomized, double-blind, placebo-controlled study. Pain 145:96-104.

Staud R, Vierck CJ, Cannon RL, Mauderli AP, Price DD (2001) Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. Pain 91:165-175.

Staud R, Craggs JG, Perlstein WM, Robinson ME, Price DD (2008) Brain activity associated with slow temporal summation of C-fiber evoked pain in fibromyalgia patients and healthy controls. Eur J Pain 12:1078-1089.

Staud R, Robinson ME, Vierck CJ, Jr., Cannon RC, Mauderli AP, Price DD (2003) Ratings of experimental pain and pain-related negative affect predict clinical pain in patients with fibromyalgia syndrome. Pain 105:215-222.

Stein N, Sprenger C, Scholz J, Wiech K, Bingel U (2012) White matter integrity of the descending pain modulatory system is associated with interindividual differences in placebo analgesia. Pain 153:2210-2217.

Stejskal EO (1965) Use of Spin Echoes in a Pulsed Magnetic-Field Gradient to Study Anisotropic Restricted Diffusion and Flow. J Chem Phys 43:3597-&.

Steriade M (2001) Impact of network activities on neuronal properties in corticothalamic systems. J Neurophysiol 86:1-39.

Steriade M (2005) Cellular substrate of brain rhythms. In: Electroencephalography: basic principles, clinical applications and related fields, 5th Edition (Niedermeyer E, ed), pp 31-83. Philadelphia, Pa. ; London: Lippincott Williams & Wilkins.

Sudhyadhom A, McGregor K, Okun MS, Foote KD, Trinastic J, Crosson B, Bova FJ (2012) Delineation of motor and somatosensory thalamic subregions utilizing probabilistic diffusion tractography and electrophysiology. Journal of magnetic resonance imaging : JMRI.

Suffczynski P, Kalitzin S, Pfurtscheller G, Lopes da Silva FH (2001) Computational model of thalamo-cortical networks: dynamical control of alpha rhythms in relation to focal attention. International journal of psychophysiology : official journal of the International Organization of Psychophysiology 43:25-40.

Suhr JA (2003) Neuropsychological impairment in fibromyalgia: relation to depression, fatigue, and pain. Journal of psychosomatic research 55:321-329.

Sullivan MJ, Martel MO, Tripp DA, Savard A, Crombez G (2006) Catastrophic thinking and heightened perception of pain in others. Pain 123:37-44.

Sullivan MJ, Thorn B, Haythornthwaite JA, Keefe F, Martin M, Bradley LA, Lefebvre JC (2001) Theoretical perspectives on the relation between catastrophizing and pain. Clin J Pain 17:52-64.

Sullivan MJL, Bishop SR, Pivik J (1995) The Pain Catastrophizing Scale: Development and validation. Psych Ass 7:524-532.

Sundgren PC, Dong Q, Gomez-Hassan D, Mukherji SK, Maly P, Welsh R (2004) Diffusion tensor imaging of the brain: review of clinical applications. Neuroradiology 46:339-350.

Sundgren PC, Petrou M, Harris RE, Fan X, Foerster B, Mehrotra N, Sen A, Clauw DJ, Welsh RC (2007) Diffusion-weighted and diffusion tensor imaging in fibromyalgia patients: a prospective study of whole brain diffusivity, apparent diffusion coefficient, and fraction anisotropy in different regions of the brain and correlation with symptom severity. Academic radiology 14:839-846.

Teutsch S, Herken W, Bingel U, Schoell E, May A (2008) Changes in brain gray matter due to repetitive painful stimulation. NeuroImage 42:845-849.

Thickbroom GW, Mastaglia FL, Carroll WM, Davies HD (1984) Source derivation: application to topographic mapping of visual evoked potentials. Electroenceph Clin Neurophysiol 59:279-285.

Thieme K, Gracely RH (2009) Are psychological treatments effective for fibromyalgia pain? Current rheumatology reports 11:443-450.

Thieme K, Turk DC, Flor H (2004) Comorbid depression and anxiety in fibromyalgia syndrome: relationship to somatic and psychosocial variables. Psychosom Med 66:837-844.

Thieme K, Spies C, Sinha P, Turk DC, Flor H (2005) Predictors of pain behaviors in fibromyalgia syndrome. Arthritis Rheum 53:343-350.

Thimineur M, Kitaj M, Kravitz E, Kalizewski T, Sood P (2002) Functional abnormalities of the cervical cord and lower medulla and their effect on pain: observations in chronic pain patients with incidental mild Chiari I malformation and moderate to severe cervical cord compression. Clin J Pain 18:171-179.

Thomas DL, De Vita E, Deichmann R, Turner R, Ordidge RJ (2005) 3D MDEFT imaging of the human brain at 4.7 T with reduced sensitivity to radiofrequency inhomogeneity. Magnetic resonance in medicine : official journal of the Society of Magnetic

Resonance in Medicine / Society of Magnetic Resonance in Medicine 53:1452-1458.

Thompson JL, Ebersole JS (1999) Long-term inpatient audiovisual scalp EEG monitoring. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 16:91-99.

Thulborn KR, Waterton JC, Matthews PM, Radda GK (1982) Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. Biochimica et biophysica acta 714:265-270.

Treede RD, Kenshalo DR, Gracely RH, Jones AK (1999) The cortical representation of pain. Pain 79:105-111.

Turk DC, Robinson JP, Burwinkle T (2004) Prevalence of fear of pain and activity in patients with fibromyalgia syndrome. The journal of pain : official journal of the American Pain Society 5:483-490.

Turk DC, Okifuji A, Sinclair JD, Starz TW (1996a) Pain, disability, and physical functioning in subgroups of patients with fibromyalgia. The Journal of rheumatology 23:1255-1262.

Turk DC, Okifuji A, Starz TW, Sinclair JD (1996b) Effects of type of symptom onset on psychological distress and disability in fibromyalgia syndrome patients. Pain 68:423-430.

Turner R, Le Bihan D, Maier J, Vavrek R, Hedges LK, Pekar J (1990) Echo-planar imaging of intravoxel incoherent motion. Radiology 177:407-414.

Tzovara A, Murray MM, Bourdaud N, Chavarriaga R, Millan Jdel R, De Lucia M (2012) The timing of exploratory decision-making revealed by single-trial topographic EEGanalyses. NeuroImage 60:1959-1969.

Usui C, Hatta K, Doi N, Nakanishi A, Nakamura H, Nishioka K, Arai H (2010) Brain perfusion in fibromyalgia patients and its differences between responders and poor responders to gabapentin. Arthritis research & therapy 12:R64.

Valet M, Gundel H, Sprenger T, Sorg C, Muhlau M, Zimmer C, Henningsen P, Tolle TR (2009) Patients with pain disorder show gray-matter loss in pain-processing structures: a voxel-based morphometric study. Psychosom Med 71:49-56.

Van Damme S, Crombez G, Eccleston C (2002) Retarded disengagement from pain cues: the effects of pain catastrophizing and pain expectancy. Pain 100:111-118.

van Middendorp H, Lumley MA, Jacobs JW, Bijlsma JW, Geenen R (2010a) The effects of anger and sadness on clinical pain reports and experimentally-induced pain thresholds in women with and without fibromyalgia. Arthritis care & research 62:1370-1376.

van Middendorp H, Lumley MA, Jacobs JW, van Doornen LJ, Bijlsma JW, Geenen R (2008) Emotions and emotional approach and avoidance strategies in fibromyalgia. Journal of psychosomatic research 64:159-167.

van Middendorp H, Lumley MA, Moerbeek M, Jacobs JW, Bijlsma JW, Geenen R (2010b) Effects of anger and anger regulation styles on pain in daily life of women with fibromyalgia: a diary study. Eur J Pain 14:176-182.

van Wijk G, Veldhuijzen DS (2010) Perspective on diffuse noxious inhibitory controls as a model of endogenous pain modulation in clinical pain syndromes. The journal of pain : official journal of the American Pain Society 11:408-419.

VanVeen BD, vanDrongelen W, Yuchtman M, Suzuki A (1997) Localization of brain electrical activity via linearly constrained minimum variance spatial filtering. Ieee T Bio-Med Eng 44:867-880.

Veldhuijzen DS, Nemenov MI, Kaeser M, Zhuo J, Gullapalli RP, Greenspan JD (2009) Differential brain activation associated with laser-evoked burning and pricking pain: an event-related fMRI study. Pain 141:104-113.

Verne GN, Himes NC, Robinson ME, Gopinath KS, Briggs RW, Crosson B, Price DD (2003) Central representation of visceral and cutaneous hypersensitivity in the irritable bowel syndrome. Pain 103:99-110.

Vierck CJ, Jr., Staud R, Price DD, Cannon RL, Mauderli AP, Martin AD (2001) The effect of maximal exercise on temporal summation of second pain (windup) in patients with fibromyalgia syndrome. The journal of pain : official journal of the American Pain Society 2:334-344.

Villarreal G, Hamilton DA, Petropoulos H, Driscoll I, Rowland LM, Griego JA, Kodituwakku PW, Hart BL, Escalona R, Brooks WM (2002) Reduced hippocampal volume and total white matter volume in posttraumatic stress disorder. Biol Psychiatry 52:119-125.

Villemure C, Bushnell MC (2009) Mood influences supraspinal processing separately from attention. J Neurosci 29:705-715.

Vincent JL, Snyder AZ, Fox MD, Shannon BJ, Andrews JR, Raichle ME, Buckner RL (2006) Coherent spontaneous activity identifies a hippocampal-parietal memory network. J Neurophysiol 96:3517-3531.

Vlaeyen JW, Linton SJ (2000) Fear-avoidance and its consequences in chronic musculoskeletal pain: a state of the art. Pain 85:317-332.

Vogt BA (2005) Pain and emotion interactions in subregions of the cingulate gyrus. Nature reviews Neuroscience 6:533-544.

Vogt BA, Derbyshire S, Jones AK (1996) Pain processing in four regions of human cingulate cortex localized with co-registered PET and MR imaging. The European journal of neuroscience 8:1461-1473.

Wakana S, Caprihan A, Panzenboeck MM, Fallon JH, Perry M, Gollub RL, Hua K, Zhang J, Jiang H, Dubey P, Blitz A, van Zijl P, Mori S (2007) Reproducibility of quantitative tractography methods applied to cerebral white matter. NeuroImage 36:630-644.

Walen HR, Cronan PA, Bigatti SM (2001) Factors associated with healthcare costs in women with fibromyalgia. The American journal of managed care 7 Spec No:SP39-47.

Watson A, El-Deredy W, Iannetti GD, Lloyd D, Tracey I, Vogt BA, Nadeau V, Jones AK (2009) Placebo conditioning and placebo analgesia modulate a common brain network during pain anticipation and perception. Pain 145:24-30.

Watson NF, Buchwald D, Goldberg J, Maravilla KR, Noonan C, Guan Q, Ellenbogen RG (2011) Is Chiari I malformation associated with fibromyalgia? Neurosurgery 68:443-448; discussion 448-449.

Weir PT, Harlan GA, Nkoy FL, Jones SS, Hegmann KT, Gren LH, Lyon JL (2006) The incidence of fibromyalgia and its associated comorbidities: a population-based retrospective cohort study based on International Classification of Diseases, 9th Revision codes. Journal of clinical rheumatology : practical reports on rheumatic & musculoskeletal diseases 12:124-128.

Westin CF, Maier SE, Mamata H, Nabavi A, Jolesz FA, Kikinis R (2002) Processing and visualization for diffusion tensor MRI. Medical image analysis 6:93-108.

White KP, Harth M (2001) Classification, epidemiology, and natural history of fibromyalgia. Current pain and headache reports 5:320-329.

White KP, Speechley M, Harth M, Ostbye T (1999) The London Fibromyalgia Epidemiology Study: comparing the demographic and clinical characteristics in 100 random community cases of fibromyalgia versus controls. The Journal of rheumatology 26:1577-1585.

White LA, Birnbaum HG, Kaltenboeck A, Tang J, Mallett D, Robinson RL (2008) Employees with fibromyalgia: medical comorbidity, healthcare costs, and work loss. Journal of occupational and environmental medicine / American College of Occupational and Environmental Medicine 50:13-24.

Whitfield-Gabrieli S, Ford JM (2012) Default mode network activity and connectivity in psychopathology. Annual review of clinical psychology 8:49-76.

Whitfield-Gabrieli S, Nieto-Castanon A (2012) Conn: a functional connectivity toolbox for correlated and anticorrelated brain networks. Brain connectivity 2:125-141.

Whitfield-Gabrieli S, Moran JM, Nieto-Castanon A, Triantafyllou C, Saxe R, Gabrieli JD (2011) Associations and dissociations between default and self-reference networks in the human brain. NeuroImage 55:225-232.

Wilke WS (2001) Can fibromyalgia and chronic fatigue syndrome be cured by surgery? Cleveland Clinic journal of medicine 68:277-279.

Williams DA, Clauw DJ (2009) Understanding fibromyalgia: lessons from the broader pain research community. The journal of pain : official journal of the American Pain Society 10:777-791.

Winkelmann A, Perrot S, Schaefer C, Ryan K, Chandran A, Sadosky A, Zlateva G (2011) Impact of fibromyalgia severity on health economic costs: results from a European cross-sectional study. Applied health economics and health policy 9:125-136.

Witting N, Kupers RC, Svensson P, Jensen TS (2006) A PET activation study of brush-evoked allodynia in patients with nerve injury pain. Pain 120:145-154.

Wolfe F, Cathey MA (1983) Prevalence of primary and secondary fibrositis. The Journal of rheumatology 10:965-968.

Wolfe F, Hauser W (2011) Fibromyalgia diagnosis and diagnostic criteria. Annals of medicine 43:495-502.

Wolfe F, Ross K, Anderson J, Russell IJ, Hebert L (1995) The prevalence and characteristics of fibromyalgia in the general population. Arthritis Rheum 38:19-28.

Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P, Russell AS, Russell IJ, Winfield JB, Yunus MB (2010) The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis Care Res 62:600-610.

Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, Tugwell P, Campbell SM, Abeles M, Clark P, et al. (1990) The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 33:160-172.

Wood PB, Glabus MF, Simpson R, Patterson JC, 2nd (2009) Changes in gray matter density in fibromyalgia: correlation with dopamine metabolism. The journal of pain : official journal of the American Pain Society 10:609-618.

Woodward ND, Rogers B, Heckers S (2011) Functional resting-state networks are differentially affected in schizophrenia. Schizophrenia research 130:86-93.

Woolf CJ (2011) Central sensitization: implications for the diagnosis and treatment of pain. Pain 152:S2-15.

Woolf CJ, Mannion RJ (1999) Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 353:1959-1964.

Yunus M, Masi AT, Calabro JJ, Miller KA, Feigenbaum SL (1981) Primary fibromyalgia (fibrositis): clinical study of 50 patients with matched normal controls. Seminars in arthritis and rheumatism 11:151-171.

Yunus MB (1992) Towards a model of pathophysiology of fibromyalgia: aberrant central pain mechanisms with peripheral modulation. The Journal of rheumatology 19:846-850.

Zarei M, Patenaude B, Damoiseaux J, Morgese C, Smith S, Matthews PM, Barkhof F, Rombouts SA, Sanz-Arigita E, Jenkinson M (2010) Combining shape and connectivity analysis: an MRI study of thalamic degeneration in Alzheimer's disease. NeuroImage 49:1-8.

Zautra AJ, Fasman R, Reich JW, Harakas P, Johnson LM, Olmsted ME, Davis MC (2005) Fibromyalgia: evidence for deficits in positive affect regulation. Psychosom Med 67:147-155.

Ziegler G, Dahnke R, Jancke L, Yotter RA, May A, Gaser C (2012) Brain structural trajectories over the adult lifespan. Hum Brain Mapp 33:2377-2389.

Zyloney CE, Jensen K, Polich G, Loiotile RE, Cheetham A, LaViolette PS, Tu PC, Kaptchuk TJ, Gollub RL, Kong JA (2010) Imaging the functional connectivity of the Periaqueductal Gray during genuine and sham electroacupuncture treatment. Molecular pain 6.

Appendices

Welcome to the Integrated Research Application System

IRAS Project Filter

The integrated dataset required for your project will be created from the answers you give to the following questions. The system will generate only those questions and sections which (a) apply to your study type and (b) are required by the bodies reviewing your study. Please ensure you answer all the questions before proceeding with your applications.

Please enter a short title for this project (maximum 70 characters)
Brain-autonomic interactions in fibromyalgia syndrome patients

1. Is your project research?

⦿ Yes ◯ No

2. Select one category from the list below:

◯ Clinical trial of an investigational medicinal product

◯ Clinical investigation or other study of a medical device

◯ Combined trial of an investigational medicinal product and an investigational medical device

◯ Other clinical trial or clinical investigation

⦿ Study administering questionnaires/interviews for quantitative analysis, or using mixed quantitative/qualitative methodology

◯ Study involving qualitative methods only

◯ Study limited to working with human tissue samples, other human biological samples and/or data *(specific project only)*

◯ Research tissue bank

◯ Research database

If your work does not fit any of these categories, select the option below:

◯ Other study

2a. Please answer the following question(s):

a) Does the study involve the use of any ionising radiation?	◯ Yes	⦿ No
b) Will you be taking new human tissue samples (or other human biological samples)?	◯ Yes	⦿ No
c) Will you be using existing human tissue samples (or other human biological samples)?	◯ Yes	⦿ No

3. In which countries of the UK will the research sites be located?(Tick all that apply)

☑ England
☑ Scotland
☑ Wales
☑ Northern Ireland

3a. In which country of the UK will the lead NHS R&D office be located:

◯ England

◯ Scotland

1   29397/84748/14/297

◯ Wales

◯ Northern Ireland

◯ This study does not involve the NHS

---

4. Which review bodies are you applying to?

☑ NHS/HSC Research and Development offices
☐ Social Care Research Ethics Committee
☑ Research Ethics Committee
☐ National Information Governance Board for Health and Social Care (NIGB)
☐ Ministry of Justice (MoJ)

---

5. Will any research sites in this study be NHS organisations?

⦿ Yes   ◯ No

---

6. Do you plan to include any participants who are children?

◯ Yes   ⦿ No

---

7. Do you plan to include any participants who are adults unable to consent for themselves through physical or mental incapacity? *The guidance notes explain how an adult is defined for this purpose.*

◯ Yes   ⦿ No

---

8. Do you plan to include any participants who are prisoners or young offenders in the custody of HM Prison Service in England or Wales?

◯ Yes   ⦿ No

---

9. Is the study, or any part of the study, being undertaken as an educational project?

⦿ Yes   ◯ No

9a. Is the project being undertaken in part fulfilment of a PhD or other doctorate?

⦿ Yes   ◯ No

---

10. Is this project financially supported by the United States Department for Health and Human Services?

◯ Yes   ⦿ No

---

11. Will identifiable patient data be accessed outside the clinical care team without prior consent at any stage of the project (including identification of potential participants)?

◯ Yes   ⦿ No

Integrated Research Application System
Application Form for Research administering questionnaires/interviews for quantitative analysis or mixed methodology study

---

NHS/HSC R&D Form (project information)

Please refer to the Submission and Checklist tabs for instructions on submitting R&D applications.

The Chief Investigator should complete this form. Guidance on the questions is available wherever you see this symbol displayed. We recommend reading the guidance first. The complete guidance and a glossary are available by selecting Help.

Short title and version number: (maximum 70 characters - this will be inserted as header on all forms)
Brain-autonomic interactions in fibromyalgia syndrome patients

PART A: Core study information

1. ADMINISTRATIVE DETAILS

A1. Full title of the research:

Brain-autonomic interactions in fibromyalgia syndrome patients

A2-1. Give details of the educational course or degree for which this research is being undertaken:

Name and level of course/ degree:

Name of educational establishment:

Name and contact details of academic supervisor:

       Title Forename/Initials Surname

Address

Post Code
E-mail
Telephone
Fax

Name and contact details of student:

       Title Forename/Initials Surname

Address

Post Code
E-mail

| Telephone |
| Fax |

A copy of a current CV for the student (maximum 2 pages of A4) must be submitted with the application.

A2-2. Who will act as Chief Investigator for this study?

○ Student

○ Academic supervisor

○ Other

A3. Chief Investigator:

	Title	Forename/Initials	Surname
	Dr.	Andrej	Stancak
Post	senior lecturer
Qualifications	PhD in physiology; Professor for normal physiology;
Employer	University of Liverpool
Work Address	Eleanor Rathbone Building
	Bedford Street South
Post Code	L69 7ZA
Work E-mail	a.stancak@liverpool.ac.uk
* Personal E-mail	
Work Telephone	01517946951
* Personal Telephone/Mobile	01517946951
Fax	0151792945

* This information is optional. It will not be placed in the public domain or disclosed to any other third party without prior consent.

A copy of a current CV (maximum 2 pages of A4) for the Chief Investigator must be submitted with the application.

A4. Who is the contact on behalf of the sponsor for all correspondence relating to applications for this project?
This contact will receive copies of all correspondence from REC and R&D reviewers that is sent to the CI.

	Title	Forename/Initials	Surname
	Mrs.	Sarah	Fletcher
Address	University of Liverpool Research Et
	hics Committee, The Foresight Bldg.
	3 Brownlow Hill
Post Code	L69 3GL
E-mail	ethics@liverpool.ac.uk
Telephone	01517948290
Fax	01517948728

A5-1. Research reference numbers. *Please give any relevant references for your study:*

Applicant's/organisation's own reference number, e.g. R & D (if available):

Sponsor's/protocol number:

Protocol Version: 1

Protocol Date: 20/08/2009

Funder's reference number:

International Standard Randomised Controlled Trial Number (ISRCTN):

ClinicalTrials.gov Identifier (NCT number):

European Clinical Trials Database (EudraCT) number:

Project website:

Ref.Number Description	Reference Number
094/H1001/92	

A5-2. Is this application linked to a previous study or another current application?

◯ Yes   ⦿ No

Please give brief details and reference numbers.

<h3 style="background:gray">2. OVERVIEW OF THE RESEARCH</h3>

To provide all the information required by review bodies and research information systems, we ask a number of specific questions. This section invites you to give an overview using language comprehensible to lay reviewers and members of the public. Please read the guidance notes for advice on this section.

A6-1. Summary of the study. *Please provide a brief summary of the research (maximum 300 words) using language easily understood by lay reviewers and members of the public. This summary will be published on the website of the National Research Ethics Service following the ethical review.*

This is a PhD research project encompassing electroencephalographic (EEG) and magnetic resonance (MR) recordings in fibromyalgia syndrome patients (FMS) and healthy control participants. The links between vegetative processes such as heart rate and sweat glands activity will be compared with ongoing EEG activity, and with structural and functional brain activation recorded using MR. The purpose of the project is to evaluate the role of a diminished central nervous control of autonomic nerve system as one of the pathophysiological mechanisms of fibromyalgia.

  The project is planned for three years and it is structured into three experimental sessions. In Session 1, psychological questionnaires and evaluation of fibromyalgia pain will be performed. In Session 2, EEG will be recorded using 32 scalp electrodes. In parallel, ECG, respiratory movements and galvanic skin response will be also recorded. These electrophysiological measures will be taken under three conditions: 1. relaxed wakeful state, 2. viewing pain and non-pain scenes, 3. periods of brushing the skin using hair brush (5 s) alternating with periods of rest (5 s). Participant's awareness of their heart beats will be evaluated. In Session 3, structural MR images of the brain and 20 min resting state functional MR data will be acquired to analyse the cerebral correlates of cardiac awareness, and EEG and vegetative responsiveness in fibromyalgia syndrome patients and healthy controls.

A6-2. Summary of main issues. *Please summarise the main ethical and design issues arising from the study and say how you have addressed them.*

The electrophysiological and MR recordings are non-invasive and frequently employed in clinical research. There are few ethical issues that deserve attention:

1. Mild pain will be elicited during brushing the skin in FMS patients. However, the pain caused by brushing the skin is mild and comparable to the pain which pain patients experience frequently during washing, dressing, or manipulation with objects. Participants will be urged to terminate the experiment if the pain associated with brushing will exceed their tolerance.

2. Electrophysiological recordings will be performed in a standard laboratory room at pain clinics in Aintree hospital. Electrodes are attached using adhesive paper disks or plastic holders mounted in a velcro cap. The adaptation of electrodes lasts 10 min and does not impose any stress to the participants. However, MR scans will be acquired in using 3-Tesla MR scanner in a confined space. The MR scanner may be a stressful experience for claustrophobic

participants. Therefore, participants who know about their claustrophoby will not be advised to participate in the study. Admission to the MR centre and to scanning can be realised only after a detailed screening procedure implemented by the brain imaging centre (MARIARC, University of Liverpool). Participants will be urged to indicate any physical or psychological discomfort, which will be followed by immediate termination of the recordings.

3. PURPOSE AND DESIGN OF THE RESEARCH

A7. Select the appropriate methodology description for this research. *Please tick all that apply:*

☐ Case series/ case note review
☐ Case control
☐ Cohort observation
☐ Controlled trial without randomisation
☐ Cross-sectional study
☐ Database analysis
☐ Epidemiology
☐ Feasibility/ pilot study
☐ Laboratory study
☐ Metanalysis
☐ Qualitative research
☑ Questionnaire, interview or observation study
☐ Randomised controlled trial
☑ Other (please specify)

A PhD project involving electrophysiological recordings (EEG, ECG, eye movements, respiration movements), questionnaires, and magnetic resonance scans.

A10. What is the principal research question/objective? *Please put this in language comprehensible to a lay person.*

Fibromyalgia syndrome (FMS) is a clinical condition associated with soft tissue pain and other symptoms such as impaired sleep, fatigue, and affective disorders. Previous research has revealed no peripheral changes in the muscles of tendons that could account for chronic pain in FMS patients. In contrast, several studies suggest alterations in the central nervous system as critical players in patients' symptom formation, e.g., deficiency of certain neurostransmitters or increased excitability of certain brain regions. In parallel to these findings, a growing body of evidence suggests alterations in the sympathetic and possibly parasympathetic control of vegetative functions. The main research question in the present project to be addressed is whether dysregulation of the vegetative system in FMS is related to impaired central nervous control of vegetative functions. To explore this issue, we will have FMS patients and healthy control subjects undergo electrophysiological recordings to designed to test whether FMS patients show greater awareness of their vegetative functions and greater autonomic changes during a set of standardised challenges. The cardiac awareness score, electrophysiological measures, and the clinical symptoms will be correlated with the structural brain images and with the resting state activity of the brain.

A11. What are the secondary research questions/objectives if applicable? *Please put this in language comprehensible to a lay person.*

A12. What is the scientific justification for the research? *Please put this in language comprehensible to a lay person.*

Fibromyalgia syndrome is a painful clinical condition characterised by widespread muscle pain, tenderness, sleep impairment, fatigue, autonomic disturbances, and alterations in affect and cognition. FMS has a prevalence of 2-4% and it occurs more frequently in females than in males. Despite intense search no convincing changes in the muscles have been found that would explain the widespread pain and associated symptoms in FMS patients. Therefore, a possible role for central nervous system involvement in the pathogenesis of FMS has been raised.
Some recent studies do suggest altered cerebral function in FMS although the picture remains mixed. Brain imaging studies reported reduced blood flow in thalamus in FMS patients and also increased activations in a number of brain

regions during painful stimulation. FMS leads to shrinkage of grey matter especially in insula and posterior cingulate cortex.

The role of elevated sympathetic nerve outflow in the pathogenesis and maintenance of FMS has been indicated by the finding that regional sympathetic blockade temporarily alleviates pain in FMS patients The sympathetic nerve response to stress, evaluated as skin conductance level is stronger in FMS patients than in control particpants. Increased sympathetic nerve tone in FMS patients also manifests in increased amplitude of the low-frequency variations in R-R intervals of ECG representing the sum of sympathetic nerve impulses impinging on the sinoatrial node. Nocturnal bursts of sympathetic activity manifested in heart rate variability patterns are frequent in FMS patients. In contrast, the cardiac orthostatic response and circadian variations of heart rate variability are blunted in FMS patients indicating dysfunctional sympathetic nerve regulation in the background of elevated sympathetic tone. Thus, the sympathetic nervous system may play a role in the maintenance of FMS, e.g., by adding to the vasomotor muscle hypoperfusion and its aberrant function could explain many common symptoms in this condition, such as fatigue, impaired sleep, mood disorders, and sensation of cold

Alterations in autonomic regulation and presence of cerebral hyperexcitability in select brain regions in FMS patients form the foundation of our hypothesis that cerebrally mediated dysregulation of the sympathetic and parasympathetic control contributes to pain and associated symptoms in FMS. The cerebral centres driving autonomic nervous activity have been investigated in healthy persons using modern brain imaging techniques in the last few years. Heart rate variations associated with pain are encoded in the mid- and posterior cingulate cortex, somatosensory regions, and dorsomedial prefrontal cortex. The sympathetic sudomotor outflow during heat pain, indexed by skin conductance changes, is controlled in primary and secondary somatosensory and anterior cingulate cortex, which in part fits the default mode regions shown earlier to bear relationships to spontaneous changes in skin conductance. Awareness of cardiac changes during various tasks correlates with activations in the cingulate cortex, insula and orbitofrontal cortex. Subjects who are aware of their autonomic changes show stronger cerebral sources of EEG heartbeat evoked potentials, fMRI activations, and even greater grey matter density in interoceptive regions of the brain than low awareness persons. In the context of FMS, there are no data either on cardiac awareness and its relevance to pain or brain centres controlling the sympathetic and parasympathetic nervous systems.

We propose to address the mechanisms of cerebral control of autonomic nervous system activity in FMS patients. The specific research questions are the following:

1. Do FMS patients show altered cardiac awareness compared to healthy subjects? Does cardiac awareness in FMS patients correlate with clinical pain (number of tender points, pain threshold at tender points), and related symptoms (fatigue, affect, sleep quality)?
2. What is the topography and amplitude of the EEG heartbeat evoked potential in FMS patients compared to healthy subjects?
3. What are the EEG and functional correlates of skin conductance level oscillations in FMS patients at rest and during periods of somatic stimulation and imagined pain? Does activity in the cortical regions showing coupling with the skin conductance level oscillations correlate with clinical pain and associated FMS symptoms?
4. Are there correlations between the grey and white matter density, cardiac awareness and clinical pain in FMS patients?

A13. Please give a full summary of your design and methodology. *It should be clear exactly what will happen to the research participant, how many times and in what order. Please complete this section in language comprehensible to the lay person. Do not simply reproduce or refer to the protocol. Further guidance is available in the guidance notes.*

Each participant will take part in three sessions:

Session 1:

a. psychological tests and questionnaires to measure certain personality characteristisc and FMS symptoms.

b. evaluation of tender points.

Session 2.

a. adaptation of 32 EEG electrodes, ECG electrodes, respiration belt and galvanic skin resistance electrodes - 10 min.

b. 5 min of electrophysiological recordings, followed by a simple mood questionnaire.

c. electrophysiological recordings during viewing 20 photographs involving pain and 20 photographs not involving pain - 20 min.

d. alternating periods of brushing the skin at right elbow using hair brush (5 s) and rest (5 s). Thirty cycles of brushing-rest will be taken in 5 blocks per 6 cycles. Subjective reports of pain will be noted after each block. Total duration of this part of the sesion is 6-7 min.

204

Session 3.

a. safety screening performed by the MARIARC radiographer.

b. T2-weighted brain scan for clinical evaluation - 2min.

c. T2-weighted functional imaging involving resting state interrupted 20 times by a visual stimulus prompting a quick motor response - 20 min.

d. T1-weighted brain scan for the analysis of structural brain differences in FMS patients and healthy participants - 10 min.

3. T2-weighted brain scan for analysis of the cerebral white matter (diffusion tensor imaging).

A14-1. In which aspects of the research process have you actively involved, or will you involve, patients, service users, and/or their carers, or members of the public?

☐ Design of the research
☐ Management of the research
☐ Undertaking the research
☐ Analysis of results
☐ Dissemination of findings
☑ None of the above

Give details of involvement, or if none please justify the absence of involvement.

---

4. RISKS AND ETHICAL ISSUES

RESEARCH PARTICIPANTS

A15. What is the sample group or cohort to be studied in this research?

Select all that apply:

☐ Blood
☐ Cancer
☐ Cardiovascular
☐ Congenital Disorders
☐ Dementias and Neurodegenerative Diseases
☐ Diabetes
☐ Ear
☐ Eye
☐ Generic Health Relevance
☐ Infection
☐ Inflammatory and Immune System
☐ Injuries and Accidents
☐ Mental Health
☐ Metabolic and Endocrine
☑ Musculoskeletal
☑ Neurological

☐ Oral and Gastrointestinal

☐ Paediatrics

☐ Renal and Urogenital

☐ Reproductive Health and Childbirth

☐ Respiratory

☐ Skin

☐ Stroke

Gender: Female participants only

Lower age limit: 19   Years

Upper age limit: 50   Years

A17-1. Please list the principal inclusion criteria (list the most important, max 5000 characters).

Inclusion criteria for FMS paients:
1. age 19-50 years
2. females
3. presence of widespread musculoskeletal pain diagnosed as fibromyalgia pain syndrome according to the criteria od American College of Rheumatology. These include
a) widespread pain in axial region and at least two contralateral body quadrants above and below the waist, and b) 11 or more tender points out of 18 points on digital palpation (4 kg pressure).

Inclusion criteria for healthy control participants:
1. age 19-50 years
2. females

A17-2. Please list the principal exclusion criteria (list the most important, max 5000 characters).

1. cardiovascular or respiratory disease such as hypertension or ischemic heart disease
2. diabetes, obesity
3. any chronic pain condition other than fibromyalgia (e.g. low back pain, arthritis).
4. any acute pain such as recent fracture, burns, surgery, or inflammation
5. psychiatric or neurologic diseases such as schisophrenia or epilepsy.
6. regular medication known or expected to have a pharmacological effect on the central nervous system

---

RESEARCH PROCEDURES, RISKS AND BENEFITS

A18. Give details of all non-clinical intervention(s) or procedure(s) that will be received by participants as part of the research protocol. *These include seeking consent, interviews, non-clinical observations and use of questionnaires.*

Please complete the columns for each intervention/procedure as follows:

    1. Total number of interventions/procedures to be received by each participant as part of the research protocol.

    2. If this intervention/procedure would be routinely given to participants as part of their care outside the research, how many of the total would be routine?

    3. Average time taken per intervention/procedure (minutes, hours or days)

    4. Details of who will conduct the intervention/procedure, and where it will take place.

Intervention or procedure	1	2	3	4
Psychological tests	6	0	30 min	performed by the PhD student; The tests will be used to evaluate the fibromyalgia symptoms and psychological state. 1. Fibromyalgia Impact Questionnaire (Bennett et al., 1991); 2. FibroFatigue Scale (Zachrisson et al., 2002). 3. Pain Catastrophising Scale (Sullivan et al., 1995). 4. The Sleep Problem Scale (Jenkins et al., 1988). 5. State and Trait Anxiety Inventory (Spielberger and Lushene, 1971).

				6. Beck Depression Inventory (Beck et al., 1961) 7. Illness Attitude Scale (Stewart and Watts, 2000)
Evaluation of tender points	1	2-3	5 min	performed by PhD student after induction by Dr. Chiu or Professor Nurmikko. Calibrated pressure of 4kg/cm2 will be applied to 18 tender points and pain reports will be obtained for each point.
Cardiac awareness test	1	0	4 min	performed by the PhD student; participant is asked to estimate the number of her heat beats during defined epochs of time (e.g. 45 s) and the estimated number of heart beats is compared with the number of QRS complexes in ECG
Resting EEG recording followed by mood questionnaire	1	0	5 min	performed by the PhD student; The participant will be asked to view the fication cross on a computer screen. Participant will rate her current mood using simple questionnaire involving 14 mood categories. Each category will be rated on a scale from 0 to 4.
Electrophysiological recordings during viewing pain and non-pain scenes	1	0	20 min	performed by the PhD student;
Alternating periods of brushing and rest	6	0	1 min	performed by the PhD student; gentle brushing of the skin on the right elbow for five seconds (navigated by the sound audible to the experimenter only) alternating with resting periods.
T2-weighted brain scan	1	0	2 min	performed by the PhD student passing induction training at MARIARC and supervised by MARIACR's radiographer. This recording will be sent to Walton Centre for radiographic evaluation.
T2-weighed, EPI functional brain scan	1	0	20 min	passing induction training at MARIARC and supervised by MARIACR's radiographer. Participants will view the projections screen and respond by pressing the switch with their right hand once a blue circle will appear on the screen. The blue circles will be presented 20 times with about 1 min intervals.
T1-weighed brain scan	1	0	10 min	performed by the PhD student passing induction training at MARIARC and supervised by MARIACR's radiographer. This scan will be used to analysed the associations between clinical pain, cardiac awareness and qutonocmic responses with brain structure in healthy participants and FMS patients.
T2-weighted brain scan (diffusion tensor imaging)	1	0	7 min	performed by the PhD student passing induction training at MARIARC and supervised by MARIARC's radiographer. The purpose of the scan is to evaluate the structure of the white matter.

A21. How long do you expect each participant to be in the study in total?

Two months.

A22. What are the potential risks and burdens for research participants and how will you minimise them?

For all studies, describe any potential adverse effects, pain, discomfort, distress, intrusion, inconvenience or changes to lifestyle. Only describe risks or burdens that could occur as a result of participation in the research. Say what steps would be taken to minimise risks and burdens as far as possible.

As some of drugs used to alleviate pain can modulate the brain activity, we suggest withdrawal of some of pain medication for 3 to 5 days before Session 2 and Session 3. Patients will be allowed to continue to use paracetamol as recommended by the doctor. If the patient uses a medication that contains paracetamol on an opioid-based drug (co-codamol, co-dydramol, co-proxamol) or an opioid medication without paracetamol (e.g., dihydrocodeine, tramadol)   the withdrawal will depend on the average daily dose she is taking. For example, if the patient usually uses 6 to 8 tablets of mild co-codamol (8/500) a day only, she will be asked to stop it 3 days before the test. If, by contrast, the patient is taking up to 8 tablets of a strong co-codamol (30/500) or high doses of dihydrocodeine, she will be asked to taper the dose over a period of 5 days. We will try to arrange tests on consecutive or nearly consecutive days so that the drug withdrawal will only happen once during participation in the study.
The exact information about when and what drug should be withdrawn will be provided in written form by the clinical team (Prof. Nurmikko or Dr. Chiu) and will be made in complete agreement with you. The patient will be able to

207

resume medication immediately after the experiments.
Medications that have no or minimal central nervous system effect will not be withdrawn. This includes paracetamol which the patient may take up to 4000 mg/day (8 tablets containing 500 mg). If the patient would be on another stable, regular medication for fibromyalgia it may be continued, depending on the dose. An example of an acceptable dose would be pregabalin 75 mg twice a day, gabapentin 300 mg twice a day or amitriptyline 10 mg at night. Prof. Nurmikko and Dr. Chiu will advice the patient on this regard.   Patients will not be asked to change any of the medication they may be on that has been prescribed for other medical conditions than fibromyalgia.

1. Opioid drugs
(a) regular daily dose of codeine or dihydrocodeine 90 mg/d or less: withdrawal over 3 days
(b) regular daily dose of codeine or dihydrocodeine 91 mg/d – 240 mg/day: withdrawal over 5 days
(c) codeine, dihydrocodeine >240 mg/day: not eligible (these patients can be reassessed if they manage to reduce their intake to 240 mg/d or less)
(d) strong opioids, including fentanyl and buprenorphine plasters: not eligible
(e) patients on prn codeine/dihydrocodeine: to refrain completely for 3 days
Specific withdrawal times for individual drugs:
1. Opioid drugs
(a) regular daily dose of codeine or dihydrocodeine 90 mg/d or less: withdrawal over 3 days
(b) regular daily dose of codeine or dihydrocodeine 91 mg/d – 240 mg/day: withdrawal over 5 days
(c) codeine, dihydrocodeine >240 mg/day: not eligible (these patients can be reassessed if they manage to reduce their intake to 240 mg/d or less)
(d) strong opioids, including fentanyl and buprenorphine plasters: not eligible
(e) patients on prn codeine/dihydrocodeine: to refrain completely for 3 days

2. Tramadol

(a) 150 mg/day or less (short-acting): withdrawal 3 days
(b) any long-acting tramadol or short-acting 180-400 mg/day: withdrawal   5 days
(c) prn use: to refrain completely for 3 days

A23. Will interviews/ questionnaires or group discussions include topics that might be sensitive, embarrassing or upsetting, or is it possible that criminal or other disclosures requiring action could occur during the study?

◯ Yes   ⦿ No

A24. What is the potential for benefit to research participants?

There is no direct benefit.

A26. What are the potential risks for the researchers themselves? *(if any)*

There are no risks for the researchers.

RECRUITMENT AND INFORMED CONSENT

In this section we ask you to describe the recruitment procedures for the study. Please give separate details for different study groups where appropriate.

A27-1. How will potential participants, records or samples be identified? Who will carry this out and what resources will be used? *For example, identification may involve a disease register, computerised search of GP records, or review of medical records. Indicate whether this will be done by the direct healthcare team or by researchers acting under arrangements with the responsible care organisation(s).*

FMS patients will be identified by Dr. Chiu and Prof. Nurmikko in their respective clinics at Arrow Park Hospital and Walton Centre. Both collaborators see a large number of patients with FMS in their Out-Patient Clinics where they are involved in the diagnosis-making and management of such patients. As patients will be recruited from the clinic, there is no specific need for any separate recruitment policy.

A27-2. Will the identification of potential participants involve reviewing or screening the identifiable personal

information of patients, service users or any other person?

○ Yes   ⦿ No

Please give details below:
Dr. Chiu and Prof. Nurmikko as NHS Consultants will have access to their patients' records as usual and in all cases will be the persons to approach the candidate patients and obtain consent. No other member of the research team or auxiliary personnel will have access to the patients' medical records.   Participants in the control group (healthy participants) will be recruited based on advert, and this procedure does not involve the use of any identifiable personal information.

A28. Will any participants be recruited by publicity through posters, leaflets, adverts or websites?

⦿ Yes   ○ No

If Yes, please give details of how and where publicity will be conducted, and enclose copy of all advertising material (with version numbers and dates).

Advertisments aimed at recruiting healthy control subjects will be placed on dedicated poster places at the University of Liverpool, including the "digital notice board"

A29. How and by whom will potential participants first be approached?

The patients are those attending the Out-Patient Clinics held by Dr. Chiu or Professor Nurmikko. Each patient will be given a brief explanation of the study and handed the Patient Information Leaflet, following which they are asked to contact the department if they are interested in participating. Once interest has been expressed, both doctors will then liaise with the PhD student to prepare an individual study timetable for the patient. (The PhD Student will have an honorary contract with both hospitals). The healthy control participants will contact the PhD student directly.

A30-1. Will you obtain informed consent from or on behalf of research participants?

⦿ Yes   ○ No

If you will be obtaining consent from adult participants, please give details of who will take consent and how it will be done, with details of any steps to provide information (a written information sheet, videos, or interactive material). Arrangements for adults unable to consent for themselves should be described separately in Part B Section 6, and for children in Part B Section 7.

If you plan to seek informed consent from vulnerable groups, say how you will ensure that consent is voluntary and fully informed.

The consent will be taken by the Consultant or the PhD Student prior to the study. This will usually happen during the initial interview (Session 1). However, we ensure that there will be at least a 7-day period between reading the participant information sheet and signing the consent form which allows plenty of time for participants to consider their participation.

If you are not obtaining consent, please explain why not.

Please enclose a copy of the information sheet(s) and consent form(s).

A30-2. Will you record informed consent (or advice from consultees) in writing?

⦿ Yes   ○ No

A31. How long will you allow potential participants to decide whether or not to take part?

We recommend that after the introduction of the possibility of study participation the patient goes home, read the Participant Information Leaflet and contact the department as appropriate. The period between reading the Participant Information Sheet and signing the consent form will be at least 7 days.

A33-1. What arrangements have been made for persons who might not adequately understand verbal explanations or

written information given in English, or who have special communication needs?*(e.g. translation, use of interpreters)*

Due to the nature of the study in which the communication between the researchers and the participant must remain accurate, people with special communication needs or inadequate language skills cannot be entered into the study.

A33-2. What arrangements will you make to comply with the principles of the Welsh Language Act in the provision of information to participants in Wales?

Not applicable.

A35. What steps would you take if a participant, who has given informed consent, loses capacity to consent during the study? *Tick one option only.*

◉ The participant and all identifiable data or tissue collected would be withdrawn from the study. Data or tissue which is not identifiable to the research team may be retained.

◯ The participant would be withdrawn from the study. Identifiable data or tissue already collected with consent would be retained and used in the study. No further data or tissue would be collected or any other research procedures carried out on or in relation to the participant.

◯ The participant would continue to be included in the study.

◯ Not applicable – informed consent will not be sought from any participants in this research.

Further details:

CONFIDENTIALITY

In this section, personal data means any data relating to a participant who could potentially be identified. It includes pseudonymised data capable of being linked to a participant through a unique code number.

Storage and use of personal data during the study

A36. Will you be undertaking any of the following activities at any stage (including in the identification of potential participants)?*(Tick as appropriate)*

☐ Access to medical records by those outside the direct healthcare team

☐ Electronic transfer by magnetic or optical media, email or computer networks

☐ Sharing of personal data with other organisations

☐ Export of personal data outside the EEA

☑ Use of personal addresses, postcodes, faxes, emails or telephone numbers

☐ Publication of direct quotations from respondents

☐ Publication of data that might allow identification of individuals

☐ Use of audio/visual recording devices

☐ Storage of personal data on any of the following:

    ☐ Manual files including X–rays

    ☐ NHS computers

    ☐ Home or other personal computers

    ☑ University computers

    ☐ Private company computers

    ☐ Laptop computers

Further details:

The names, birth dates, postal addresses, and phone numbers will be noted and stored in locked file cabinets. The purpose for noting birth dates is that published research reports necessitate descriptions of age of research samples. The names and addresses will be obtained to allow contact of participants and arrange their visits.

A37. Please describe the physical security arrangements for storage of personal data during the study?

Any papers (e.g. questionnaires, forms) will be stored in a locked file cabinet in the office of the principal investigator, Dr. A. Stancak.

Electronic data (MR images, EEG recordings) will be saved under unique code numbers in the University of Liverpool password protected computer.

A38. How will you ensure the confidentiality of personal data? *Please provide a general statement of the policy and procedures for ensuring confidentiality, e.g. anonymisation or pseudonymisation of data.*

Throughout the study the NHS Code of Confidentiality will be followed.

As for anonymisation of data the following procedure will be used: Every participant will receive a code. The coding will follow the system implemented in the brain imaging centre (MARIARC) of University of Liverpool.   For instance, data from MRI recordings will be named Tddmmyy_N (N = order of the recording at particular day), or the EEG files will be named Eddmmyy_N. All subsequent files generated during data processing will be expanded by appending additional letters to the generic code. The data will be linked anonymous, with the principal investigator being able to access and link personal information.

A40. Who will have access to participants' personal data during the study? *Where access is by individuals outside the direct care team, please justify and say whether consent will be sought.*

Dr. Yee Chiu, Professor T. Nurmikko as Consultants in charge of the participant patients' care will have access to their personal data as shown in the medical records. As for healthy volunteers, no attempt will be made to access their medical records,

Dr. Stancak and the PhD student will have access to names, birth dates, and phone numbers of participants. Participants' consents with sharing their personal data under these terms will be obtained.

Storage and use of data after the end of the study

A41. Where will the data generated by the study be analysed and by whom?

Data will be analysed by the PhD. student and by the principal investigator at School of Psychology, University of Liverpool, Eleanor Rathbone Building, room 243.

A42. Who will have control of and act as the custodian for the data generated by the study?

	Title	Forename/Initials	Surname
	Dr.	Andrej	Stancak
Post	Senior Lecturer		
Qualifications	BSc. in psychology, PhD. in physiology, senior lecturer in psychobiology of pain, track record of clinical and experimental research		
Work Address	School of Psychology		
	Bedford Street South		
	Liverpool		
Post Code	L69 7ZA		
Work Email	a.stancak@liverpool.ac.uk		
Work Telephone	01517946951		
Fax	01517942954		

A43. How long will personal data be stored or accessed after the study has ended?

○ Less than 3 months
○ 3 – 6 months
◉ 6 – 12 months
○ 12 months – 3 years
○ Over 3 years

A44. For how long will you store research data generated by the study?

Years: 5
Months: 0

A45. Please give details of the long term arrangements for storage of research data after the study has ended. *Say where data will be stored, who will have access and the arrangements to ensure security.*

Paper data will be stored in a locked file cabinet in principal investigator's office.

Digital data will be stored on a password protected university computer.

INCENTIVES AND PAYMENTS

A46. Will research participants receive any payments, reimbursement of expenses or any other benefits or incentives for taking part in this research?

◉ Yes   ○ No

If Yes, please give details. For monetary payments, indicate how much and on what basis this has been determined.
Participant will receive £60 to cover their travel expenses associated with visiting MARIARC and/or Aintree hospital.

A47. Will individual researchers receive any personal payment over and above normal salary, or any other benefits or incentives, for taking part in this research?

◉ Yes   ○ No

If Yes, please indicate how much and on what basis this has been decided:
The PhD student's living costs and tuition will be covered by the studentship funded by the Pain Relief Foundation.

A48. Does the Chief Investigator or any other investigator/collaborator have any direct personal involvement (e.g. financial, share holding, personal relationship etc.) in the organisations sponsoring or funding the research that may give rise to a possible conflict of interest?

○ Yes   ◉ No

NOTIFICATION OF OTHER PROFESSIONALS

A49-1. Will you inform the participants' General Practitioners (and/or any other health or care professional responsible for their care) that they are taking part in the study?

◉ Yes   ○ No

If Yes, please enclose a copy of the information sheet/letter for the GP/health professional with a version number and date.

A49-2. Will you seek permission from the research participants to inform their GP or other health/ care professional?

⦿ Yes   ○ No

It should be made clear in the participant's information sheet if the GP/health professional will be informed.

PUBLICATION AND DISSEMINATION

A50. Will the research be registered on a public database?

○ Yes   ⦿ No

Please give details, or justify if not registering the research.
Not a clinical trial. No appropriate registry exists.

This study will be listed in the Annual Report of the Pain Relief Foundation .

A51. How do you intend to report and disseminate the results of the study? *Tick as appropriate:*

☑ Peer reviewed scientific journals

☐ Internal report

☑ Conference presentation

☐ Publication on website

☐ Other publication

☐ Submission to regulatory authorities

☐ Access to raw data and right to publish freely by all investigators in study or by Independent Steering Committee on behalf of all investigators

☐ No plans to report or disseminate the results

☐ Other (please specify)

A52. If you will be using identifiable personal data, how will you ensure that anonymity will be maintained when publishing the results?

We will not use any identifiable data in data analysis, and therefore final results will not contain any links to a specific participant.

A53. Will you inform participants of the results?

⦿ Yes   ○ No

Please give details of how you will inform participants or justify if not doing so.
A letter describing results of the study will be sent to every participant.

---

5. Scientific and Statistical Review

A54. How has the scientific quality of the research been assessed? *Tick as appropriate:*

☑ Independent external review

☐ Review within a company

☐ Review within a multi−centre research group

☐ Review within the Chief Investigator's institution or host organisation

☐ Review within the research team

☐ Review by educational supervisor

☐ Other

Justify and describe the review process and outcome. If the review has been undertaken but not seen by the researcher, give details of the body which has undertaken the review:
The project has been reviewed by two international experts and by the board of experts selected by Pain Relief Foundation.

For all studies except non-doctoral student research, please enclose a copy of any available scientific critique reports, together with any related correspondence.

For non-doctoral student research, please enclose a copy of the assessment from your educational supervisor/ institution.

A56. How have the statistical aspects of the research been reviewed? *Tick as appropriate:*

☐ Review by independent statistician commissioned by funder or sponsor

☐ Other review by independent statistician

☐ Review by company statistician

☐ Review by a statistician within the Chief Investigator's institution

☐ Review by a statistician within the research team or multi–centre group

☐ Review by educational supervisor

☐ Other review by individual with relevant statistical expertise

☑ No review necessary as only frequencies and associations will be assessed – details of statistical input not required

In all cases please give details below of the individual responsible for reviewing the statistical aspects. If advice has been provided in confidence, give details of the department and institution concerned.

Title Forename/Initials Surname

Department
Institution
Work Address

Post Code
Telephone
Fax
Mobile
E-mail

Please enclose a copy of any available comments or reports from a statistician.

A57. What is the primary outcome measure for the study?

There is no single outcome measure in this study. Variables of interest are:
1. Cardiac awareness score
2. EEG power in 8-13 Hz and 15-30 Hz band and band power changes during viewing pain photographs and brushing.
3. Resting state EEG rhythms.
4. Structural images of the brain that will be processed in group analysis using voxel based morphometry method.
5. Functional brain images that will be processed using independent component analysis.

A58. What are the secondary outcome measures? *(if any)*

A59. What is the sample size for the research? *How many participants/samples/data records do you plan to study in total? If there is more than one group, please give further details below.*

Total UK sample size: 50

Total international sample size (including UK):

Total in European Economic Area:

Further details:
Twenty-five patients with FMS, and twenty-five age- and sex matched healthy controls.

A60. How was the sample size decided upon? *If a formal sample size calculation was used, indicate how this was done, giving sufficient information to justify and reproduce the calculation.*

The group sizes were determined based on previous clinical experimental studies in pain patients and particularly in FMS patients.

A61. Will participants be allocated to groups at random?

◯ Yes   ⦿ No

A62. Please describe the methods of analysis (statistical or other appropriate methods, e.g. for qualitative research) by which the data will be evaluated to meet the study objectives.

EEG data will be analysed using event-related desynchronisation method and by low-resolution electromagnetic tomography methods.

Resting cardiovascular and respiratory signals will be analysed by spectral analysis of time series.

Structural and functional brain images will be analysed using voxel based morphometry and by independent component analysis.

Final data will be analysed using STATISTICA v. 6 (analysis of variance, correlation analysis, multiple regression analysis).

---

6. MANAGEMENT OF THE RESEARCH

A63. Other key investigators/collaborators. *Please include all grant co-applicants, protocol co-authors and other key members of the Chief Investigator's team, including non-doctoral student researchers.*

	Title Forename/Initials Surname
	Prof. Turo J. Nurmikko
Post	Professor of Pain Science; Honorary Consultant in Pain Relief
Qualifications	MD, PhD
Employer	School of Clinical Sciences, University of Liverpool
Work Address	Lower Lane
	Liverpool
Post Code	L9 7AL
Telephone	015 1529 5820
Fax	015 1529 5821

Mobile   015 1529 5820
Work Email   tjn@liverpool.ac.uk

                Title   Forename/Initials Surname
                M.D., PhD. Yee Ho   Chiu
Post   clinical rheumatologist
Qualifications medical doctor; rheumatology specialist; record of previous clinical pain research
Employer   Wirral University Teaching Hospital NHS Foundation Trust
Work Address   Arrow Park Road, Upton, Wirral

Post Code   CH49 5PE
Telephone   0151 678 5111
Fax
Mobile   0151 678 5111
Work Email   yeechiu@liverpool.ac.uk

A64. Details of research sponsor(s)

A64-1. Sponsor

Lead Sponsor

Status: ○ NHS or HSC care organisation   Commercial status: Non-Commercial
          ◉ Academic
          ○ Pharmaceutical industry
          ○ Medical device industry
          ○ Local Authority
          ○ Other social care provider (including voluntary sector or private organisation)
          ○ Other

          If Other, please specify:

Contact person

Name of organisation University of Liverpool
Given name   Sarah
Family name   Fletcher
Address   Foresight Building, Brownlow Street
Town/city   Liverpool
Post code   L69 3GL
Country   UNITED KINGDOM
Telephone   0151 7948290
Fax
E-mail   ethics@liverpool.ac.uk

Is the sponsor based outside the UK?
○ Yes   ○ No

Where the lead sponsor is not established within the UK, a legal representative in the UK may need to be appointed. Please consult the guidance notes.

Co-Sponsor

Status: ○ NHS or HSC care organisation   Commercial status:

        ○ Academic

        ○ Pharmaceutical industry

        ○ Medical device industry

        ○ Local Authority

        ○ Other social care provider (including voluntary sector or private organisation)

        ○ Other

      If Other, please specify:

Contact person

Name of organisation

Given name

Family name

Address

Town/city

Post code

Country

Telephone

Fax

E-mail

Is the sponsor based outside the UK?

○ Yes   ○ No

Where the lead sponsor is not established within the UK, a legal representative in the UK may need to be appointed. Please consult the guidance notes.

A64-2. Please explain how the responsibilities of sponsorship will be assigned between the co-sponsors listed in A64-1

The University of Liverpool will be the sole sponsor for this project.

A65. Has external funding for the research been secured?

☑ Funding secured from one or more funders

☐ External funding application to one or more funders in progress

☐ No application for external funding will be made

Please give details of funding applications.

Organisation	Pain Relief Foundation
Address	Clinical Sciences Centre
	University Hospital Aintree

217

Lower Lane
Post Code   L9 7AL
Telephone   01515295820
Fax
Mobile   01515295820
Email   administrator@painrelieffoundation.org.uk

Funding Application Status: ◉ Secured ◯ In progress

Amount: £ 60.0000

Duration
Years: 3
Months: 0

If applicable, please specify the programme/ funding stream:

What is the funding stream/ programme for this research project?

What type of research project is this?

◯ Standalone project

◯ Project that is part of a programme grant

◯ Project that is part of a fellowship/ personal award/ research training award

◯ Other

Other – please state:

A66. Has responsibility for any specific research activities or procedures been delegated to a subcontractor (other than a co-sponsor listed in A64-1) ? *Please give details of subcontractors if applicable.*

◯ Yes   ◉ No

A67. Has this or a similar application been previously rejected by a Research Ethics Committee in the UK or another country?

◯ Yes   ◉ No

Please provide a copy of the unfavourable opinion letter(s). You should explain in your answer to question A6-2 how the reasons for the unfavourable opinion have been addressed in this application.

A68. Give details of the lead NHS R&D contact for this research:

	Title Forename/Initials Surname
	Dr. Andrew Pennington
Organisation	The Walton Centre NHS Foundation Trust
Address	Lower Lane, Fazakerley

Post Code   L9 7LJ
Work Email   andrew.pennington@thewaltoncentre.nhs.uk
Telephone   0151 529 5718
Fax

Mobile   0151 529 5718

Details can be obtained from the NHS R&D Forum website: http://www.rdforum.nhs.uk

---

A69-1. How long do you expect the study to last in the UK?

Planned start date: 01/08/2010
Planned end date: 31/12/2012
Total duration:
Years: 3 Months: Days:

A71-1. Is this study?

◉ Single centre
◯ Multicentre

A71-2. Where will the research take place? *(Tick as appropriate)*

☑ England
☑ Scotland
☑ Wales
☑ Northern Ireland
☐ Other countries in European Economic Area

Total UK sites in study 3

Does this trial involve countries outside the EU?
◯ Yes   ◉ No

A72. What host organisations (NHS or other) in the UK will be responsible for the research sites? *Please indicate the type of organisation by ticking the box and give approximate numbers of planned research sites:*

☑ NHS organisations in England   2
☐ NHS organisations in Wales
☐ NHS organisations in Scotland
☐ HSC organisations in Northern Ireland
☐ GP practices in England
☐ GP practices in Wales
☐ GP practices in Scotland
☐ GP practices in Northern Ireland
☐ Social care organisations
☐ Phase 1 trial units
☐ Prison establishments
☐ Probation areas
☐ Independent hospitals
☑ Educational establishments   1
☐ Independent research units
☐ Other (give details)

Total UK sites in study: 3

A73-1. Will potential participants be identified through any organisations other than the research sites listed above?

◯ Yes   ⦿ No

A74. What arrangements are in place for monitoring and auditing the conduct of the research?

Usual monitoring measures apply. This is likely to entail annual reporting by the Principal Investigator to the relevant Ethics and Research Governance Committees on the progress of the study.

A76. Insurance/ indemnity to meet potential legal liabilities

Note: in this question to NHS indemnity schemes include equivalent schemes provided by Health and Social Care (HSC) in Northern Ireland

A76-1. What arrangements will be made for insurance and/or indemnity to meet the potential legal liability of the sponsor(s) for harm to participants arising from the <u>management</u> of the research? *Please tick box(es) as applicable.*

<u>Note</u>: *Where a NHS organisation has agreed to act as sponsor or co-sponsor, indemnity is provided through NHS schemes. Indicate if this applies (there is no need to provide documentary evidence). For all other sponsors, please describe the arrangements and provide evidence.*

☐ NHS indemnity scheme will apply (NHS sponsors only)

☑ Other insurance or indemnity arrangements will apply (give details below)

University clinical trials and professional liability insurances will apply to the project as appropriate. This is on the assumption that no part of the study will take place outside of the UK.

Please enclose a copy of relevant documents.

A76-2. What arrangements will be made for insurance and/ or indemnity to meet the potential legal liability of the sponsor(s) or employer(s) for harm to participants arising from the <u>design</u> of the research? *Please tick box(es) as applicable.*

<u>Note</u>: *Where researchers with substantive NHS employment contracts have designed the research, indemnity is provided through NHS schemes. Indicate if this applies (there is no need to provide documentary evidence). For other protocol authors (e.g. company employees, university members), please describe the arrangements and provide evidence.*

☐ NHS indemnity scheme will apply (protocol authors with NHS contracts only)

☑ Other insurance or indemnity arrangements will apply (give details below)

University clinical trials and professional liability insurances will apply to the project as appropriate. This is on the assumption that no part of the study will take place outside of the UK.

Please enclose a copy of relevant documents.

A76-3. What arrangements will be made for insurance and/ or indemnity to meet the potential legal liability of investigators/collaborators arising from harm to participants in the <u>conduct</u> of the research?

<u>Note</u>: *Where the participants are NHS patients, indemnity is provided through the NHS schemes or through professional indemnity. Indicate if this applies to the whole study (there is no need to provide documentary evidence). Where non-NHS sites are to be included in the research, including private practices, please describe the arrangements which will be made at these sites and provide evidence.*

☑ NHS indemnity scheme or professional indemnity will apply (participants recruited at NHS sites only)

☑ Research includes non-NHS sites (give details of insurance/ indemnity arrangements for these sites below)

University clinical trials and professional liability insurances will apply to the project as appropriate. This is on the assumption that no part of the study will take place outside of the UK.

Please enclose a copy of relevant documents.

A78. Could the research lead to the development of a new product/process or the generation of intellectual property?

○ Yes ◉ No ○ Not sure

PART C: Overview of research sites

Please enter details of the host organisations (Local Authority, NHS or other) in the UK that will be responsible for the research sites. *For NHS sites, the host organisation is the Trust or Health Board. Where the research site is a primary care site, e.g. GP practice, please insert the host organisation (PCT or Health Board) in the Institution row and insert the research site (e.g. GP practice) in the Department row.*

Research site		Investigator/ Collaborator/ Contact	
Institution name	University of Liverpool	Title	
Department name	School of Psychology	First name/ Initials	Andrej
Street address	Bedford Street South		
Town/city	Liverpool	Surname	Stancak
Post Code	L69 7ZA		
Institution name	The Walton Centre NHS Foundation Trust	Title	
Department name	Neurosurgery, Pain & Critical Care	First name/ Initials	Turo
Street address	Lower Lane		
Town/city	Liverpool	Surname	Nurmikko
Post Code	L9 7AL		
Institution name	Wirral University Teaching Hospital NHS Trust	Title	
Department name		First name/ Initials	Yee
Street address	Arrow Park Road		
Town/city	Upton, Wirral	Surname	Chiu
Post Code	CH49 PE		

221

PART D: Declarations

D1. Declaration by Chief Investigator

1. The information in this form is accurate to the best of my knowledge and belief and I take full responsibility for it.

2. I undertake to abide by the ethical principles underlying the Declaration of Helsinki and good practice guidelines on the proper conduct of research.

3. If the research is approved I undertake to adhere to the study protocol, the terms of the full application as approved and any conditions set out by review bodies in giving approval.

4. I undertake to notify review bodies of substantial amendments to the protocol or the terms of the approved application, and to seek a favourable opinion from the main REC before implementing the amendment.

5. I undertake to submit annual progress reports setting out the progress of the research, as required by review bodies.

6. I am aware of my responsibility to be up to date and comply with the requirements of the law and relevant guidelines relating to security and confidentiality of patient or other personal data, including the need to register when necessary with the appropriate Data Protection Officer. I understand that I am not permitted to disclose identifiable data to third parties unless the disclosure has the consent of the data subject or, in the case of patient data in England and Wales, the disclosure is covered by the terms of an approval under Section 251 of the NHS Act 2006.

7. I understand that research records/data may be subject to inspection by review bodies for audit purposes if required.

8. I understand that any personal data in this application will be held by review bodies and their operational managers and that this will be managed according to the principles established in the Data Protection Act 1998.

9. I understand that the information contained in this application, any supporting documentation and all correspondence with review bodies or their operational managers relating to the application:

    - Will be held by the main REC or the GTAC (as applicable) until at least 3 years after the end of the study; and by NHS R&D offices (where the research requires NHS management permission) in accordance with the NHS Code of Practice on Records Management.
    - May be disclosed to the operational managers of review bodies, or the appointing authority for the main REC, in order to check that the application has been processed correctly or to investigate any complaint.
    - May be seen by auditors appointed to undertake accreditation of RECs.
    - Will be subject to the provisions of the Freedom of Information Acts and may be disclosed in response to requests made under the Acts except where statutory exemptions apply.

10. I understand that information relating to this research, including the contact details on this application, may be held on national research information systems, and that this will be managed according to the principles established in the Data Protection Act 1998.

11. I understand that the summary of this study will be published on the website of the National Research Ethics Service (NRES), together with the contact point for enquiries named below. Publication will take place no earlier than 3 months after issue of the ethics committee's final opinion or the withdrawal of the application.

Contact point for publication *(Not applicable for R&D Forms)*

NRES would like to include a contact point with the published summary of the study for those wishing to seek further information. We would be grateful if you would indicate one of the contact points below.

☑ Chief Investigator

☐ Sponsor

☐ Study co-ordinator

☐ Student
☐ Other – please give details
☐ None

Access to application for training purposes *(Not applicable for R&D Forms)*
Optional – please tick as appropriate:

☐ I would be content for members of other RECs to have access to the information in the application in confidence for training purposes. All personal identifiers and references to sponsors, funders and research units would be removed.

Signature: ....

Print Name: Andrej Stancak

Date: 30/09/2009   *(dd/mm/yyyy)*

D2. Declaration by the sponsor's representative

If there is more than one sponsor, this declaration should be signed on behalf of the co–sponsors by a representative of the lead sponsor named at A64-1.

I confirm that:

1. This research proposal has been discussed with the Chief Investigator and agreement in principle to sponsor the research is in place.

2. An appropriate process of scientific critique has demonstrated that this research proposal is worthwhile and of high scientific quality.

3. Any necessary indemnity or insurance arrangements, as described in question A76, will be in place before this research starts. Insurance or indemnity policies will be renewed for the duration of the study where necessary.

4. Arrangements will be in place before the study starts for the research team to access resources and support to deliver the research as proposed.

5. Arrangements to allocate responsibilities for the management, monitoring and reporting of the research will be in place before the research starts.

6. The duties of sponsors set out in the Research Governance Framework for Health and Social Care will be undertaken in relation to this research.

7. I understand that the summary of this study will be published on the website of the National Research Ethics Service (NRES), together with the contact point for enquiries named in this application. Publication will take place no earlier than 3 months after issue of the ethics committee's final opinion or the withdrawal of the application.

Signature: ....

Print Name: Mrs. Sarah Fletcher

Post: Research Governance officer

Organisation: University of Liverpool

Date: 30/09/2009   *(dd/mm/yyyy)*

D3. Declaration for student projects by academic supervisor

1. I have read and approved both the research proposal and this application. I am satisfied that the scientific content of the research is satisfactory for an educational qualification at this level.

2. I undertake to fulfil the responsibilities of the Chief Investigator and the supervisor for this study as set out in the Research Governance Framework for Health and Social Care.

3. I take responsibility for ensuring that this study is conducted in accordance with the ethical principles underlying the Declaration of Helsinki and good practice guidelines on the proper conduct of research, in conjunction with clinical supervisors as appropriate.

4. I take responsibility for ensuring that the applicant is up to date and complies with the requirements of the law and relevant guidelines relating to security and confidentiality of patient and other personal data, in conjunction with clinical supervisors as appropriate.

Signature: ....

Print Name:

Post:

Organisation:

Date: (dd/mm/yyyy)

---

14 January 2010

Dr. Andrej Stancak
senior lecturer
University of Liverpool
Eleanor Rathbone Building
Bedford Street South
L69 7ZA

Dear Dr. Stancak

Study Title:	**Brain-autonomic interactions in fibromyalgia syndrome patients**
REC reference number:	**09/H1001/92**
Protocol number:	**1.0**

Thank you for your letter of 17th December 2008, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered by the Chair.

Confirmation of ethical opinion

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

Ethical review of research sites

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

Conditions of the favourable opinion

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

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

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System

226

or at http://www.rdforum.nhs.uk. *Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.*

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

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

Approved documents

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

Document	Version	Date	
Covering Letter		12 October 2009	
REC application	2.3		
Protocol	1.0	28 August 2009	
Investigator CV	Dr Andrej Stancak	28 August 2009	
Participant Information Sheet	1.0	20 August 2009	
Participant Consent Form	1	16 September 2009	
Letter from Sponsor		25 September 2009	
Questionnaire			
Questionnaire: illness Attitude Scale			
Questionnaire: Sleep Disorders Questinnaire			
Questionnaire: Pain catastrophizing Scale			
Questionnaire: The FIQ Directions and Questions			
C.V. - Yee Ho Chiu			
C.V. - turo Juhani Nurmikko		24 September 2009	
Letter from Funder		17 May 2009	
Questionnaire: BDI II			
Covering Letter	2	17 December 2009	
Participant Information Sheet	2		
Participant Information Sheet	2		

Participant Consent Form	2		
Participant Consent Form	2		
Response to Request for Further Information			

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

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

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

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

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

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H1001/92	**Please quote this number on all correspondence**

Yours sincerely

Dr Peter Owen
Chair

Email: sue.culshaw@liverpoolpct.nhs.uk

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Mrs. Sarah Fletcher*
R&D office

North West 4 Research Ethics Committee Liverpool North
LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	09/H1001/92	Issue number:	1	Date of issue:	14 January 2010

Chief Investigator:	Dr. Andrej Stancak

Full title of study:	Brain-autonomic interactions in fibromyalgia syndrome patients

This study was given a favourable ethical opinion by North West 4 Research Ethics Committee Liverpool North on 25 November 2009. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	Post	Research site	Site assessor	Date of favourable opinion for this site	Notes [1]
Dr Andej Stancak		.University of Liverpool	North West 4 Research Ethics Committee Liverpool North	14/01/2010	

Approved by the Chair on behalf of the REC:

…………………………………………… (Signature of Chair/Co-ordinator)
(delete as applicable)

………………………………………… (Name)

(1)   *The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded*

Dr. A. Stancak
School of Psychology
Liverpool
L69 7ZA
Tel :0151 794 6951
email: a.stancak@liv.ac.uk

Volunteer Identification Number:

PARTICIPANT CONSENT FORM

Study: Brain-autonomic interactions in fibromyalgia syndrome patients

Investigators: Prof. A Stancak, Prof. T. Nurmikko, Dr. Y. Chiu

Please tick each box

1. I confirm that I have read and understood the Participant Information Sheet for the above study and have had the opportunity to ask questions.

2. I understand that if an incidental abnormality is picked up on my scan I agree to the procedure as set out in the information sheet.

3. I give permission for the researchers to routinely contact my GP during the course of the research

4. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.

5. I agree that personal data relating to myself (as defined by the Data Protection Act, 1998), being used for research purposes only. I understand that my personal information will be kept for up to five years and then will be destroyed.

6. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the University of Liverpool, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

7. I agree to take part in the above study.

_____   _____   _____
Name of Volunteer   Date   Signature

_____   _____   _____
Name of person taking consent   Date   Signature

(if different from researcher)

_____   _____   _____
Name of Researcher   Date   Signature

UNIVERSITY OF
LIVERPOOL

Professor Andrej Stancak
School of Psychology
Eleanor Rathbone
Building,
Bedford Street South,
Liverpool.
L69 7ZA.
Tel: 0151-794-2961

Participant Information Leaflet

Brain-autonomic interactions in fibromyalgia syndrome patients

You are being invited to take part in a research study. Before you decide whether to take part, it is important to understand why the research is being done and what it will involve. Please take some time to read the following information carefully and feel free to discuss it with others if you wish. Ask the researcher if there is anything that is not clear or if you would like more information. Take your time to decide whether or not you wish to take part.

What is the purpose of the study?
The study aims to investigate differences in brain activity and structure in people with fibromyalgia and healthy volunteer subjects.

Why have I been chosen to take part?
We have chosen you to take part in the study because you have been diagnosed with fibromyalgia and are otherwise healthy, or because you are a suitable healthy volunteer.

Do I have to take part?
Your participation in this study is completely voluntary, and you are free to refuse to take part or withdraw from the study at any time.

What will happen to me if I take part?

You will be invited for three sessions that will take place approximately two weeks apart. Sessions one and two can be completed on the same day for your convenience.

Will my GP be informed?

Your GP will be notified of your participation in the study and any relevant information may be shared with your GP during the course of the research.

Session 1:

The purpose of Session 1 is to collect information about your mood and psychological traits as well as any pain symptoms you may have. You will be asked to fill in a number of questionnaires, including: Sleep Problem Questionnaire, Pain Catastrophising Scale, State and Trait Anxiety Scale, Beck Depression Inventory, Illness Attitude Scale, and Fibromyalgia Impact Questionnaire.

At the end of Session 1, we will examine the level of tenderness in 18 preselected points in your body (neck, shoulders, elbows, wrists, back, hips, knees and ankles) by exerting mild-to-moderate pressure on them. This mimics the clinical examination made by a doctor to diagnose a chronic pain condition known as fibromyalgia. The exam uses a simple hand-held device to deliver a standard pressure on these points. You will be asked to indicate on a scale from 0 to 4 any pain felt during each pressure stimulus.

Session 1 will last about 45 minutes and it will take place in the Sensory-Motor Laboratory, a dedicated pain measurement laboratory facility, in the Pain Relief Foundation, Clinical Sciences Centre, University Hospital Aintree, Lower Lane, L9 7AL.

Session 2:

The purpose of Session 2 is to collect data attributed to electrical activity in the brain and other physiological data, using methods similar to those found in common medical tests, to better understand how the brain reacts to various challenges. Electroencephalography (EEG) is a standard method for recording electrical currents of the brain. We will use a system that enables us to adapt 64 electrodes onto your head quickly (10 min) through a cap which you will wear. At the same time, heart rate (EEG), respiratory movements and activity of the sweat glands on your fingers will be recorded. Throughout these tests you will sit in a comfortable chair and view a computer screen.

There will be four experimental conditions in Session 2:

1. Resting recording: you will rest with eyes closed for about 10 min. Before and after the recording, you will indicate your mood by attributing a number from 0 to 4 to various moods such as "joy" or "sadness".
2. Cardiac awareness test. You will be asked to estimate the number of your heart beats occurring during a defined period of time. Your estimate should not be based, however, on counting your heart beats e.g. on your wrist. This condition will last about 4 minutes.
3. Viewing 50 photographs containing pain scenes and 50 photographs with a similar content and setup, but not containing pain. The photographs will be presented in random order with 15 second intervals. Each photograph will be displayed for 4 seconds. You will also be asked to rate the intensity of pain you would associate with each photograph on a computerised scale ranging from 0 (no pain) to 7 (worst possible pain).
4. Periods of brushing and rest. The researcher will brush your right elbow for the period of 4 seconds and wait for another 4 seconds (rest). There will be 20 brushing-rest cycles.

232

Sesssion 2 will last about 1 hour and also take place in the Sensory-Motor Laboratory, Pain Relief Foundation, University Hospital Aintree, Lower Lane, L9 7AL.

Session 3:

The purpose of Session 3 is to use an fMRI (functional magnetic resonance imaging) scan to analyse the structure and function of the brain. You will first be interviewed by Mrs. Valerie Adams in MARIARC to be sure that magnetic resonance scanning is feasible. You will change into a hospital gown, and be asked to lie down on a scanner table. The MR session consists of four scans:
1. One anatomical scan (2 min) will be taken for diagnostic purposes.
2. One functional scan (20 min) will be acquired to analyse the dynamics of brain activity at rest. During functional scanning, you will rest with eyes closed and press a button with your right hand whenever you hear a beep.
3. One anatomical scan (10 min) for fine structural analysis of the brain's cortical matter.
4. One anatomical scan (7 min) to image the structure of the brain white matter.

The total imaging time will be 39 minutes, and the whole session with assessment and instructions will not last longer than 1 hour. Session 3 will take place in the brain imaging centre of University of Liverpool, MARIARC, Pembroke Place, L69 3GE.

Will I have to withdraw medication?

 As some of drugs used to alleviate pain can modulate the brain activity, we may suggest withdrawal of some of your pain medication for 3 to 5 days before Session 2 and Session 3. You are allowed to continue to use paracetamol as recommended by our doctor. If you use a medication that contains paracetamol on an opioid-based drug (co-codamol, co-dydramol, co-proxamol) or an opioid medication without paracetamol (e.g., dihydrocodeine, tramadol) the withdrawal will depend on the average daily dose you are taking. For example, if you usually use 6 to 8 tablets of mild co-codamol (8/500) a day only, you will be asked to stop it 3 days before the test. If, by contrast, you are taking up to 8 tablets of a strong co-codamol (30/500) or high doses of dihydrocodeine, you will be asked to taper the dose over a period of 5 days. We will try to arrange tests on consecutive or nearly consecutive days so that the drug withdrawal will only happen once during your participation.

The exact information about when and what drug should be withdrawn will be provided in written form by the clinical team (Prof. Nurmikko or Dr. Chiu) and will be made in complete agreement with you. You will be able to resume medication immediately after the experiments.

Medications that have no or minimal central nervous system effect will not be withdrawn.This includes paracetamol which you may take up to 4000 mg/day (8 tablets containing 500 mg). If you are on another stable, regular medication for fibromyalgia it may be continued, depending on the dose. An example of an acceptable dose would be pregabalin 75 mg twice a day, gabapentin 300 mg twice a

day or amitriptyline 10 mg at night. Prof. Nurmikko and Dr. Chiu will advice you on this regard. You will not be asked to change any of the medication you may be on that has been prescribed for other medical conditions than fibromyalgia.

What are the possible disadvantages and risks of taking part?

The fMRI scan is very noisy but otherwise it causes no harm or long-term effects. High quality disposable earplugs will be provided to protect against the possibility of hearing loss. Some people may experience a feeling of claustrophobia in the scanner. If you do feel uncomfortable while being scanned you will be able to notify us immediately by pressing an alarm button and we will remove you from the scanner without delay.

There are no known risks from properly conducted magnetic resonance scanning. As it involves a strong magnetic field, certain standard precautions must be observed. Most importantly, we will NOT study you if you are fitted with a heart pacemaker, mini-defbrillator or a neurostimulator; if you have surgical clips in your head; if you have suffered injuries which may have left metal particles in your eye or head, or elsewhere in your body; or if you have an artificial heart valve. We will also ask about other kinds of surgery and metal implants which might affect your suitability. Some people find the scanner a claustrophobic or uncomfortable environment, and we will ask you about this.

Occasionally research studies using magnetic resonance imaging reveal significant unexpected abnormalities which require medical follow-up, either for further investigation or (more rarely) treatment. The scans we do are for research purposes, but we review them carefully to avoid missing such an abnormality. We will spend a few extra minutes taking high-quality images which we will routinely have reviewed by a consultant radiologist. If any significant abnormality is found, we will send the report to your GP, who will be able to take it further with you. Please note that this is not a substitute for a 'medical' magnetic resonance scan that a doctor might order to make a diagnosis. It should therefore not be seen as a 'health check'.

Will information about me be kept confidential?
All information that is collected about you during the course of the research will be kept strictly confidential by the researchers. Your data related to the MR study will be treated in an anonymous way. Your personal information that is collected on the safety screening form, will be kept for up to 15 years, and then confidentially destroyed. You have a legal right to view your personal information stored with us. If you wish to view your personal information, please write to the University of Liverpool Data Protection Officer, Computing Services Department, University of Liverpool.

Will my taking part be covered by an insurance scheme?

This research is sponsored by the University of Liverpool, and therefore the insurance cover is provided by the University of Liverpool.

If you have concerns about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your questions.

Please contact Dr Andrej Stancak on: **(0151) 794 6951**.

If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure (Details can be obtained from the PALS team, Ground Floor, University Hospital Aintree, phone: (0151) 5293287) or you can use the complaints procedure at the University of Liverpool, addressed to the Research Governance Officer in Legal Services (ethics@liv.ac.uk, 0151 794 8920).

The study does not involve any therapeutic interventions or potentially hazardous procedures. In the unlikely event that you become ill or suffer any injury as a direct result of a procedure of the study, the study doctor will arrange for the correct treatment. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against University of Liverpool but you may have to pay your legal costs.

What will happen to the results of the study?
Once analysed, the results of the research study will be presented at research meetings and published in scientific literature, so that other researchers can benefit from the sharing of information. The study will take at least three months to conduct and the results longer to analyse fully, but we would be happy to supply you with our final results after this time.

What will happen if I want to stop taking part?
During the course of the study, you are able to withdraw at any time without explanation. Your results up to the period of withdrawal may be used, if you are happy for this to be done. Otherwise you may request that they are destroyed and no further use is made of them.

What if I am unhappy or if there is a problem?
If you are unhappy, or if there is a problem, please feel free to let us know by contacting Prof Andrej Stancak on 0151-794-2961, and we will try to help. If you remain unhappy or have a complaint which you feel you cannot come to us with then you should contact the Research Governance Officer on 0151-794-8290 (ethics@liv.ac.uk). When contacting the Research Governance Officer, please provide details of the name or description of the study (so that it can be identified), the researcher(s) involved, and the details of the complaint you wish to make.

Thank you for taking time to read this information.